JHeimbach LLC

April 21, 2011

Paulette Gaynor, Ph.D.
Supervisory Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Paulette:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Ocean Nutrition Canada, Ltd., through me as its agent, herby provides notice of a claim that the use of refined tuna oil as a source of docosahexaenoic acid in infant formula when accompanied by a source of arachidonic acid as described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Ocean Nutrition Canada has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, three copies of the notification are provided. Each copy includes the GRAS monograph with appendices and the Conclusion of the Expert Panel with signatures of the four members of the GRAS expert panel.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5548 or jh@jheimbach.com.

Sincerely,

(b) (6)

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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Determination of the GRAS Status of Refined Tuna Oil as a Source of Docosahexaenoic Acid In Infant Formula When Accompanied by a Source of Arachidonic Acid

Prepared for
Ocean Nutrition Canada Ltd.
Dartmouth, Nova Scotia, Canada

Prepared by
JHeimbach LLC
Port Royal, Virginia, USA.
and
Write-Tox Consulting
Spruce Grove, Alberta, Canada

April 2011

Table of Contents **Pg**

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1. GRAS Exemption Claim

Ocean Nutrition Canada Ltd. (ONC) is a world leader in fish oil refining and processing. ONC supplies refined fish oil to the food and dietary supplement markets globally. With its head office in Dartmouth Nova Scotia, Canada, ONC also has fish oil refining plants in Peru and Mulgrave Nova Scotia and a fish oil microencapsulation plant in Arcadia Wisconsin. Fish oils used in ONC products are primarily sourced from species fished from the South Pacific Ocean off the coasts of Peru and Ecuador but tuna oils may derive from tuna caught in other parts of the world. ONC manufactures a wide variety of fish oils with various concentrations and ratios of the omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

ONC, through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration that the use of ONC's refined tuna oil described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because ONC has determined that such use is generally recognized as safe (GRAS) through scientific procedures.

(b) (6)

4/20/11

James T. Heimbach, Ph.D., F.A.C.N.
President, JHEIMBACH LLC

Date

1.1. Name and Address of Notifier

Ocean Nutrition Canada Ltd.
101 Research Drive
Dartmouth, Nova Scotia B2Y 4T6
Canada
Contact: Paul Browner, Regulatory Affairs Manager
Telephone: (902) 480-3179
Facsimile: (902) 480-3212
E-mail: pbrowner@ocean-nutrition.com

1.2. Name of GRAS Substance

The substance that is the subject of this GRAS determination is refined tuna oil derived from crude oil that is extracted from various species of tuna including but not limited to skipjack (*Katsuwonas pelamis*), yellowfin (*Thunnus albacares*), and bigeye (*Thunnus obesus*). This extracted raw tuna oil is refined and processed by ONC to produce the final product. Refined tuna oil produced by ONC includes a mixture of triacylglycerols with DHA and EPA predominating, DHA at not less than 25% and not more than 30%; EPA at not less than 5% and not more than 8%; and a ratio of DHA to EPA of not less than 3:1.

1.3. Intended Use and Consumer Exposure

Tuna oil refined by ONC is intended to be added as a source of DHA to both preterm and term infant formulas, along with a source of arachidonic acid. The intended level of addition will result in a DHA level up to 0.5% of the total fatty acids in both preterm and term formulas.

According to tables of daily energy intake by formula-fed infants provided by Fomon (1993), the subpopulation of infants with the highest intake per kg body weight is boys age 14–27 days. The 90th percentile energy intake by this group is 141.3 kcal/kg bw/day. Among girls, the highest energy intake is found in the same age group, 14–27 days, and is nearly as high as boys: 138.9 kcal/kg bw/day. Assuming that approximately 50% of calories in infant formula are provided by fats, this indicates intake of about 70 kcal from fat/kg bw/day, or about 8 g fat/kg bw/day. In infant formulas for which DHA provides 0.5% of the fatty acids, the 90th percentile intake of DHA would be 40 mg/kg bw/day.

Since ONC's refined tuna oil has a DHA:EPA ratio of at least 3:1, the 90th percentile intake of EPA would be no more than a third of that of DHA, or 13 mg/kg bw/day.

Finally, since DHA is present at a minimum of 25% in ONC's refined tuna oil, the 90th percentile intake of refined tuna oil itself would not exceed four times the intake of DHA, or 160 mg/kg bw/day.

1.4. Basis for GRAS Determination

Determination of the safety and GRAS status of ONC's refined tuna oil for addition to infant formula under the intended conditions of use (including addition of a source of arachidonic acid at appropriate levels) has been made through the deliberations of an Expert Panel comprising Anthony P. Bimbo, Joseph F. Borzelleca, Ph.D., Berthold V. Koletzko, M.D., and George H. Pauli, Ph.D. These individuals are qualified by scientific training and experience to evaluate the processing methods employed to extract and refine tuna oil and the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, as well as other information available to them, and have concluded:

> *No evidence exists in the available information on ONC's refined tuna oil, or on EPA and DHA, that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when ONC's refined tuna oil, along with an approved source of arachidonic acid, is added to infant formula intended for consumption by preterm and term infants at the intended levels.*

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, ONC's refined tuna oil is safe and is GRAS based on scientific procedures for addition to infant formula when this addition is accompanied by addition of an appropriate source of arachidonic acid.

1.5. Availability of Information

The data and information that serve as the basis for the GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHEIMBACH LLC, 923 Water Street, P.O. Box 66, Port Royal VA 22535, telephone 804-742-5548 or e-mail jh@jheimbach.com.

