Docosahexaenoic Acid (DHA) Algal Oil

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
DHA Algal Oil – Includes Docosahexaenoic acid (DHA) and other triglycerides, including: myristic acid, palmitic acid, oleic acid, lauric acid, and capric acid in varying percentages. This review discusses both DHA Algal Oil, and DHA, where relevant. (Kyle, et al., 1995; Wyeth Nutritionals, 1998)

Other Names (DHA):
(4E,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid; DHA; cervonic acid; doconexent; (NLM, 2011b)

Trade Names: DHA Algal Oil: DHA Single-cell Oil, DHASCO®; **DHA:** Marinol D 50TG; Martek DHA HM; Ropufa 60; AquaGrow Advantage; AquaGrow Advantage; Doconexent; Martek DHA HM; Marinol D 50TG; Ropufa 60; (NLM, 2011b)

CAS Numbers:
6217-54-5 (USDA, 2010b); 25167-62-8 (Martek, 2010)

Other Codes:
CCRIS 7670; CCRIS 8534 (NLM, 2011b)

Characterization of Petitioned Substance

Composition of the Substance:

Docosahexaenoic acid (DHA) Algal Oil is the oil obtained from processing of the dinoflagellate *Crypthecodinium cohnii* or the thraustochytrid *Schizochytrium* species. DHA Algal Oil is composed mainly of triglycerides, and contains DHA at 35-45% concentration (Kyle, et al., 1995). DHA Algal Oil from *C. cohnii* contains other triglycerides including: myristic acid (13-20%), palmitic acid (12-25%), oleic acid (10-25%), lauric acid (2-6%), and capric acid (1%) (Kyle, et al., 1995). DHA Algal Oil from *Schizochytrium* species contains DHA (approx. 35%), palmitic acid (approx. 24%), docosapentaenoic acid (approx. 13.5%), myristic acid (approx. 10%), and eicosapentaenoic acid (EPA) (approx. 3%) (FDA, 2004b). DHA is an omega-3 fatty acid, which is a type of polyunsaturated fatty acid (PUFA) with 6 carbon double bonds. The last carbon double bond is located after the third carbon atom from the end of the carbon chain. The basic formula of DHA is $C_{22}H_{32}O_2$. The molecular structure of DHA is presented in Figure 1.

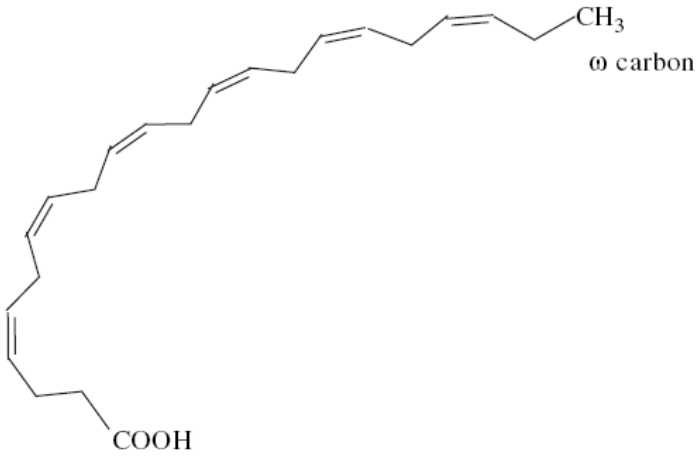


Figure 1: Chemical Structure of Docosahexaenoic Acid (Ruxton et al., 2004)

Properties of the Substance:

The physical and chemical properties of the substance are presented in Table 1. The properties presented in Table 1 describe either DHA Algal Oil or free DHA, as indicated.

Table 1. Chemical Properties of Docosahexaenoic Acid:

Chemical or Physical Property	Value
Color	DHA Algal Oil: yellow to light orange (Martek, 2010) DHA: clear to faintly yellow
Physical State	DHA Algal Oil: semi-solid to liquid (Martek, 2010), DHA: oily liquid
Molecular Weight	DHA: 328.49 (NLM, 2011b)
Odor	DHA Algal Oil: "Characteristic" odor, free from rancidity (Wyeth Nutritionals, 1998)
Melting Point	DHA: -44.5° to -44.1° C (Matreya, LLC, 2004)
Boiling Point	DHA: >300° C (Matreya, LLC, 2004)
Solubility	DHA: DMSO to 100 mM (Tocris Bioscience, 2010)
Stability	DHA: Stable (Matreya, LLC, 2004)
Reactivity	DHA: Not reactive (Matreya, LLC, 2004)
Oxidizing or Reduction Action	DHA: Incompatible with strong oxidizers – no inherent oxidizing or reduction action (Matreya, LLC, 2004)
Flammability	DHA: Not flammable (Matreya, LLC, 2004)
Hazardous Combustion/ Decomposition	DHA: May emit carbon dioxide, carbon monoxide, or nitrogen oxide if burned or decomposed by heat (Tocris Bioscience, 2010)

Specific Uses of the Substance:

The petitioned use of DHA Algal Oil is as an ingredient as a source of DHA in foods, beverages, infant formulas, and as a dietary supplement. Some of the foods and products the petitioner lists as intended or current foods to supplement with DHA Algal Oil include: cookies and crackers, breads and rolls, meat products, condiments, beverages (including flavored milk and milk products, soy milk, other dairy products, and juices), pasta, dietary supplements, and infant formula. DHA Algal Oil is added to these foods for nutritional purposes, as a source of DHA in the foods (Martek, 2010).

DHA is manufactured and sold as a dietary supplement. The DHA in dietary supplements may be obtained from fish oil or from DHA Algal Oil. Fish oil supplements commonly contain both DHA and EPA, as both PUFAs are components of fish oil. Dietary supplements such as DHA do not need specific approval from FDA before they are marketed and sold, but the manufacturer is required to determine that the supplement is "safe" before the supplement is marketed (FDA, 2009b). Supplements containing DHA may make the following health claim, as permitted by FDA: "Supportive but not conclusive research shows that consumption of EPA and DHA omega-3 fatty acids may reduce the risk of coronary heart disease. One serving of [name of food] provides [x] grams of EPA and DHA omega-3 fatty acids. [See nutrition information for total fat, saturated fat and cholesterol content.]" (FDA, 2004c).

Aside from its use as a food additive and dietary supplement, no information on other uses of DHA Algal Oil in agricultural handling or processing was found. DHA is considered an accessory nutrient by the USDA. The term "accessory nutrient" has not been written into law, but the term has been used to refer loosely to substances that are not specifically classified as vitamins or minerals but are found to promote optimal health (NOSB, 2011). Accessory nutrients can be contrasted with the essential nutrients such as the fatty acids linoleic acid (LA) and ALA, which cannot be synthesized by the body (Jump, 2009).

Approved Legal Uses of the Substance:

Omega-3 fatty acids are considered Generally Recognized as Safe (GRAS) by the FDA. Specifically, DHA Algal Oil), as a source of DHA, is GRAS when used alone or in combination with arachidonic acid (ARA) or EPA according to FDA GRAS Notices No. GRN 000041 and No. GRN 000137 (FDA, 2004b; FDA, 2001). The GRAS notices state that FDA has no objection to the use of DHA Algal Oil under the conditions of use (FDA, 2001).

Although DHA Algal Oil has achieved GRAS status, FDA noted that the incorporation of DHA Algal Oil into infant formula by a formula manufacturer would require the submission of documentation to FDA under section 412 of the Federal Food, Drug, and Cosmetic Act (FFDCA). Section 412 of FFDCA describes the additional statutory and regulatory requirements that apply to infant formula as compared to the regulation of other foods (FDA, 2006). Manufacturers of infant formula are not currently required to list on the label the amounts of DHA added to infant formula. However, most infant formula manufacturers provide this information (Institute of Medicine, 2005).

Action of the Substance:

Triglycerides, cholesterol, and phospholipids that enter the body through the diet are hydrolyzed by enzymes in the pancreas to produce fatty acids. Once in the digestive tract, bile salts help to incorporate the fatty acids and other products of digestion into micelles, or a spherical collection of lipids. The micelles are absorbed throughout the small intestine and incorporated into the tissues. The human body can then synthesize the longer chain PUFAs such as DHA and EPA from the relatively shorter fatty acids such as linoleic acid (LA) and α-linolenic acid (ALA) (Jump, 2009).

Synthesis of DHA from ALA occurs through multiple desaturation (carbon double bond addition) and elongation (two-carbon addition) reactions (Jump, 2009). Synthesis of DHA and EPA is inefficient in the body and it is estimated that DHA conversion from ALA less than about 5% for men and up to 9% for women (Birch et al., 2010; Jump, 2009). The low conversion rate of DHA from ALA may mean that DHA is an essential nutrient under some dietary conditions (Jump, 2009).