1.6. Abbreviations and Acronyms

AA = arachidonic acid
AI = adequate intake
ARA = arachidonic acid
ALA = α-linolenic acid
BAEP = brainstem acoustic evoked potential
BRS = behavioral rating scale
CA = corrected age
DHA = docosahexaenoic acid
DLC = dioxin-like compounds
EEG = electroencephalogram
EPA = eicosapentaenoic acid
ERG = electroretinogram
FFA = free fatty acids
GRAS = generally recognized as safe
GRN = GRAS Notice
HDL = high-density lipoprotein
IQ = intelligence quotient
LA = linoleic acid
LCPUFA = low-density polyunsaturated fatty acids
LDL = low-density lipoprotein
MDI = mental development index
NEC = necrotizing enterocolitis
ONC = Ocean Nutrition Canada
PAH = polycyclic aromatic hydrocarbons
PBDE = polybrominated diphenylethers
PCA = postconceptional age
PCB = polychlorinated biphenyls
PDI = psychomotor development index
PMA = postmenstrual age
SIDS = sudden infant death syndrome
VEP = visual evoked potential

2. Identity of the Substance

2.1. Name and Description of the Substance

The substance that is the subject of this GRAS determination is refined tuna oil derived from crude fish oil that is extracted from various species of tuna including but not limited to skipjack (*Katsuwonas pelamis*), yellowfin (*Thunnus albacares*), and bigeye (*Thunnus obesus*). Ocean Nutrition Canada may receive raw crude tuna oil or semi refined tuna oil that has been alkali refined. The crude tuna oil and/or semi refined tuna oil is further refined and processed by ONC to produce the final product. Refined tuna oil produced by ONC includes a mixture of triacylglycerols with DHA and EPA predominating, DHA at not less than 25% and not more than 30%; EPA at not less than 5% and not more than 8%; and a ratio of DHA to EPA of not less than 3:1

2.2. CAS Registry Number

No Chemical Abstracts Service (CAS) Registry Number exists specifically for tuna oil, although CAS Number 8016-13-5 has been assigned to generic fish oil. The CAS Registry Numbers for DHA and EPA, the primary components of tuna oil, are 25167-62-8 and 10417-94-4, respectively.

2.3. Molecular and Structural Formulas

Because tuna oil is a mixture, no single molecular or structural formula exists for this substance. The molecular formula for DHA is $C_{22}H_{32}O_2$, while the molecular formula for EPA is $C_{20}H_{30}O_2$. The structural formulas for these two major components of tuna oil are shown in Figures 1 and 2.

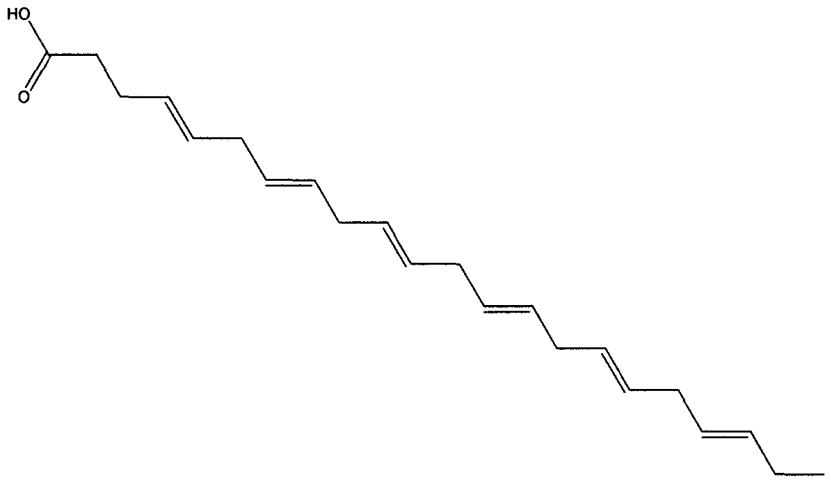


Figure 1. Docosahexaenoic Acid (DHA).

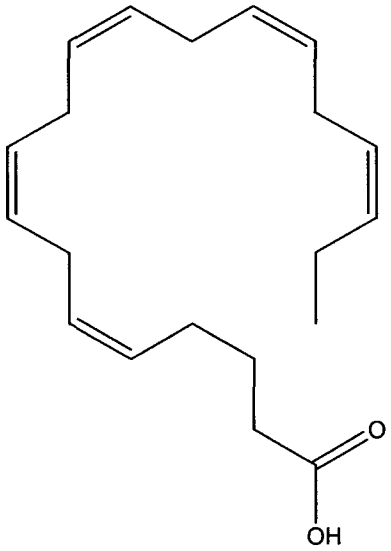


Figure 2. Eicosapentaenoic Acid (EPA).

2.4. Processing

The manufacturing methods used by ONC and its suppliers from fish oil extraction through fish oil refining are outlined below. These methods are widely used in the industry; are effective in producing a safe, food grade fish-oil product that conforms with current industry standards for edible oil manufacturing; and are recognized as appropriate for production of food-grade fish-oil products in both the scientific and oil-processing communities based on published information. The general recognition of ONC's processing methods is discussed in detail in Section 7.3.1.

2.4.1. Crude Fish Oil Extraction

Tuna oil is a by-product of the tuna canning and fish-meal industries. Thus, the primary goal of many of the methods described in the literature is to separate fish muscle and/or fish meal from liquid fish oil with the intent of producing dry fish meal. Nevertheless, not all of the steps commonly used to produce fish meal are necessary to produce fish oil. As one example, most descriptions of extraction of fish oil include drying of the fish meal, which is not part of the process relevant to oil extraction.

Tuna oil refined by ONC is sourced from the food-grade-tuna canning industry. The canning facilities from which fish oil is derived must comply with strict current good manufacturing practice (cGMP) guidelines from the time whole tuna fish enters the canning facility until fish are split and all edible meat is extracted from the carcass for canning. The fins, heads, and remainder of the bodies are not used for canned tuna. This is where fish oil extraction begins.