As mentioned in the "Specific Uses" section, DHA and other accessory nutrients are beneficial to health, but are not considered essential because they can be synthesized by the body from essential nutrients. Long chain PUFAs, including DHA, are found in high concentrations in the central nervous system (Birch et al., 2010). Specifically, DHA serves an important function as a structural membrane lipid, especially in the retina and nerve tissue, affecting the permeability, flexibility, fluidity, and the activity of enzymes in the membrane (Jump, 2009). Animal studies have shown that DHA is critical for the normal function and development of the retina. Research has shown that DHA plays a main role in the regeneration of rhodopsin, a visual pigment that functions to convert light to images received by the brain. When dietary omega-3 intake is low, the retina conserves and recycles DHA (Jump, 2009).

The last trimester of pregnancy has been identified as a critical time for accumulation of DHA in the brain and retina. For this reason, infants born preterm are at risk for incomplete visual and neurological development if they do not receive enough DHA in the diet. Human breast milk and infant formulas both contain ALA, but not all infant formulas contain DHA. Preterm infants can synthesize DHA, but not enough to prevent declines in the plasma levels of DHA that may be detrimental to development. The results of several randomized controlled trials of preterm and term infants fed formula enriched with DHA have been mixed. It is unclear from the trials whether DHA-enriched infant formula enhances neurological development or visual acuity in full term or preterm infants (Jump, 2009).

Combinations of the Substance:

DHA is a component of fish oil, and is listed on the National List of Allowed and Prohibited Substances (hereafter referred to as the National List) as such, with CAS number 25167-62-8. Fish oil is listed as a "nonorganically produced agricultural product allowed as an ingredient in or on processed products

labeled as 'organic'." (7 CFR § 205.606(f)). DHA is usually found in fish oil in combination with EPA, another long-chain PUFA which is also included in the listing for fish oil. The full listing from 7 CFR § 205.606(f) for fish oil is as follows:

> Fish oil (Fatty acid CAS #'s: 10417–94–4, and 25167–62–8)—stabilized with organic ingredients or only with ingredients on the National List, §§205.605 and 205.606.

When DHA is added to infant formula, ARA is also commonly added. ARA is an omega-6 fatty acid that can be synthesized by humans from LA. DHA is not a precursor to any substance identified on the National List.

A preservative (including tocopherols, ascorbyl palmitate, or others) can be added to DHA Algal Oil to prevent oxidation and related adverse effects on the nutritional quality, odor, and flavor of the oil (Bartee et al., 2007; Jacobsen, 2010). High oleic sunflower oil, tocopherols, rosemary extract or other antioxidants are generally added to DHA Algal Oil in varying amounts to achieve consistent DHA potency across batches and products (Martek, 2010).

DHA Algal Oil is petitioned for addition to infant formula, which contains a number of nutrients included on the National List by inference to FDA requirements for nutrient vitamins and minerals, in accordance with 21 CFR 104.20, Nutritional Quality Guidelines For Foods (7 CFR 205.605). Furthermore, a mixture of food ingredients comprising carbohydrates, proteins, fats, and stabilizers are expected to be included in infant formula and other foods to which DHA is added. These ingredients will vary significantly with the type of product and manufacturer.

Status

Historic Use:

The patent for production of DHA Algal Oil from *C. cohnii* was filed in 1990 and registered in 1995 (Kyle, et al., 1995). As cited in the patent application, marine dinoflagellates such as *C. cohnii* were a known source of PUFA in 1970. However, a method for culturing *C. cohnii* with the purpose of extracting the oil was not known until the registration of patents 5,374,657 and 5,407,957 by Martek in 1994 and 1995, respectively (Kyle, 1994; Kyle, et al., 1995). The patents describe the production of an edible oil containing predominantly DHA as the end product. The intent of the manufacturer of the oil was to include the oil in infant formulas, baby foods, and as dietary supplements in capsule form or by parenteral administration (injection) (Kyle, et al., 1995).

A study of DHA infant formula supplementation reports that both DHA and ARA have been added to U.S. infant formulas since 2002 (Birch et al., 2010). Martek reports that they began adding DHA and ARA oils to infant formula as early as 1994 (Martek, 2010). DHA and ARA were allowed to be included in organic foods, including infant formula, according to a 2006 decision by the NOP that interpreted 7 CFR, §205.605(b) and 21 CFR §104.20, which relied on the FDA GRAS determination for ARA and DHA. After consulting with FDA, NOP concluded in April of 2010 that their interpretation of §104.20 was incorrect, and requested that NOSB re-evaluate the classification of ARA and DHA as "nutrient vitamins and minerals" under 21 CFR §104.20. Producers of the chemicals in question, including ARA and DHA, were invited to petition for those chemicals to be added directly to the National List (USDA, 2010a).

OFPA, USDA Final Rule:

As discussed in the "Combinations of the Substance" sections, DHA is specifically listed by CAS number on the National List as a component of fish oil in 7 CFR § 205.606(f): "Nonorganically produced agricultural products allowed as ingredients in or on processed products labeled as 'organic'." It is further stipulated on the National List that the fish oil "…must be stabilized with organic ingredients or only with

ingredients on the National List, §§205.605 and 205.606." As discussed in the "Combinations of the Substance" section, the listing for DHA in the National List is as follows:

> Fish oil (Fatty acid CAS #'s: 10417–94–4, and 25167–62–8)—stabilized with organic ingredients or only with ingredients on the National List, §§205.605 and 205.606.

The CAS number for DHA in the National List is 25167-62-8. This CAS number is described in ChemIDplus (NLM, 2011b) as pertaining to "C22 unsaturated fatty acids found predominantly in fish oil." The CAS number 6217-54-5 is listed by USDA (2011b) for DHA, but the source of the DHA is not specifically identified. DHA Algal Oil is not listed on the National List.

As described in the "Historic Use" section, DHA was allowed to be included in organic foods, including infant formula, between 2006 and April of 2010. This was based on the NOP interpretation of 7 CFR, §205.605(b) and 21 CFR §104.20. In April of 2010, NOP stated that their interpretation was incorrect, and requested that NOSB re-evaluate the classification of ARA and DHA as "nutrient vitamins and minerals" under 21 CFR §104.20. Producers of the chemicals in question, including ARA and DHA, were able to petition for those chemicals to be added directly to the National List (USDA, 2010a).

International

The Canadian General Standards Board (CGSB) does not specifically list DHA or DHA Algal Oil as food ingredients or food processing ingredients. According to Standard 72 (1981) published by the CODEX Alimentarius Commission, DHA may be added to infant formula. The standard was adopted as a world-wide standard in 1981 and revised in 2007. The standard states that if DHA is added to infant formula, the content of ARA must be at least the same amount as that of DHA. In addition, the content of EPA cannot exceed the content of DHA. An exception is noted that, "National authorities may deviate from the above conditions, as appropriate for the nutritional needs." The guidance upper level (GUL) for DHA is 0.5% of the total fatty acids in the formula (Codex Alimentarius Commission, 1981).

DHA is not listed or described specifically in the Japan Agricultural Standard (JAS) for Organic Production. Plant lecithin, a processing ingredient and a source of omega-3 and omega-6 fatty acids, is listed as an allowed ingredient in processing, with the stipulation that it may not be treated with organic solvents. "Plant and animal oils" are also listed in the standard, with the stipulation that they may not be used for pest control in plants (MAFF, 2006).

Evaluation Questions for Substances to be used in Organic Handling

Evaluation Question #1: 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)).

DHA-rich oil can be made from the marine dinoflagellate species *Crypthecodinium cohnii* and the marine algae *Schizochytrium* species. *C. cohnii* and *Schizochytrium* species can be cultivated in a fermenter using similar techniques. The algae are cultivated in a nutrient solution for which natural, filtered or artificial seawater can be used. After addition of the seawater solution to the fermenter, the fermenter is sterilized and cooled. Next, nutrients and a seeding population of microorganisms are added to the fermenter. Typically, fermentation is initiated with about 10-20 g/liter glucose or other sugars as a carbon source for the fermentation. Yeast extract is added to the fermenter as a nitrogen source that also contains some micronutrients (Kyle, 1994; Kyle, et al., 1995; Martek, 2010).

To cause increased DHA production, a nitrogen deficiency is induced later in the fermentation process. The yeast extract is allowed to be depleted while the glucose remains. Once oil production begins, it is allowed to continue for approximately 24 hours. Total fermentation time is in the range of 60 to 90 hours

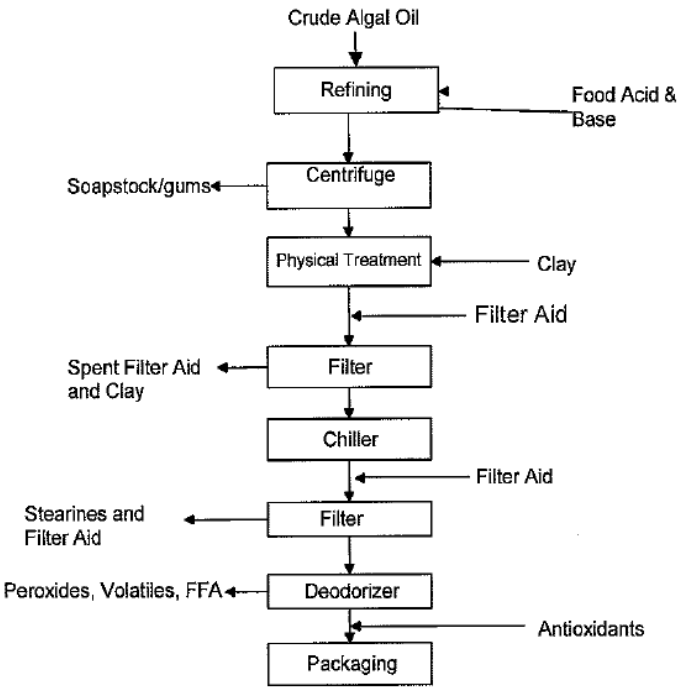
(Kyle, 1994; Kyle, et al., 1995). When the fermentation process is finished, 20% to 30% of the final biomass is oil, depending on the strain of algae that is used. Of the extracted oil, 35 to 45 percent is DHA (Kyle, et al. 1995; FDA, 2004b).

The algal biomass is harvested by centrifugation, flocculation or filtration, and can be processed immediately or dried for reuse in future processing. In either event, the oil can be extracted readily with an effective amount of solvent, which is usually pure hexane. The ratio of hexane to dry algal biomass in the extraction process is about 4 liters of hexane per kilogram of dry biomass. The hexane is mixed with the biomass in a reaction vessel for about 2 hours at a temperature of about 50 °C. After mixing, the biomass is filtered and separated from the hexane-containing oil. The hexane is then removed from the oil by distillation and the hexane is recycled. Conventional oilseed processing equipment has been used to perform filtering, separation, and distillation of algal biomass (Kyle, 1994; Kyle, et al., 1995; FDA, 2004b).

In the manufacturer's petition, the petitioner notes that in addition to the use of hexane to extract the oil, citric or oleic acid must be added to decrease the pH and precipitate undesirable residues from the oil. Following this step, sodium hydroxide is used to raise the pH of the oil and aid in removal of "soaps" and "gums" from the mixture, which is completed by heat and centrifugation. The oil is further treated with citric acid, silica, clay and filtration processes to adsorb and chelate any remaining polar compounds, trace metals, and oxidation products (Martek, 2010).

The treated oil may also be chilled to remove high-melting point compounds for clarity, then heated and cooled again. The second heating and cooling causes crystallization of the high-melting triglycerides. These compounds are filtered using diatomaceous earth. A deodorizing step using heating and vacuum is used following the chill filtration process to remove peroxides and any other compounds that might cause off-flavors and odors. The oil is cooled again, and various compounds such as rosemary extract, tocopherols, and ascorbyl palmitate are added to the oil for flavor and/or oxidative stability. High oleic sunflower oil may also be added to adjust the final DHA content of the oil (Martek, 2010). The refining process is illustrated in Figure 2, below.

Figure 2: Schematic of the Refining Process for DHA Algal Oil (Martek, 2010**)**



The patents and manufacturer's petition describe methods for purifying and extracting the desired oils from a heterogeneous matrix of other materials. Neither the patents nor the manufacturer's petition

describe any chemical changes in the oils themselves. The manufacturer's petition explicitly states that no chemical changes occur as a result of processing the oils.

Evaluation Question #2: Is the substance synthetic? 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)).

DHA Algal Oil is produced by a naturally occurring biological process through fermentation of *C. cohnii*. Following fermentation, hexane extraction, a chemical process, is typically used to extract the oil from the cells. As discussed in the response to Evaluation Question #1, two U.S. patents describe the production process of DHA Algal Oil on behalf of Martek Biosciences Corporation, the petitioner. The crude oil extracted using hexane is often further purified to clarify and deodorize the oil for use as a food additive. The petitioner reports that no detectable residues of hexane, at a detection limit of <0.3 ppm, remains in the oil at the completion of the manufacturing and purification process. Both the patents and the petition discuss that processes are employed to remove any extraction and purification solvents for recycling and reuse (Martek, 2010).

In its April 2010 guidance to the National Organic Program (NOP), the National Organic Standards Board (NOSB) Joint Materials and Handling Committee sought to clarify the definition of synthetic with the following statement: "extraction with a synthetic not on the National List would not result in a material being classified as synthetic unless either the extraction resulted in chemical change or the synthetic remained in the final material at a significant level"(NOSB, 2010).

As discussed in the response to Evaluation Question #1, according to the petitioner and given the absence of any evidence to the contrary, the hexane used during processing of DHA Algal Oil does not appear to alter the chemical identity of the DHA Algal Oil. The petitioner also states that hexane is removed from the oil, leaving no detectible concentrations at or above 0.3 ppm. In light of the clarification provided in NOSB (2010) on the definition of "synthetic," the substance should be considered non-synthetic.

Evaluation Question #3: Provide a list of non-synthetic or natural source(s) of the petitioned substance (7 CFR § 205.600 (b) (1)).

DHA Algal Oil and DHA are available from two natural sources in addition to *C. cohnii*: an algal source (DHA Algal Oil) and oily fish and shellfish (DHA). DHA Algal Oil can be obtained from *Schizochytrium* species, another species of marine algae (Doughman et al., 2007). The extraction process is very similar to that used to extract algal oil from *C. cohnii* (see Evaluation Question #1), and the oil is considered GRAS by FDA (FDA, 2004b). DHA Algal Oil extracted from *Schizochytrium* species would be considered non-synthetic based on the same criteria as *C. cohnii*, as described in the response to Evaluation Question #2.

The DHA Algal Oil derived from *Schizochytrium* species is produced by Martek Biosciences Corporation, the petitioner for DHA Algal Oil derived from *C. cohnii*, which is the subject of the current review. The DHA Algal Oil from *C. cohnii* contains a different mixture of fatty acids than the oil from *Schizochytrium* species. As discussed in the "Composition of the Substance" section, oil from *C. cohnii* contains DHA at 38-45 percent, as well as other triglycerides including: myristic acid (13-20%), palmitic acid (12-25%), oleic acid (10-25%), lauric acid (2-6%), and capric acid (1%) (Kyle, et al., 1995; Wyeth Nutritionals, 1998). DHA Algal Oil from *Schizochytrium* species is somewhat different and includes: palmitic acid (24%), docosapentaenoic acid (13.5%), myristic acid (10%), and EPA (3%) (FDA, 2004b).

The petitioner has noted that DHA Algal Oil from *Schizochytrium* species was not developed for use in infant formula and the substance has not been "reviewed by the FDA" for that application. However, Martek Biosciences does plan to add the oil to various food products, including all of the food product categories which are currently listed in 21 CFR 184.1472(a)(3) as enriched with menhaden (fish) oil. In addition, Martek plans to add the DHA Algal Oil from *Schizochytrium* species to foods in additional categories such as: soy protein bars; processed vegetable drinks; hard and soft candies; non-dairy and powdered cream substitutes; jams and jellies; non-dairy milk, imitation milk, and soy milk (FDA, 2004b).

Natural sources of DHA include oily fish and shellfish such as: herring, salmon, sardines, mussels, oysters, caviar, mackerel, anchovies, shrimp, trout, tuna, crab, pollock, squid, halibut, and others (Jump, 2009 ;FDA, 2007). Farm-raised and wild-caught fish of the same species have been found to contain comparable levels of DHA and EPA (Gebauer et al., 2006). Fish oil is extracted by a wet steam process in which the fish is first cooked, then strained and pressed to extract the oil and other liquids. The liquid is separated using a centrifuge and "polished" in a series of hot water washes, then treated with an alkaline solution to remove free fatty acids which are precipitated as soaps. The oil is then bleached and deodorized with steam (EPA, 1995).

Phospholipids extracted from hen's eggs are naturally occurring sources of DHA, though the diet of chickens producing the eggs must be supplemented to produce increased amounts of DHA. Egg yolk lipids contain large amounts of cholesterol, so egg phospholipids are preferred to egg yolk lipids as additions to infant formula. The ratio of DHA to ARA in egg yolk lipids may be different from that in human milk, so the diet of the hens producing the eggs may need to be carefully manipulated by supplementation with DHA to obtain the desired ratio (FSANZ, 2003).

Evaluation Question #4: Specify whether the petitioned substance is categorized as generally recognized as safe (GRAS) when used according to FDA's good manufacturing practices (7 CFR § 205.600 (b)(5)). If not categorized as GRAS, describe the regulatory status. What is the technical function of the substance?

Menhaden oil, a source of the omega-3 fatty acids DHA and EPA, is considered GRAS by the FDA. Specifically, docosahexaenoic acid-rich single-cell oil (DHASCO), as a source of DHA, is GRAS according to FDA GRAS Notice No. GRN 000041. FDA began permitting DHASCO as an ingredient in infant formula in 2001. Although DHASCO has achieved GRAS status, FDA noted in their petition that the incorporation of DHASCO in infant formula by a manufacturer of infant formula would require that manufacturer to submit documentation to FDA under section 412 of FFDCA (FDA, 2001). Manufacturers of infant formula are not currently required to list on the label the amounts of DHA added to infant formula. However, most infant formula manufacturers provide this information (Institute of Medicine, 2005).