The remaining fish parts (bones, fins, skins and offals) are ground and cooked to denature the protein and separate the crude oil, which is then separated from the solid matter by filtration and centrifugation. This crude oil is then subjected to refining as described below.

2.4.2. Fish Oil Refining

This section details the steps used by ONC to refine crude fish oil. The refining process is shown in schematic form in Figure 3 at the end of the section.

Fish oil refining encompasses the processes used to transform crude fish oil into safe, edible fish oil through a variety of steps designed to purify the oil. The safety and purity of fish oil can be measured by the levels of contaminants present in the oil after refining.

ONC incorporates industry-accepted oil refining techniques using state-of-the-art equipment to ensure that the fish oil end product is of appropriate quality and safety for inclusion in the food and dietary-supplement markets.

Fish oil refining is performed at ONC's Mulgrave (Nova Scotia) facility. The following processing steps are undertaken to refine ONC fish oil. Figure 3 provides a summary of the process.

2.4.2.1. Neutralization (Alkali Refining)

Neutralization and alkali refining are terms used interchangeably. This step involves adding an alkali (sodium hydroxide) to the crude oil and heating the mixture. The purpose of this is for the alkali to react with free fatty acids in the crude oil to form a soap, which is then centrifuged out. This step is completed with water washes to ensure the complete removal of unreacted sodium hydroxide.

2.4.2.2. Deodorization

Each incoming lot of crude fish oil is tested for contaminants. Deodorization is designed to reduce the level of contaminants in fish oil. Fish oil passes under a vacuum and through a deodorizer at a high temperature and reduced pressure. The vacuum and elevated temperature reduces polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins and furans (DLCs), free fatty acids, and some sterols and other volatile components. The remaining deodorized oil enters the next step in the process.

2.4.2.3. Decolorization/Adsorbing (Bleaching)

Various methods of processing can cause darkening of the fish oil; therefore, it is 'bleached' in order to lighten the color. Food-grade bleaching clay is added to the fish oil. The fish oil and clay mixture is heated to and held under reduced pressure. The mixture is then filtered until there is no bleaching clay left in the mixture. This process reduces

colored compounds, PAHs and other contaminants, and removes the products of oil oxidation (peroxides, aldehydes, etc.). This process stabilizes the oil.

2.4.2.4. Antioxidant Blending

This step involves the addition of an antioxidant blend to the oil. The blend consists of food-grade mixed natural tocopherols in vegetable oil with food-grade citric acid. The fish oil with the antioxidant blend is then homogenized. Ascorbyl palmitate may be added in addition to the antioxidant blend or as a stand along antioxidant in combination with mixed natural tocopherols. Ascorbyl palmitate does not use vegetable oil as a carrier.

The antioxidant blend is added to the oil prior to deodorization due to the high temperature the oil is heated to during deodorization. High temperatures greatly accelerate oxidation of fish oil. It is virtually impossible to remove all traces of oxygen in the oil. The presence of an antioxidant combats oxidation of the oil while being deodorized. It is important that the antioxidant blend is added at a level that will be effective. We have determined that there is an optimal addition level for our antioxidant blend in tuna oil. At higher levels the antioxidants become a prooxidant, thus the relationship between the antioxidant activity and the dose follows a bell-shaped curve. We are able to demonstrate that the rate of antioxidant addition prior to deodorization is effective in preventing oxidation of our tuna oil via multiple stability studies which indicate that our oil lives up to its shelf life.