Evaluation Question #5: Describe whether the primary function/purpose of the petitioned substance is a preservative. If so, provide a detailed description of its mechanism as a preservative (7 CFR § 205.600 (b)(4)).

The primary function of the substance is to provide added nutritional value to foods. Omega-3 fatty acids such as DHA have been found to be important for visual and neurological development and have been associated with reductions in cardiovascular disease risk (FDA, 2001). DHASCO is included in foods to deliver DHA, which acts as a nutritional supplement. DHA is not a preservative. In fact, when DHA from fish oil is added to some foods such as milk, the fatty acids can become rancid and produce off-flavors following oxidation. In some cases, preservatives such as antioxidant tocopherols have been used in DHA-enriched milk to decrease the presence of off-flavors caused by oxidation (Jacobsen, 2010).

Evaluation Question #6: Describe whether the petitioned substance will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law) and how the substance recreates or improves any of these food/feed characteristics (7 CFR § 205.600 (b)(4)).

DHA Algal Oil is not petitioned to be used to recreate or improve flavors, colors, or nutritive values lost in processing. DHA Algal Oil has been used as an addition to infant formula, as a means of delivering DHA, a supplemental (non-essential) nutrient (Institute of Medicine, 2005; Jump, 2009).

Evaluation Question #7: Describe any effect or potential effect on the nutritional quality of the food or feed when the petitioned substance is used (7 CFR § 205.600 (b)(3)).

The petitioned substance, DHA Algal Oil, is added to foods to increase the level of DHA, the predominant compound in DHA Algal Oil (Martek, 2010). Given that DHA is a fatty acid, the addition of DHA Algal Oil to foods could provide a small increase in the fat content of the food product. The recommended daily intake of DHA is 500 mg per day, so a small increase in the fat content of foods would be due to the addition of DHA (Jump, 2009; Kris-Etherton et al., 2009). The purpose of adding DHA Algal Oil to foods is simply to increase the DHA content of those foods. No information was found that discussed the effect of DHA on the bioavailability of other nutrients in the enriched foods.

In a recent FDA GRAS notification, Spherix, Inc (2010) on behalf of Cargill Inc. compared the fatty acid profiles of an infant formula with no added DHA or ARA to two infant formulas with different DHA and ARA supplementation methods. One infant formula was supplemented with DHA-rich tuna oil and SUNTGA40S (Suntory, Ltd.), an ARA-rich oil. The other infant formula was supplemented with DHA-rich tuna oil and another refined arachidonic acid-rich oil (Cargill, Inc). The authors reported "virtually no effect" on the final formula fatty acid composition for the supplemented formulas compared to the un-supplemented formula, with the exception of the intentional increase in levels of DHA and ARA (Spherix, Inc., 2010).

Evaluation Question #8: List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in the petitioned substance (7 CFR § 205.600 (b)(5)).

The petitioner states that DHA Algal Oil does not contain marine pollutants or environmental contaminants by virtue of the fact that the oil is produced by fermentation under closed, aseptic conditions (Martek, 2010). Mendes and colleagues (2009) note that "…PUFAs from cultured microalgae are cholesterol free, contaminant free [e.g. heavy metals, polychlorobiphenyls (PCBs)], and taste good," and that DHA from algal oil is a "…contaminant-free resource."

In a GRAS claim submitted by Wyeth Nutritionals International to the FDA which was denied based on an unrelated concern, Wyeth Nutritionals International reported that DHA Algal Oil (called by its trade name DHASCO®) did not contain any contaminants present above the levels of detection. The contaminants on the screening list included: 74 pesticide chemicals, residual hexane solvent (<0.3 ppm), iron (<1 ppm), silicon (<500 ppm), sulfur (<40 ppm), phosphorous (<10 ppm), copper (<0.1 ppm), lead (<0.1 ppm), cadmium (<0.2 ppm), and mercury (<0.2 ppm) (Wyeth Nutritionals, 1998). According to U.S. Patent 5,407,957, the hexane used in the solvent extraction of DHA Algal Oil is removed from the resulting oil by distillation techniques. The data presented by Wyeth Nutritionals International shows that hexane does not appear in DHA Algal Oil at levels above the limit of detection of 0.3 ppm (Wyeth Nutritionals, 1998). As reported by the petitioner, the recovered hexane from the extraction process is recycled and used again for extraction purposes (Martek, 2010).

No other research reports or purity analyses were available which specifically addressed the presence of heavy metals or other contaminants in DHA Algal Oil obtained from *C. cohnii*. No information was found that described DHA Algal Oil containing detectable levels of any of the substances listed in the FDA Action Levels for Poisonous or Deleterious Substances in Human Food, nor the Food Chemicals Codex.

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

C. cohnii is produced in closed, aseptic environments by fermentation in a nutrient broth. *C. cohnii* grows naturally in marine environments throughout the world, so it must be harvested from those environments (Mendes et al., 2009). However, given that fermentation is used to produce large populations of *C. cohnii* for DHA Algal Oil production, it is unlikely that excessive harvesting of *C. cohnii* would be required to sustain production of the oil.

The nutrient broth historically used to culture *C. cohnii* was enriched seawater (Mendes et al., 2009), but according to U.S. Patent 5,407,957, an enriched artificial seawater mix is the preferred media. *C. cohnii* requires nutrient sources for laboratory cultivation which includes an organic carbon substrate and a nitrogen source. Depending on the strain, carbon substrates that work well for *C. cohnii* include glucose, dextrose, ethanol, acetic acid, sodium acetate, and carob pulp syrup, with glucose being the most common carbon source. Nitrogen sources used for culturing *C. cohnii* include peptone, yeast extract, meat extract, glutamic acid, waste molasses, and corn steep liquor (Mendes et al., 2009).

The solvent hexane is a potential environmental contaminant from the production process for DHA Algal Oil. As discussed in the response to Evaluation Question 8, U.S. Patent 5,407,957 describes the process by which the hexane used in the solvent extraction of DHA Algal Oil is removed from the resulting oil by distillation techniques. According to the petitioner, the hexane is then recycled and used again for extraction purposes (Martek, 2010). If the hexane solvent is recycled in the process, then hexane wastes are not a likely environmental contaminant.

Once the oil is extracted from the *C. cohnii* biomass, the residual biomass is used as animal feed for livestock, as a feed material for aquaculture, or for pet foods and treats (Mendes et al., 2009). The use of the biomass as a food material eliminates the potential for biomass waste from the production process to adversely impact the environment. No information was found on the potential for the production of DHA Algal Oil to negatively impact biodiversity.

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)).

Adverse Health Effects Attributed to Excessive Amounts of DHA:

Consumption of high levels of DHA (in the form of fish oil) in excess of 3 g per person per day, in addition to EPA, may increase bleeding time, levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics, as noted in the GRAS final rule for menhaden oil (FDA, 1997). Increased incidence of hemorrhagic stroke and excessive bleeding times have been reported in Greenland Eskimos with intake levels of 6.5 g/day of combined DHA and EPA. However, it is not known whether the high intakes of EPA and DHA were the sole cause of the increased stroke incidence (Jump, 2009). High intakes of omega-3 fatty acids may be necessary to obtain clinically relevant blood pressure reductions, and at high dose levels there is an increased risk of bleeding. Therefore, a qualified healthcare provider should be consulted prior to starting treatment with supplements (NLM, 2011a).

In its review of the Martek Biosciences Corporation notification for DHA Algal oil from *C. cohnii*, FDA discussed some adverse effects that were observed in studies and panel reports that evaluated infant consumption of DHA and ARA from sources such as fish oil and egg phospholipid. Some studies of infants that consumed formula containing long-chain PUFAs showed unexpected deaths, which were attributed to necrotizing colitis, sepsis, or Sudden Infant Death Syndrome (SIDS). Other studies have reported increased flatulence, diarrhea, apnea, and jaundice in infants that were fed formulas with long-chain PUFAs (FDA, 2001).

Increased omega-3 fatty acid intake, mainly from DHA and EPA, may decrease inflammatory responses in individuals with autoimmune or inflammatory diseases, but could also decrease the potential of the immune system to destroy pathogens (Jump, 2009). Some immunosuppressive effects from supplementation with EPA and DHA have been observed in studies comparing immune cell function outside of the body (*ex vivo*) at doses as low as 0.6 grams DHA per day. It remains unclear whether the *ex vivo* evidence would translate to effects within living systems (*in vivo*) (Institute of Medicine, 2005).