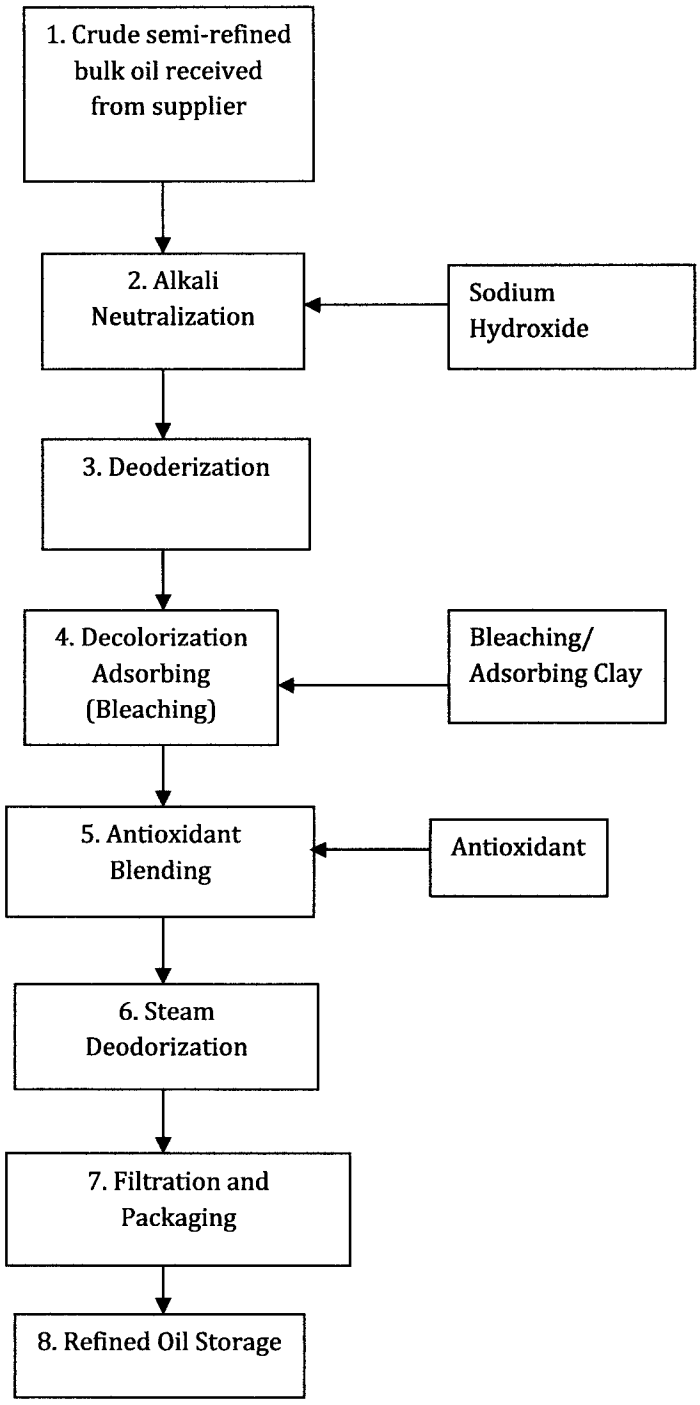
2.4.2.5. Steam Deodorization

During steam deodorization, the oil is heated again under a vacuum. After which steam is injected into the oil to further ensure that contaminants are reduced to acceptable levels. The product is tested to assure that it meets established internal specifications, including EPA and DHA content and maximum levels of contaminants. No further antioxidant is added after steam deodorization.

2.4.2.6. Filtration, Drumming and Oil Storage

The oil is then filtered and packaged in FDA-approved containers. A nitrogen blanket with a purity of not less than 99.98% pure is added to protect against oxidation. Final oil is then stored for future use or sold on the global market.

Figure 3. Production Flow Chart for ONC's Refined Tuna Oil.



2.5. Tuna Oil Specifications

The product specifications for ONC's refined tuna oil are shown in Table 1. Certificates of analyses are provided in Appendix A.

Table 1. Product Specifications for ONC Refined Tuna Oil.

Parameter	Specification	Analytical Method
ANALYSIS		
Color and clarity (Gardner)	NMT[1] 7	AOCS Td 1a-64 (09)
Appearance	Clear yellow-amber	N/A
Flavor and odor	Bland	N/A
Free fatty acids (as % oleic)	NMT 0.5%	AOCS CD 3D-63 modified
Acid value (mg KOH/g)	NMT 1.0	AOCS CD 3D-63 modified
p-Anisidine value*	NMT 20	AOCS CD 18-90
Peroxide value (meq/kg)	NMT 1.0	AOCS CD 8-53
Totox number	NMT 22	N/A
Moisture (%)	NMT 0.1	AOCS CA 2E-84 modified
FATTY ACID PROFILE		
EPA (area[3] %)	NLT 5 and NMT 8	EP 2003:1352, 2.4.29 modified
DHA (area %)	NLT 25 and NMT 30	EP 2003:1352, 2.4.29 modified
EPA (mg/g as TG[4])	NLT 45	EP 2003:1352, 2.4.29 modified
DHA (mg/g as TG)	NLT 220	EP 2003:1352, 2.4.29 modified
EPA (mg/g as FFA[5])	NLT 40	EP 2003:1352, 2.4.29 modified
DHA (mg/g as FFA)	NLT 210	EP 2003:1352, 2.4.29 modified
DHA:EPA ratio	NLT 3:1	EP 2003:1352, 2.4.29 modified
Total n-3 fatty acids (area %)	NLT 32 and NMT 40	EP 2003:1352, 2.4.29 modified
Total n-3 fatty acids (mg/g as TG)	NLT 280	EP 2003:1352, 2.4.29 modified
RESIDUES AND CONTAMINANTS		
Cadmium (mg/kg)	NMT 0.1	US EPA 200.7 & 200.8 modified
Arsenic (mg/kg)	NMT 0.1	US EPA 200.7 & 200.8 modified
Lead (mg/kg)	NMT 0.1	US EPA 200.7 & 200.8 modified
Mercury (mg/kg)	NMT 0.01	US EPA 245.6
PCB[6] (mg/kg)	NMT 0.09	US EPA 1668 modified
Benzo(a)pyrene (µg/kg)***	NMT 2.0	In accord with NEN-ISO-15302
Dioxin and furans[7] (pg WHO-PCDD/FTEQ/g)	NMT 1.5	N/A
Pesticides[8] (mg/kg)**	<0.05 ppm	NA
MICROBIOLOGICAL		
Standard aerobic plate count (cfu[9]/g)	NMT 100	NA
Enterbacteriaceae (cfu/g)	NMT 100	NA
E. coli (in 1 g)	Not detected	NA
Salmonella spp. (in 10 g)	Not detected	NA
Yeast and mold (cfu/g)	NMT 100	NA

1. NMT = not more than 2. NLT = not less than 3. Area under the curve of a chromatogram
4. TG = triacylglycerol 5. FFA = free fatty acids 6. Total of IUPAC nos. 28, 52, 101, 118, 138, 153, 180
7. Includes PCDD and PCDF 8. Includes DDT, DDE, HCB, Lindane 9. cfu= colony forming units

*p-anisidine value is a measure of aldehydes (principally 1-alkenals), equal to 100x increase in absorbance measured at a wavelength of 350 nm in a 10-mm cell of a test solution when reacted with *p*-anisidine.

** 4 pesticides (DDT, DDE, HCB, Lindane) are assayed individually with limit of not more than 0.05 ppm.
*** Benzo(a)pyrene is analyzed as an indicator of PAH levels; it was not detected in analyses of refined oils.

2.6. DHA and EPA Content of ONC's Refined Tuna Oil

The compositional analyses of ONC's refined tuna oil indicate the following contents of DHA and EPA:

DHA content: mean = 26.5% (±1.0); minimum and maximum = 26% and 28%
EPA content: mean = 8.0% (±0.0); minimum and maximum = 8% and 8%
DHA:EPA ratio: mean = 3.3 (±0.1); minimum and maximum = 3.3 and 3.5

2.7. Pesticide Residues in ONC's Refined Tuna Oil

In addition to the analyses discussed above, five lots of ONC's refined tuna oil were analyzed for the presence of any pesticide residues. No pesticides were detected in any of the five lots. Copies of the laboratory reports are in Appendix C, while the results, including the limit of detection of each analysis, are shown in Table 2a.