As of 2010, a dose-response study has not been conducted on DHA supplementation in infant formula. Clinical trials that have fed formulas containing relatively higher DHA content, for longer periods of time, or using more sensitive assessment mechanisms, have been more likely to show a benefit of DHA

supplementation (Birch et al., 2010). Randomized clinical trials found that DHA supplementation in infants was associated with positive effects on visual and cognitive maturation, especially in preterm infants. Worldwide, the range of DHA concentrations in human milk are much broader than the ranges that have been evaluated in clinical trials. (Birch et al., 2010)

The Role of DHA in the Body and Health Benefits Attributed to DHA:

DHA is an integral part of the cell membranes of neurons and retinal cells, which suggests it plays an important role in normal vision and nervous system function (Institute of Medicine, 2005; Jump, 2009). There are high proportions of DHA and ARA in the brain's gray matter, indicating both compounds are important to normal central nervous system function. Animal studies have shown that depletion of DHA in the brain can result in learning deficits, underscoring the importance of DHA in normal brain function. DHA accumulates in the brain during pre- and post-natal development and throughout the first two years of life (Institute of Medicine, 2005). Although it is not clear exactly how DHA affects brain function, a lack of DHA in the cell membranes of neuron cells could affect the way that ion channels or receptors function, and may impact the availability of neurotransmitters (Jump, 2009).

Supplementation with omega-3 fatty acids such as DHA could potentially help prevent or treat neurological disorders associated with memory loss, like Alzheimer's disease. DHA appears to be protective against the development of Alzheimer's disease and other types of dementia. Conversely, cognitive decline has been linked to decreased levels of DHA in the brain (Jump, 2009). It is not currently known whether DHA supplementation could be used to treat Alzheimer's disease, but some laboratory studies in animals have shown evidence to that effect(Jump, 2009). A placebo-controlled trial with 295 patients with Alzheimer's disease found that DHA supplementation (2 grams/day) for 18 months was not effective in slowing cognitive decline (Jump, 2009).

The Impact of DHA Deficiency:

Phospholipids of specific brain regions are enriched with DHA and ARA, so an omega-3 or omega-6 PUFA deficiency during brain development could have long-term effects on cognitive and visual function. Studies in laboratory rodents have found that a deficiency in dietary omega-3 PUFA impaired measured cognitive performance by affecting the dopamine neurotransmitter system in the frontal cortex region of the brain (Jump, 2009). Deficiencies in omega-3 PUFA during fetal development have also been shown to have adverse effects on visual function (Jump, 2009).

<u>Evaluation Question #11:</u> Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR § 205.600 (b)(1)).

DHA is added to infant formula and other foods in the form of fish oils, egg yolk lipids, and egg phospholipids (Institute of Medicine, 2005; FSANZ, 2003). Before the large-scale production of DHA Algal Oil for fatty acid supplementation of infant formula, fish oil was the primary source of fatty acids to formula-fed infants (Carlson et al., 1999). Fish oils are the most common source of DHA for enrichment of foods and supplementation and provide the highest amounts of DHA (Institute of Medicine, 2005). Some fish oils were found to have higher levels of EPA than DHA, and supplementation of infant formula with these oils was associated with adverse effects on infant growth (FSANZ, 2003). Though fish oil is not an organic agricultural product *per se*, fish oil is on the National List as a non-organically produced agricultural product allowed for use in organic production (7 CFR § 205.606(f)).

As discussed in the response to Evaluation Question #3, materials extracted from hen's eggs are potential alternatives for DHA Algal Oil, though the chickens and eggs would have to be managed according to organic production methods to be eligible. Egg yolk lipids contain large amounts of cholesterol, so egg phospholipids are preferred to egg yolk lipids as additions to infant formula. The composition of egg yolk lipids (e.g., ratio of DHA to ARA) may be different from that in human milk, so the diet of the hens producing the eggs may need to be manipulated by supplementation with DHA to obtain the desired ratio (FSANZ, 2003). Research has shown that by varying the diet of chickens, eggs with virtually any desired

ratio of DHA to ARA can be produced (Carlson, 1997). The biomass of single-cell organisms is often used to supplement the chicken feed to produce the desired level of DHA in the egg. The lipid fraction of an egg yolk is about 31%, of which about 29% is phospholipids (Ahn et al., 2006).

A patent exists for an aqueous extraction method to remove phospholipid from egg yolks (Merkle & Ball, 2001). In general, an aqueous method is used to separate the majority of proteins from the egg yolk using ionic strength, pH, and gravitational centrifuge forces. First, the egg yolks are separated from the albumen (i.e., egg white) by hand or using mechanical methods, and the egg whites are discarded or used for other purposes. Egg yolks are then diluted with water, and the pH of the diluted egg yolk material is adjusted by the addition of food-grade acids, bases, or salts. The adjusted and mixed egg yolk material is then exposed to gravitational separation via centrifugation, and a viscous precipitate is removed, leaving the supernatant fraction containing most of the egg phospholipids. The precipitate can be discarded or reused for other purposes. Viscosity agents such as algin or carboxy methylcellulose are then added to the supernatant fraction and again exposed to gravitational separation forces for separation into a cream fraction and an aqueous subnatant fraction. The cream and subnatant of the algin separation contain approximately 35.5% and 1.3% fat, respectively, with the cream layer accounting for approximately 13% of the total volume (Merkle & Ball, 2001). Other manufacturing methods are described that use ethanol, a food-grade solvent, to initiate the separation of the lipid and protein fractions of the egg yolk (Nielson, 2007; Schneider, 2010). Using these methods, eggs from hens fed a diet rich in DHA could produce eggs containing phospholipids that are a substantial source of DHA.

Additional Questions Specific to DHA Algal Oil

The following additional questions were posed by the NOSB Handling Committee to aid the National List review for DHA Algal Oil use in handling (USDA, 2011).

Additional Question #1: Describe the FDA approval process for the use of DHA Algal Oil in foods and infant formula.

Infant formula is considered a food by FDA; therefore, infant formula and other foods are subject to the same FDA approval process for the inclusion of DHA Algal Oil as an ingredient. Under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (FFDCA), "any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive" (FDA, 2004a). Infant formula is subject to additional statutory and regulatory requirements provided in 21 CFR 106 and 107 to ensure the nutritional quality and safety of what is considered the "sole source of nutrition by a vulnerable population during a critical period of growth and development" (FDA, 2009a).

Omega-3 fatty acids, including DHA, are considered GRAS by the FDA. Specifically, DHA Algal Oil, as a source of DHA, is GRAS according to FDA GRAS Notice No. GRN 000041. FDA began permitting DHA Algal Oil as an ingredient in infant formula in 2001. Manufacturers of infant formula are not currently required to list on the label the amounts of DHA added to infant formula. However, most infant formula manufacturers provide this information.

Because DHA Algal Oil is GRAS for human consumption, even for vulnerable infant populations, it is not statutorily required under FFDCA for a premarket review and approval to be completed before DHA Algal Oil can be added to infant formula and other foods. Manufacturers of a food ingredient intended for specific use (e.g., DHA Algal Oil as an ingredient in baked goods) may submit a GRAS notice to FDA that includes a "GRAS exemption claim" comprising a short description of the substance, the applicable conditions of use, and the statutory basis for the GRAS determination (i.e., through scientific procedures or through experience based on common use in food) (FDA, 2004a). A GRAS notice also includes information about the identity and properties of the notified substance and a discussion of the notifier's reasons for

concluding that the substance is GRAS for its intended use. However, this program is voluntary for inclusion of GRAS food additives in most food items.

Pre-market requirements do exist for addition of macroingredients to infant formula. Manufacturers that wish to market new or reformulated infant formulas are required to register with FDA, submit a notification 90 days before marketing the formula, and submit a statement that summarizes the test results that verify that the product complies with the FFDCA (FDA, 2009a). This 90-day notification for a new infant formula must include (1) the quantitative formulation of the infant formula, (2) a description of any reformulation of the formula or change in processing of the infant formula, (3) assurances that the infant formula will not be marketed unless it meets the quality factors and the nutrient requirements of the FFDCA, and (4) assurances that the processing of the infant formula complies with good manufacturing practices, including quality control procedures. The manufacturer can market the new infant formula without providing these assurances to FDA, but the formula is then defined as adulterated under section 412(a)(1) of the FFDCA, and FDA has the authority to take compliance action (FDA, 2009a).

The CFSAN Office of Nutritional Products, Labeling, and Dietary Supplements (ONPLDS) evaluates whether the manufacturer of the formula has met the requirements in section 412 of the FFDCA. The ONPLDS then consults with the Office of Food Additive Safety (OFAS) regarding the safety of the ingredients in the formula and the packaging materials for the formula. OFAS evaluates the safety of the ingredients in the formula according to sections 201(s) and 409 of FFDCA. The manufacturer can market a new infant formula without providing a pre-market notification to FDA, but the formula is then automatically defined as adulterated under section 412(a)(1) of the FFDCA, and FDA has the authority to take compliance action (FDA, 2009a). Compliance actions may range from sanctions to removal of products from the market.

Additional Question #2: Describe how the FDA approves ingredients to be considered essential, required, and/or allowed in foods and infant formula. Does FDA consider DHA to be essential, required, and/or allowed in foods and infant formula?

The FDA Fortification Policy of 1980 was established as a way for essential nutrients to be identified and listed for the "rational fortification of food," (21 CFR § 104.20). FDA only considers "essential nutrients" to be within the scope of its Fortification Policy (21 CFR § 104.20).The list of essential nutrients includes vitamins and minerals that are essential to human nutrition, for which there is an established Reference Daily Intake (RDI). The group of essential nutrients has been updated since it was first established in 1980, and it is possible for additional nutrients to be added to the group. In order to be added to the group under the Fortification Policy, the substance would need to: maintain a balanced nutrient profile; correct a dietary insufficiency; improve the quality of a replacement food; restore nutrients to certain levels; or be added as required or permitted by another FDA regulation. In addition, the nutrient must be an approved food additive or GRAS under the conditions of its intended use to be considered an essential nutrient. In light of these criteria, FDA does not consider omega-3 fatty acids (and DHA, by inclusion) to be within the scope of the Fortification Policy. As a result, these nutrients when added to food would be categorized as food additives and would be allowed in food products following premarket review and approval by FDA or determination of GRAS status, as required in sections 201(s) and 409 of the FFDCA (Schneeman, 2010). USDA considers nutrients that are not essential to be "accessory nutrients," which means that the nutrients are not "…specifically classified as a vitamin or mineral but found to promote optimal health." However, FDA does not use the term "accessory nutrients." FDA has stated that omega-3 fatty acids (which includes DHA) are not considered to be essential nutrients according to 21 CFR § 101.9(c)(8)(iv) and, again, are not within the scope of FDA's Fortification Policy (Schneeman, 2010).

The Infant Formula Act of 1980 was enacted after the FDA Fortification Policy and the recommended daily values of essential nutrients in the policy were established for children aged 4 years and above, not for younger children and infants (Schneeman, 2010). The nutrient requirements of infant formula are therefore considered to be outside of the scope of the Fortification Policy. Minimum amounts for 29 specified nutrients are required in infant formulas, and maximum amounts are provided for 9 of those nutrients in 21 CFR Part 107. Any infant formula ingredient not specified in 21 CFR Part 107 is subject to the same

regulations as a food additive and would be allowed in infant formula following premarket review and approval by FDA or determination of GRAS status, as required in sections 201(s) and 409 of the FFDCA (FDA, 2006).

Additional Question #3: Describe how the FDA regulates the use of DHA in foods and infant formula. What is the maximum amount of DHA that is permitted? How does the FDA regulate what foods can be fortified with DHA?

As discussed in Additional Question #1, infant formula is considered a food by FDA; therefore, infant formula and other foods which are enriched with DHA Algal Oil are subject to the same FDA approval process. Under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (FFDCA), "any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive" (FDA, 2004a). Since DHA Algal Oil is a substance which is considered GRAS (FDA, 2001), then it may be added to food and those foods are not subject to premarket review by FDA.

As discussed in "Additional Question #1," manufacturers of a food ingredient intended for specific use (e.g., DHA Algal Oil as an ingredient in baked goods) may submit a GRAS notice to FDA that includes a "GRAS exemption claim" comprising a short description of the substance, the applicable conditions of use, and the statutory basis for the GRAS determination (i.e., through scientific procedures or through experience based on common use in food) (FDA, 2004a). A GRAS notice also includes information about the identity and properties of the notified substance and a discussion of the notifier's reasons for concluding that the substance is GRAS for its intended use. However, this program is voluntary for inclusion of GRAS food additives in most food items.

FDA does not set a maximum amount of DHA that can be added to food products. According to 21 CFR 184.1472(a)(3), menhaden oil (a source of fish oil containing DHA and EPA), may be added to various food products in varying amounts, as long as the total intake of EPA and DHA does not exceed 3.0 grams per person, per day. However, DHA and EPA are *components* of fish oil, but are not specifically regulated under 21 CFR 184.1472(a)(3). DHA is considered GRAS independently of the determination for menhaden oil, so no specific requirements for maximum levels are made. Instead, the manufacturer of the DHA must show through their petition that adding the DHA to the given foods at the levels proposed would be safe (FDA, 2004a).

With regard to the types of foods to which DHA can be added, the GRAS notification from Martek for DHA Algal Oil from *Schizochytrium* species identified several food categories to which Martek planned to add DHA Algal Oil (FDA, 2004b). The food categories listed by Martek in the GRAS notice were based on those listed in the CFR entry for menhaden oil (21 CFR § 184.1472(a)(3)), as well as other categories listed in another GRAS notice, GRN 000105, for fish oil (FDA, 2004b). In their GRAS Notice for DHA Algal Oil derived from *Schizochytrium* species, Martek noted that the proposed levels of use for the given food categories were 50 percent of the use levels specified by GRN 000105, and would result in a mean dietary exposure of not more than 1.5 g DHA per person, per day (FDA, 2004b).

Infant formula is subject to additional statutory and regulatory requirements provided in 21 CFR 106 and 107 to ensure the nutritional quality and safety of what is considered the "sole source of nutrition by a vulnerable population during a critical period of growth and development" (FDA, 2009a). The FDA Center for Food Safety and Applied Nutrition (CFSAN) is responsible for regulating infant formula in the U.S. Within CFSAN, the Office of Nutritional Products, Labeling, and Dietary Supplements (ONPLDS) evaluates whether the manufacturer of the formula has met the requirements in section 412 of the FFDCA. The ONPLDS then consults with the Office of Food Additive Safety (OFAS) regarding the safety of the ingredients in the formula and the packaging materials for the formula. OFAS evaluates the safety of the ingredients in the formula according to sections 201(s) and 409 of FFDCA. Section 201(s) defines the term

"food additive" as any substance that is intended to be a component or affect the characteristics of any food (FDA, 2009a).

FDA is not required to approve infant formulas before they can be marketed and sold, but all formulas have to meet federal requirements for basic nutrients. As discussed in the response to "Additional Question #1," manufacturers of infant formulas also have to notify FDA 90 days before they market a new formula. Nutrient requirements for infant formula are stipulated in section 412(d) of FFDCA and in 21 CFR 107.100. The only exception to these rules are "exempt infant formulas" which are specially formulated for infants with "…an inborn error of metabolism or low birth weight, or who otherwise has an unusual medical or dietary problem." Substances that can be used in infant formulas are GRAS substances for use in infant formula and those substances used in accordance with FFDCA sections 201(s) and 409. (FDA, 2006)

Additional Question #4: What is the recommended daily allowance of DHA for humans at various stages of growth and maturity?

In 2002, the Food and Nutrition Board of the U.S. Institute of Medicine (IOM) set adequate intake (AI) levels for omega-3 fatty acids, by life stage and age group (See Table 2, below). The AI levels set for infants are based on the average values observed from studies of infants fed primarily human milk. The AI levels set by the IOM may be met by a combination of DHA, EPA, and ALA. No AI was set by the IOM for DHA alone.

Table 2: Adequate Intake Levels for Omega-3 Fatty Acids

Age	Source	Males (g/day)	Females (g/day)	Reference
Infants, 0-6 months	DHA, EPA, ALA	0.5	0.5	(Institute of Medicine, 2005)
Infants, 7-12 months	DHA, EPA, ALA	0.5	0.5	(Institute of Medicine, 2005)

A workshop titled, "Towards Establishing Dietary Reference Intakes for Eicosapentaenoic and Docosahexaenoic Acids" sponsored by the Technical Committee on Dietary Lipids of the International Life Sciences Institute North America was held in June, 2008 (Harris et al., 2009). The International Life Sciences Institute is a nonprofit science organization whose members are mainly agricultural, food, beverage, chemical, and pharmaceutical companies. The petitioner, Martek Biosciences, was a member of the technical committee as of 2008. The workshop participants concluded that evidence from multiple research paradigms shows an inverse relationship between EPA+DHA intake and the risk of coronary heart disease. Based on these findings, the workshop participants recommended a dietary reference intake (DRI) for EPA+DHA between 250 and 500 mg per day and noted that "…there is no evidence that intakes of EPA+DHA in these recommended ranges are harmful."(Harris et al., 2009)

In an article by Kris-Etherton and colleagues (2009), many recommended DRI values were listed for DHA and EPA. Table 3 below summarizes the values reported in that article, unless otherwise cited.

Additional Question #5: What are the effects on humans if more than the recommended amount of DHA is consumed at various stages of growth and maturity?

As discussed in the response to Evaluation Question #10, consumption of high levels of DHA in excess of 3 g per person per day, in addition to EPA, may have adverse effects including an increase in bleeding time, levels of low-density lipoprotein cholesterol, and may affect glycemic control in non-insulin dependent diabetics (FDA, 1997). An increased incidence of hemorrhagic stroke and excessive bleeding times have been reported in Greenland Eskimos with combined intake levels of 6.5 g/day of DHA and EPA. It is not currently known whether the high intakes of EPA and DHA were the sole cause of the increased stroke incidence in the population observed (Jump, 2009). High intakes of omega-3 fatty acids may be necessary

to obtain clinically relevant blood pressure reductions, and at high dose levels there is an increased risk of bleeding. Therefore, a qualified healthcare provider should be consulted prior to starting treatment with supplements (NIH Medline-DHA).