Table 2a. Results of Analyses of Pesticide Residues in ONC Refined Tuna Oil.

Pesticide	Pesticide Residue Concentrations (mg/kg) in 5 Lots of ONC Refined Tuna Oil				
	Lot 20954	Lot 21653	Lot 19843	Lot 20438	Lot 22310
Azinphos-methyl	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Bromophos-ethyl	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Bromophos-methyl	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Chlorfenvinphos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Chlorpyriphos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Coumaphos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Demeton-S	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Diazinon	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Dibrom	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Dichlorvos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Disulfoton	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Ethion	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fenchlorphos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fenitrothion	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fensulphothion	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fenthion	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Malathion	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Methidathion	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Mevinphos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Naled	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Parathion-ethyl	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Parathion-methyl	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

Pesticide	Pesticide Residue Concentrations (mg/kg) in 5 Lots of ONC Refined Tuna Oil				
	Lot 20954	Lot 21653	Lot 19843	Lot 20438	Lot 22310
Phosphamidon	< 0.01*	< 0.01	< 0.01	< 0.01	< 0.01
Phorate	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pirimiphos-ethyl	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pirimiphos-methyl	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Prophos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Sulfotep	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002
Tetrachlorvinphos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Tokuthion	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Tributyl phosphorotrioite	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Trichloranat	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Trichlorphon	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Dichlorbenil	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Diclofop-methyl	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Captafol	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Captan	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Procymidon	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Vinclozolin	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Propoxur	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Amitraz	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Aldrin	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Chlordane	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Dieldrin	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Endosulfan 1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Endosulphan 2	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Endosulfan sulfate	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Endrin	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Endrin aldehyde	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
PCB	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
HCH alpha	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
HCH beta	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
HCH delta	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
HCH gamma	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Heptachlor	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Heptachlorepoxide	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Methoxychlor	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
op DDD	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
op DDE	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

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Pesticide	Pesticide Residue Concentrations (mg/kg) in 5 Lots of ONC Refined Tuna Oil				
	Lot 20954	Lot 21653	Lot 19843	Lot 20438	Lot 22310
op DDT	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
pp DDD	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
pp DDE	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
pp DDT	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Toxaphene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Mirex	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Di-isobutyl phthalate	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Di-n-butyl phthalate	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Di-(2-ethylexyl)-phthalate	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Alpha-BHC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
PCB 1254 (Arochlor 1254)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Beta-BHC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Chlorothalonil	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
DCNA	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
DCPA	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Delta-BHC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Dichlorofenthion	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Dicofol	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
EPN	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Folpet	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fonofos	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Gamma-BHC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Oxadiazon	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
PCNB	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Phosalone	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Phosmet	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Propetamphos	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Propyzamide	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Prothiophos	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Ronnel	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Technical chlordane	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Tecnazene	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Tetradifon	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Thimet	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Trithion	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Vapona	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
* The limit of detection is indicated by the number following <.					

000018

2.8. Polybrominated Diphenylethers in ONC's Refined Tuna Oil

In addition to the analyses discussed above, four lots of ONC's refined tuna oil were analyzed for concentrations of polybrominated diphenyl ethers (PBDEs). Levels were consistently very low and most congeners were either absent or present below the level of detection. The results, including the limit of detection of each analysis, are shown in Table 2b. Copies of the laboratory reports are in Appendix B.

Table 2b. Results of Analyses of PBDEs in ONC Refined Tuna Oil.

PBDE Congener	PBDE Concentrations (ng/g) in 4 Lots of ONC Refined Tuna Oil			
	Lot 19843	Lot 20954	Lot 21653	Lot 22310
2,2',4-triBDE	<0.030*	<0.020	<0.030	<0.030
2,4,4'-triBDE	<0.025	<0.019	<0.025	<0.022
2,2',4,4'-tetraBDE	0.078	0.083	0.115	0.079
2,2',4,5'-tetraBDE	<0.049	<0.049	<0.050	<0.049
2,3',4,4'-tetraBDE	<0.054	<0.049	<0.050	<0.049
2,3',4',6- tetraBDE	<0.054	<0.049	<0.050	<0.049
3,3',4,4'- tetraBDE	<0.048	<0.049	<0.050	<0.049
2,2',3,4,4'-pentaBDE	<0.048	<0.052	<0.054	<0.049
2,2',4,4',5- pentaBDE	<0.048	<0.049	0.055	<0.049
2,2',4,4',6- pentaBDE	<0.048	<0.049	<0.050	<0.049
2,3',4,4',6- pentaBDE	<0.048	<0.053	<0.054	<0.049
3,3',4,4',5- pentaBDE	<0.048	<0.049	<0.050	<0.049
2,2',3,4,4',5'-hexaBDE	<0.077	<0.080	<0.087	<0.078
2,2',4,4',5,5'- hexaBDE	<0.077	<0.078	<0.091	<0.078
2,2',4,4',5,6'- hexaBDE	<0.077	<0.078	<0.080	<0.078
2,3,3',4,4',5- hexaBDE	<0.077	<0.112	<0.122	<0.078
2,2',3',4,4',5,6'-heptaBDE	<0.097	<0.097	<0.100	<0.097
2,2',3,4,4',6,6'- heptaBDE	<0.097	<0.097	<0.100	<0.097
2,3,3',4,4',5',6- heptaBDE	<0.097	<0.097	<0.100	<0.097
2,2',3,4,4',5,5',6-octaBDE	<0.242	<0.243	<0.249	<0.243
2,2',3,3',4,4',6,6'-octaBDE	<0.242	<0.243	<0.249	<0.243
2,2',3,3',4,4',5,5',6-nonaBDE	<0.483	<0.487	<0.499	<0.486
2,2',3,3',4,4',5,6,6'-nonaBDE	<0.483	<0.487	<0.499	<0.486
Total decaBDE	<1.930	<4.070	<1.990	<1.940
* The limit of detection is indicated by the number following <.				

3. Intended Use and Exposure

3.1. Intended Use.

Tuna oil refined by ONC is intended to be added as a source of DHA to both preterm and term infant formulas, along with a source of arachidonic acid. The intended level of addition will result in a DHA level up to 0.5% of the total fatty acids in both preterm and term formulas.

3.2. Estimated exposure

According to tables of daily energy intake by formula-fed infants provided by Fomon (1993), the subpopulation of infants with the highest intake per kg body weight is boys age 14–27 days. The 90th percentile energy intake by this group is 141.3 kcal/kg bw/day. Among girls, the highest energy intake is found in the same age group, 14–27 days, and is nearly as high as boys: 138.9 kcal/kg bw/day. Assuming that approximately 50% of calories in infant formula are provided by fats, this indicates intake of about 70 kcal from fat/kg bw/day, or about 8 g fat/kg bw/day. In infant formulas for which DHA provides 0.5% of the fatty acids, the 90th percentile intake of DHA would be 40 mg/kg bw/day.

Since ONC's refined tuna oil has a DHA:EPA ratio of at least 3:1, the 90th percentile intake of EPA would be no more than a third of that of DHA, or 13 mg/kg bw/day.

Finally, since DHA is present at a minimum of 25% in ONC's refined tuna oil, the 90th percentile intake of refined tuna oil itself would not exceed four times the intake of DHA, or 160 mg/kg bw/day.

As the infant grows, formula intake increases, but more slowly than weight gain, so that consumption assessed as ml formula per kg body weight is lower for infants older than 27 days. As a result, intake per kg body weight decreases as the infant grows older and larger and the estimates above represent the highest intakes (per kg bw) that will occur during infant growth.

The intake estimates above are generally similar to those offered by Martek in GRN 000041 (30 mg DHA/kg bw/day based on DHA addition at 0.5% of total fatty acids) and somewhat higher than those provided by Ross in GRN 000094 (7.31-20.3 mg DHA/kg bw/day based on DHA addition at 0.15-0.25% of total fatty acids).

4. Review of Safety Data

4.1. Human Studies in Infants

Clinical studies prior to 2001 were evaluated in detail in a GRAS evaluation entitled "GRAS Determination for Docosahexaenoic Acid Rich Oil Derived from Tuna and Arachidonic Acid Rich Oil Derived from *Mortierella alpina*," submitted to FDA on December 18, 2001, and designated GRN No. 000094. Key studies are included and briefly described below. An updated literature search was conducted through May 2010 to identify more recent clinical trials in which preterm or term infants were given fish oils or other sources of DHA or LCPUFA to supplement their diets.

4.1.1. Studies with Fish Oil as the Source of DHA

Table 3 at the end of this section summarizes the studies using fish oil as the source of DHA.

4.1.1.1. Preterm Infants

The content of LCPUFA in plasma lipids was studied in preterm infants weighing at least 1300 g fed formulas with or without supplementation with LCPUFA (Koletzko et al. 1989). Infants were fed either a commercially available formula (Pre-Aptamil, Milupa AG, Germany) without LCPUFA (control; n=10) or a formula equivalent to Pre-Aptamil supplemented with 0.5% LCPUFA of the n-6 and n-3 series from egg lipids and fish oil (supplemented group; n=8) from day 4 to 21 of life. DHA and AA levels (0.1 and 0.2% of total fatty acids, respectively) in the supplemented group were about half of that found in human milk. A reference group of 11 infants was breastfed (receiving 1.7% LCPUFA of total fatty acids). Blood samples were taken on days 4 and 21. Growth, tolerance, gestational age, and other clinical characteristics (e.g., Apgar scores) did not differ between groups. Breastfed infants showed no changes in LCPUFA levels in plasma lipids between days 4 and 21; however, these levels decreased significantly in controls. Infants fed the supplemented formula had levels higher than controls but lower than breastfed infants. The authors reported that "All infants tolerated the feeds well, and no side effects of the LCPUFA-formula were noted."

Koletzko et al. (1995) examined the antioxidant status of 32 preterm infants (8 controls, 9 supplemented, and 15 breastfed) from their previous clinical trial (Koletzko et al. 1989). Plasma and erythrocyte total lipid concentrations were related to plasma and erythrocyte α-tocopherol concentrations to determine vitamin E status. The ratio of erythrocyte membrane α-tocopherol/total lipid did not change between day 4 and 21 for controls and breastfed infants, but there was a significant decrease in this ratio for infants fed the supplemented formula. No differences were noted in plasma α-tocopherol/ total lipid ratios between the groups. Plasma α-tocopherol concentrations significantly increased (140%) from day 4 to 21 in the breastfed group but not in any other group. The results

indicate that when infant formulas are supplemented with LCPUFA, vitamin E status also should be considered.