Increased omega-3 fatty acid intake, mainly from DHA and EPA, may decrease inflammatory responses in individuals with autoimmune or inflammatory diseases, but could also decrease the potential of the immune system to destroy pathogens (Jump, 2009). Some immunosuppressive effects from supplementation with EPA and DHA have been observed in studies comparing immune cell function outside of the body (*ex vivo*) at doses as low as 0.6 grams DHA per day. It remains unclear whether the *ex vivo* evidence would translate to effects within living systems (*in vivo*) (Institute of Medicine, 2005).

One GRAS notification for DHA Algal Oil states that "five independent studies have shown that very high acute oral doses (up to 20 grams of DHASCO or ARASCO/kg body weight) did not have any major toxicological consequences in rats," (FDA, 2001). The only adverse effect noted at high doses in rats was an impaired concentrating ability of the kidneys at 3650 mg/kg body weight per day in combination with 4900 mg ARASCO/kg body weight/day in a subchronic study (FSANZ 2003).

774

775 **Table 3: Dietary Reference Intakes for DHA and EPA from USA/Canada**

1. Recommendations for Primary Prevention of Coronary Disease			
Recommending Body	**Recommendation**	**Omega-3 Fatty Acid**	**Source**
American Dietetic Association/ Dieticians of Canada	500 mg/day, from two, 4-ounce servings of fatty fish per week.	EPA+DHA	(Kris-Etherton et al., 2009)
American Diabetes Association	2 or more servings of fish per week (except commercially fried filets)	Omega-3 Fatty Acids	(Kris-Etherton et al., 2009)
American Heart Association	2 servings per week of fish, preferably fatty fish. People with documented coronary heart disease advised to consume 1g/day EPA+DHA preferably from oily fish, or consider supplements of EPA+DHA. People who need to lower serum cholesterol may take 2-4 g/day EPA+DHA, under physician's care.	EPA+DHA, Omega-3 Fatty Acids in general	(Jump, 2009; Kris-Etherton et al., 2009)
2005 Dietary Guidelines Advisory Committee Report	2 servings of fish per week, preferably high omega-3 fish.	Omega-3 Fatty Acids	(Kris-Etherton et al., 2009)
Dietary Guidelines for Americans 2005 Report	2 servings of fish per week (~8 oz. total)	Omega-3 Fatty Acids	(Kris-Etherton et al., 2009)
Australia/New Zealand National Health and Medical Research Council	Men (ages 19-70): 610 mg/day Women (ages 19-70): 430 mg/day	DHA/EPA/DPA (Docosapentaenoic acid)	(Kris-Etherton et al., 2009)
France: AFFSA, CNERNA, CNRS	500 mg/day EPA/DHA; 120 mg/day DHA minimum	EPA+DHA	(Kris-Etherton et al., 2009)
Dutch Health Council	450 mg/day omega-3 fatty acids from fish twice per week; one meal should be oily fish	Omega-3 Fatty Acids	(Kris-Etherton et al., 2009)
Superior Health Council of Belgium	≥0.3% of energy from EPA+DHA; approx. 667 mg/day	EPA+DHA	(Kris-Etherton et al., 2009)
European Commission	200 mg/day of EPA and DHA	EPA+DHA	(Jump, 2009)
International Society for the Study of Fatty Acids and Lipids	minimum of 500 mg/day EPA+DHA	EPA+DHA	(Kris-Etherton et al., 2009)
Japan Society for Lipid Nutrition	1 g/day of EPA+DHA, 2.6 g omega-3 fatty acids	EPA+DHA	(Jump, 2009)

UK Scientific Advisory Committee on Nutrition	≥ 2 portions of fish per week, one of which should be oily to provide 450 mg/day of EPA+DHA	EPA+DHA	(Kris-Etherton et al., 2009)
World Health Organization	1-2% of total energy intake	Omega-3 Fatty Acids	(Jump, 2009)
2. Recommendations for Intake of Long-chain PUFA in Pregnancy, Lactation, and Infancy			
Recommending Body	**Recommendation**	**Omega-3 Fatty Acid**	**Notes**
World Association of Perinatal Medicine, Early Nutrition Academy, Child Health Foundation	Pregnant and lactating women: 200-300 mg of DHA/day; Breastfeeding infants is recommended. When not possible, choose formula with DHA at levels between 0.2% and 0.5% by weight total fat with minimum amount of ARA equivalent to DHA.	DHA, ARA	(Koletzko et al., 2008)
American Dietetic Association/Dieticians of Canada	DHA in infant formula should be at least 0.2% of total fatty acids and the level of ARA should not be lower than DHA. Levels of DHA to ARA ranging from 1.4:1 to 2:1 are beneficial for visual and cognitive development of low-birth-weight infants and possibly normal birth weight infants.	DHA, ARA	(Kris-Etherton et al., 2009)

Additional Question #6: Where is added DHA listed on the nutrition panel for products?

Manufacturers of infant formula are not currently required to list on the label the amounts of DHA added to infant formula (Jump, 2009) (21 CFR §107.10). However, most infant formula manufacturers provide this information, but it is not typically listed in the Nutrition Facts panel (Jump, 2009). Earth's Best Organic Infant Formula lists DHA and ARA in the ingredients list and on the front label of the product, also listing the content of DHA (label located at: http://www.earthsbest.com/products/product/2392310040).

Only the nutrients listed by FDA as mandatory or voluntary in 21 CFR 101.9(c) may be listed in the nutrition panel for foods intended for adults and children over age four (FDA, 2009b). DHA may not be listed on the nutrient panel of infant formulas because neither the National Academy of Sciences nor the FDA have established recommended daily intake levels for DHA. Furthermore, DHA is not on FDA's list of mandatory or voluntary nutrients provided in the FDA nutrition regulations.

Several labels for products enriched with DHA Algal Oil are provided by Martek in their petition (Martek, 2010). For a Horizon Organic chocolate milk product, DHA is listed in the ingredients list, as well as a side panel describing the source and need for DHA in the diet. The trademarked name Life'sDHA™ is on the label of a Soy on the Go product, the chocolate milk product, and the label of Spectrum Essentials Flax Oil with DHA supplement. The Life'sDHA™ name and the total amount of DHA per serving is located on a side panel near the bottom of the label for each product. The words "DHA" and "Omega-3" are prominently listed on the front of each product label.

Additional Question #7: What assumptions are made to determine the amount of DHA permitted for addition to products, such as fluid milk, infant formula, and cookies?

The amount of DHA permitted for addition to products such as infant formula, fluid milk, and cookies is based on the notices provided to the FDA by the manufacturer of the DHA oil – in the case of this petition, Martek Biosciences Corporation. The notices were reviewed by FDA and at the time of submission, FDA had no questions about the proposed supplementation levels of DHA or the rationale behind adding DHA to the specific food products. In the case of DHA added to cookies and fluid milk products, the notice submitted by Martek Biosciences Corporation describes the intended use and amounts of the DHA Algal Oil from *Schizochytrium* species for those food products (FDA, 2004b). In the case of infant formula, Martek Biosciences Corporation described the amounts of DHA added to infant formula, including the ratio of ARA to DHA, or ARA Single-cell oil to DHA Algal Oil.

The Institute of Medicine has set adequate intake (AI) for DHA, which is based on the amount of omega-3 PUFAs, total fat, and energy found in human milk. It is assumed that human milk meets the requirements of omega-3 fatty acids for infants fed human milk, so the levels of omega-3 PUFAs found in human milk are used to inform the AI (Institute of Medicine, 2005). The AI of 0.5 g per day of omega-3 PUFAs (including DHA and EPA) represents approximately 1 percent of the total energy intake for infants aged 0 through 6 months, and 0.67 percent of total energy intake for infants aged 7 through 12 months (Institute of Medicine, 2005). As discussed in "Additional Question #3," according to 21 CFR § 184.1472(a)(3), the total intake of EPA and DHA from fish oil may not exceed 3.0 grams per person, per day. The intake of DHA Algal Oil for cookies, fluid milk, and infant formula discussed in the Martek GRAS Notices would likely contribute to a total omega-3 PUFA intake that falls between 0.5 g per day and 3.0 g per day (FDA, 2004b; FDA, 2001).

Additional Question #8: What foods naturally provide DHA to the human diet?

Fatty fish are the predominant, natural source of DHA in the human diet. Smaller amounts of DHA are also found in meat and eggs (Institute of Medicine, 2005). As discussed in the response to "Evaluation Question #3", fish sources of DHA include oily fish and shellfish such as: herring, salmon, sardines, mussels, oysters, caviar, mackerel, anchovies, shrimp, trout, tuna, crab, pollock, squid, halibut, and others (Jump, 2009); (Institute of Medicine, 2005);FDA, 2007). Farm-raised and wild-caught fish of the same species have been found to contain comparable levels of DHA and EPA (Gebauer et al., 2006).

Additional Question #9: Describe the commercial availability of naturally occurring sources of DHA, such as fish oils.

Fish oil supplements are widely available in grocery and health food stores. According to a 2010 survey by ConsumerLab.com, fish oil/omega-3 supplements are the top multivitamin among people who use multiple dietary supplements, and were more popular than general multivitamins with that group. In the survey, 74 percent of the 6,012 respondents reported that they used fish oil/omega-3 supplements (Consumerlab.com, 2010).