Healthy low-birth-weight preterm infants (940-2250 g) were studied for growth, regional body fat, and body composition in a randomized blinded clinical trial (Ryan et al. 1999) in which the infants were fed either unsupplemented formula (control; n=45) or formula supplemented with DHA (0.2 % of total fatty acids; n=45). The infants were fed preterm formula provided in 4 oz bottles containing 828-842 kcal/L with or without DHA from 7-10 days post-menstrual age (PMA) until 43 weeks PMA and then were fed a term infant formula provided in 32 fl. oz cans containing 682-686 kcal/L with or without DHA from 43 to 59 weeks PMA. Fish oil providing a DHA/EPA ratio of 5:1 was used as the source of DHA. Body composition was determined by total body electrical conductivity (fat-free mass) using an EM-SCAN HP2 at 43, 51, and 59 weeks PMA. Fat-free mass estimates were based on the average of 10 readings per infant. Total body fat (kg) was calculated as the difference between total body weight and fat-free mass. Anthropometric measurements were taken at birth, at enrollment into the study, and at 37, 39, 43, 47, 51, and 59 weeks PMA. Parents recorded tolerance and formula intake over a 3-day period prior to each study visit. Blood samples were taken at 43 and 51 weeks PMA for determination of plasma phospholipid concentrations of AA and DHA.

A total of 63 infants (32 controls and 31 supplemented) completed the study. The formulas were well tolerated. Four SIDS deaths were reported in DHA-supplemented infants (supplemented for 9-120 days). One death occurred one week after the infant stopped the study and followed an episode of bronchiolitis. The remaining deaths were attributed to severe immaturity. The incidence was considered a statistically significant increase over controls (0 SIDS deaths); however, the SIDS deaths were independently reviewed by a safety panel and were considered unrelated to study participation. No differences between control and DHA-supplemented female infants were noted in any of the parameters tested. DHA-supplemented males, however, showed significantly decreased growth (as measured by weight, length, and head circumference) over the study period when compared to controls. Energy intake from formula was significantly lower in DHA-supplemented males than controls at 51 and 59 weeks PMA; however, when adjusted for body weight, mean energy intake did not differ between groups. Also, when expressed as a percentage of total body weight, fat-free mass and total body fat of DHA-supplemented males did not differ from controls. Plasma phospholipid DHA levels were significantly higher in DHA-supplemented infants compared to controls. In males only, there was an inverse correlation between plasma phospholipid DHA levels and recumbent length.

In a randomized, blinded trial, 470 preterm infants (<33 weeks gestational age) weighing 750-1800 g were assessed for growth, visual acuity, and development after receiving formula with or without AA+DHA supplementation (O'Connor et al. 2001). The formula groups were given unsupplemented formula (control; n=144); formula supplemented with 0.26% DHA and 0.42% AA from fish/fungal oil (n=140); or formula supplemented with 0.26% DHA and 0.42% AA from egg-derived triglyceride/fish oil

(n=143). A reference group of 43 infants breastfed exclusively until term-corrected age (CA) also was assessed. Formula groups were fed human milk and/or preterm formula (modified version of Similac Special Care) until term CA and then term formula (modified version of NeoSure powder) and/or human milk until 12 months CA. The oils providing AA and DHA were added at the expense of coconut oil to keep total fat content constant. Blood samples were taken for blood fatty acid analysis at enrollment, at hospital discharge, and at 4 and 12 months CA. Growth (weight, length, and head circumference measured at enrollment, at term CA, and at 2, 4, 6, 9, and 12 month CA), behavioral visual acuity (using the Teller Acuity Card Procedure at 2, 4, and 6 months CA), VEP acuity (using VEP procedure at 4 and 6 months CA), information processing (using the Fagan Test of Infant Intelligence at 6 and 9 months CA), general development level (using the Bayley Scales of Infant Development at 12 months CA), and language (using the MacArthur Communicative Development Inventories at 9 and 14 months) were assessed. In-hospital feeding tolerance, clinical problems, and serious and/or unexpected adverse events were recorded.

Three hundred seventy-six (376) infants from the formula and breastfed groups completed the study. Fifteen deaths were reported over the course of the study (6 controls, 3 AA+DHA from fish/fungal, 6 AA+DHA from egg triglyceride/fish, and 0 breastfed). Investigators concluded that "no infant deaths were related to study feedings," but the causes of death were not reported. Reasons for leaving the study were similar between groups. AA and DHA plasma and red blood cell phospholipid levels were significantly higher in supplemented groups than controls except for AA red blood cell phosphatidylethanolamine levels at 4 and 12 months CA. In addition, infants fed formulas supplemented with AA+DHA from fish/fungal sources, but not from egg triglyceride/fish sources, had higher AA red blood cell phosphatidylethanolamine levels than controls. Anthropometric measurements, in-hospital feeding tolerance, clinical problems, serious and/or unexpected adverse events, and behavioral visual acuity of supplemented infants showed no consistent differences from controls. VEP acuity of supplemented infants at 4 months CA was similar to controls but by 6 months CA was significantly greater in supplemented infants than in controls. Infants supplemented with AA+DHA from egg triglyceride/fish source scored higher in the Fagan test of novelty preference at 6 months CA, but not 9 months CA, than infants supplemented with AA+DHA from fish/fungal source or controls. The Bayley Mental Development Index was similar between groups at 12 months CA but was higher for infants weighing ≤1250 g and supplemented with AA+DHA from fish/fungal source when compared to corresponding controls. At 14 months CA, control infants had lower vocabulary comprehension than supplemented infants. The authors concluded that supplementation with AA+DHA provided some benefits in visual acuity and development when fed to preterm infants while "no difference among study formula groups was found with respect to indicators of feeding tolerance" or serious adverse events.