In addition, a large variety of fish and fish products, as well as meat, dairy, margarine, and egg products containing DHA are available in grocery stores and supermarkets (Dieticians of Canada, 2010). Whereas fish and fish products contain the highest amounts of DHA, eggs, milk, and margarine can provide some DHA to the diet.

Additional Question #10: What is the trend in the marketplace for foods fortified with DHA?

The market for DHA and EPA in supplements, infant formula, and functional foods was expected to grow by 15 to 20 percent on top of a current market value of approximately $286 million according to a 2006 report by DSM, an international nutritional products company (van Doesum, 2006). A press release from the recent acquisition of Martek Biosciences Corporation by DSM discusses the "fast growing market" for DHA and ARA. The press release also states that microbial PUFAs are an "attractive growth segment" for

the company moving forward, and that the acquisition would be "…immediately earnings per share accretive for DSM by €0.15 to €0.20," ($0.21 to $0.29) on a full year basis (DSM, 2011).

Additional Question #11: What are the naturally occurring levels of omega-3 fatty acids, including DHA, in milk from cows on concentrated grain diets versus cows consuming pasture only? Is there a correlation between rate of grain supplementation and DHA content in milk?

A study compared the milk composition from three groups of cows raised predominantly on pasture, using organic production methods, or using conventional methods (Slots et al., 2009). The organic herd was fed on 16% pasture, while the conventional herd was fed on 4% pasture and the "extensive" herd was fed on 94% pasture. The organic herd was fed significantly more cereals and grass silage, while the conventional herd was fed significantly more by-products and maize silage. A summary of milk characteristics for cows fed three different diets is presented in Figure 2, as extracted from (Slots et al., 2009).

Figure 2: Summary of Milk Characteristics from Cows Fed Three Different Diets (Slots et al., 2009)

Table 2. Daily milk yield, fat concentration, fatty acid distribution, ratio between n-6 and n-3 fatty acids, and the concentration of fat-soluble antioxidants in milk samples from conventional (CPS), organic (OPS), and extensive milk production system (EPS)[1]

Item	CPS	OPS	EPS	Degrees of freedom	GLM[2] (*P*-value)
n[3]	15 (75)	10 (50)	5 (17)		
Daily milk yield (kg of milk/cow per day)	29.3 ± 0.3[a]	24.6 ± 0.4[b]	17.9 ± 0.6[c]	2	<0.0001
n	75	50	18		
Fat concentration (g/100 g of milk)	4.12 ± 0.03[b]	4.05 ± 0.04[b]	4.57 ± 0.06[a]	2	<0.0001
Fatty acids[4] (g/kg of fatty acids)					
n	75	50	20		
CLA (*cis*-9,*trans*-11)	6.8 ± 0.4[b]	8.2 ± 0.5[b]	17.5 ± 0.7[a]	2	<0.0001
Trans-11-vaccenic acid	20 ± 1[b]	22 ± 1[b]	37 ± 2[a]	2	<0.0001
ALA	4.6 ± 0.2[b]	9.4 ± 0.2[a]	9.0 ± 0.4[a]	2	<0.0001
LA	19.7 ± 0.4[a]	17.3 ± 0.5[b]	9.2 ± 0.7[c]	2	<0.0001
SAT-FA	692 ± 3[a]	706 ± 4[a]	659 ± 6[b]	2	<0.0001
MUFA	275 ± 3[b]	258 ± 4[c]	304 ± 6[a]	2	<0.0001
PUFA	33.1 ± 0.6[b]	36.6 ± 0.7[a]	37.0 ± 1.2[a]	2	0.0003
n-6:n-3 ratio	4.7 ± 0.2[a]	1.9 ± 0.2[b]	1.0 ± 0.3[c]	2	<0.0001
Fat-soluble antioxidants (mg/kg of milk fat)					
n	75	50	20		
α-tocopherol	20.3 ± 0.4[b]	21.0 ± 0.5[b]	32.0 ± 0.8[a]	2	<0.0001
RRR-α-tocopherol	16.2 ± 0.4[c]	18.6 ± 0.5[b]	30.2 ± 0.8[a]	2	<0.0001
β-carotene	3.7 ± 0.2[b]	4.3 ± 0.3[b]	9.3 ± 0.5[a]	2	<0.0001

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Means ± standard error.

[2]GLM = general linear model in SAS (SAS Institute Inc., Cary, NC).

[3]n = the number of dairy herds and samples (in parentheses) in each production system.

[4]CLA = conjugated linoleic acid; ALA = α-linolenic acid; LA = linoleic acid; SAT-FA = saturated fatty acids; MUFA = monounsaturated fatty acids; and PUFA = polyunsaturated fatty acids.

Milk from the organic herds had significantly higher ALA and PUFA than milk from the conventional herd (Slots et al., 2009). Milk from the extensive (pasture-raised) herds had a lower concentration of saturated fatty acids than the than milk from both the conventional and organic herds. The authors concluded that the extensive, pasture-based system results in higher concentrations of mono- and poly-unsaturated fatty acids and antioxidants, while having lower concentrations of saturated fatty acids than both conventional and organic management systems. Of note is that the ratio of omega-6 to omega-3 fatty acids in milk from the extensive system was 1.0 (±0.3), while the ratios for milk from the organic and conventional systems were 1.9 (±0.2) and 4.7 (±0.2), respectively (Slots et al., 2009). DHA was not specifically identified in the study as one of the omega-3 fatty acids found in the milk.

Several studies have shown that Milk from pasture-grazed cows has significantly more conjugated linoleic acid (CLA) and unsaturated fatty acids compared to milk from cows fed a mixed-ration diet containing grains (Croissant et al., 2007). In one study, pasture-based milk showed a higher concentration of CLA and a lower amount of saturated fatty acids compared to unsaturated fatty acids (Croissant et al., 2007). DHA was not found in milk from cows fed a control diet consisting of mixed silage, hay and grains, but was detected in significantly higher amounts in the milk of cows fed algae supplements from *Schizochytrium* species (Franklin et al., 1999). No research was found that observed a correlation between grain supplementation and DHA content in milk. Methods for increasing DHA content in milk include adding

fish oil to the diets of cows or adding algae to the diets of cows (Franklin et al., 1999; Nelson & Martini, 2009).

Additional Question #12: How much fish oil can be added to milk before an "off flavor" is noted?

Studies or reports that evaluated fish oil additive best practices with regard to "off flavors" were not found during the literature search. Research was found that addressed issues of analyzing and preventing off-flavors in milk enriched with fish oil. Though the amount of fish oil added does influence the presence or absence of "off flavors" in milk, factors such as the type and quality of the oil, the degree of oxidation of the oil, storage conditions, temperature, and pressure all influence the presence and amount of "off flavors" detected in enriched milk (Jacobsen, 2010; Venkateshwarlu et al., 2004).

Pure milk and fish oil-enriched milk (containing 0.5% cod liver oil by weight) were evaluated for volatile compounds using gas chromatographic methods by Venkateshwarlu and colleagues (2004). The resulting chromatograms showed 14 volatile compounds present for the fresh milk, and 60 volatile compounds for the fish oil-enriched milk. The volatile compounds found in the enriched milk, but not in the pure milk were assumed to be due to the oxidation of the added fish oil. Sensory evaluation of the milk samples showed that the enriched milk had a distinctly fishy taste one day after the milk was enriched. The intensity of the fishy odor and taste increased each day, and was significantly higher than the pure milk at days four and eight of the evaluation period. These results indicate that at the levels tested, oxidation of fish oils in milk over the time of storage can increase fishy off-flavors in milk, and that off-flavors can be detected at 0.5% fish oil by weight (Venkateshwarlu et al., 2004). Studies that incorporated fish oil into milk at less than 0.5% by weight were not found.

The type and quality of fish oil added to milk can affect the potential for off-flavors. Fish oil quality is usually measured by peroxide value (PV), and the PV can significantly affect oxidative flavor deterioration in milk. In a study with 0.5% fish oils added to milk, two fish oils were compared, cod liver oil and tuna oil. The cod liver oil had a PV of 1.5 meq/kg and the tuna oil had a PV of 0.1 meq/kg. The cod liver oil oxidized significantly faster than the tuna oil and had significantly more fishy off-flavors. Temperature and pressure of processing can also affect oxidation and the production of off-flavors. Several antioxidants have been investigated for use as additives in fish oil-enriched milk to prevent oxidation and development of off-flavors (Jacobsen, 2010).

In a petition to the FDA by Unilever United States, Inc., the "Future Intended Use Levels" of fish oil in milk products is 2.9% by weight (FDA, 2002). According to 21 CFR 184.1472(a)(3), menhaden oil (a source of fish oil), may be added to milk at a maximum level of 5.0% to ensure that the intake of EPA and DHA does not exceed 3.0 grams per person, per day. Krill oil, a substitute for fish oil, has "a strong taste that begins to be detected at levels between 300 and 500 milligrams per serving, depending on the type of food," according to an FDA agency response letter to a notice from GRAS Associates, LLC (FDA, 2008).

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