Preterm infants <35 weeks of age and weighing ≤2000 g were studied in a prospective, randomized, double-blind trial in which the infants were fed formulas supplemented with LCPUFA for up to 9 months and followed for an additional 9 months

(Fewtrell et al. 2004). Borage oil providing γ-linolenic acid and tuna oil providing DHA with a DHA/ EPA ratio of 5:1 were used as the sources of LCPUFA. A total of 238 formula-fed infants were stratified by birth weight of ≤1200 g or >1200 g and randomized to receive either LCPUFA-supplemented formula (n=122) or unsupplemented formula (control; n=116). Two formulas were used: preterm formula (prior to hospital discharge) and post-discharge formula. The infants selected for study did not have congenital malformations known to affect growth or neurodevelopment and received some enteral feedings while still in the hospital. During the hospital stay, infants were observed daily and clinical condition, body weight, enteral and parenteral feed volumes, and feed tolerance were recorded. Length and head circumference were measured weekly. Following discharge, qualified nurses examined infants at weeks 6, 12, and 26 after term and weight, length, and head circumference were recorded. Information also was collected on feeding, safety, and tolerance. Nine and 18 months after term, the infants were assessed for development and underwent anthropometry. At 18 months after term, infants were assessed using the Bayley Scales of Infant Development II and the Mental and Psychomotor Development Indexes (MDI and PDI) were derived based on post-term age. In addition, infants were assessed at 9 months using the Knobloch, Passamanick, and Sherrards' Developmental Screening Inventory (i.e., adaptive, gross motor, fine motor, language, and personal-social) and at 9 and 18 months for neurologic impairment (as diagnosed by a pediatrician). Infants also were observed for incidences of infection (e.g., skin sepsis and systemic infection), necrotizing enterocolitis, hemorrhagic events (e.g., intracranial and pulmonary), and requirement for respiratory support. Any adverse symptoms were recorded including stool consistency, abdominal distension, gastroenteritis, upper respiratory tract infection, chest infections, eczema, wheeze, and asthma. Results were statistically analyzed.

By 9 months, 9 infants from the LCPUFA-supplemented group and 25 infants from the control group no longer received the formula for various reasons. The specific reasons were not reported, but the change in formula was parent-initiated (rather than physician-initiated) for 18 of the 25 control infants and all 9 of the LCPUFA-supplemented infants. The reasons did not appear to be treatment-related. At the 18-month follow-up, 106 infants from the LCPUFA-supplemented group and 93 from the control group were available for assessment. There was no significant difference between groups in MDI and PDI at 18 months or in the proportion of infants considered to have equivocal or abnormal neurological status at 9 and 18 months. Body weight and length were similar between groups during the hospital stay and at discharge. LCPUFA-supplemented infants tended to be heavier by 195 g and 242 g at 12 and 26 weeks after term, respectively, compared to controls (statistical significance not stated). LCPUFA-supplemented infants also were longer by 0.5 cm at 26 weeks compared to controls. The LCPUFA-supplemented infants were still heavier (by 260 g) and longer (by 0.68 cm) at 9 months but the differences were not statistically significant. Weight and length gain between birth or first measurement obtained and 9 months were significantly greater in LCPUFA-supplemented infants compared to controls by 310 g (P=0.03) and 0.9 cm (P=0.05), respectively. By 18 months, the differences between the groups were insignificant. There were no differences in adverse clinical events between LCPUFA-supplemented infants and controls with the exception that

LCPUFA-supplemented infants required ventilation and supplemental oxygen for significantly longer (median of 4 days versus 2) and had umbilical catheters *in situ* for longer periods (median of 3 days versus 4) than controls. Tolerance of formula was similar between LCPUFA-supplemented infants and controls. When assessed for the effect of gender, LCPUFA-supplemented males had significantly (P=0.04) higher Bayley MDI scores at 18 months. LCPUFA-supplemented males also were 0.45 kg heavier than controls and their greater weight gain (difference of 0.51 kg) from birth to 9 months was statistically significant (P=0.02). By 18 months, these differences were no longer significant, but LCPUFA-supplemented males showed a larger length gain (difference of 1.8 cm) between first measurement and 18 months (P=0.03). It was concluded that LCPUFA-supplementation of infant formula for up to 9 months produced no adverse events and showed some benefits for growth in both sexes and neurodevelopment in males. The authors stated, "The trial formulas were well tolerated, and there was no evidence for any adverse effect of LCPUFA supplementation on clinical outcome. ... In conclusion, supplementation of infant formula with LCPUFA from tuna oil and borage oil up to 9 months after term proved to be a safe strategy with benefits for growth for the cohort as a whole and for mental development in boys."

Formulas supplemented with DHA from algae (Martek Biosciences) or tuna oil (Roche Vitamins Inc.) plus AA from fungal oil (Martek Biosciences) were given to preterm infants until 92 weeks postmenstrual age (PMA) in a prospective, randomized, double-blind, placebo-controlled trial (Clandinin et al. 2005). The trial was conducted in 2 phases: phase 1 (start of treatment until 40 weeks PMA) and phase 2 (40-92 weeks PMA). The infants ≤35 weeks PMA received <10 days enteral feedings of >30 ml/kg bw/day prior to study. Infants were observed until 118 weeks PMA. Many of the infants had medical conditions related to prematurity, but infants with congenital abnormalities of the gastrointestinal tract, hepatitis, hepatic or biliary pathology, necrotizing enterocolitis, or congenital malformations likely to interfere with the study were excluded. A total of 361 infants were randomized into 3 test groups: (1) control without DHA + AA supplementation (n=119); (2) supplementation with 17 mg algal DHA/100 kcal + 34 mg AA/100 kcal (n=112); and (3) supplementation with 17 mg tuna DHA/100 kcal + 34 mg AA/100 kcal (n=130). These amounts of DHA and AA were selected to be consistent with median amounts reported in mature human milk (approximately 0.3% by weight of fatty acids as DHA and 0.6% as ARA). Each group received 3 formula variations: premature (24 kcal/oz; recommended feeding until hospital discharge), discharge (22 kcal/oz; recommended feeding to 53 weeks PMA), and term (24 kcal/oz; recommended feeding to 92 weeks). A reference group consisted of healthy appropriate-for-gestational-age (38-42 weeks) breastfed infants (n=105).

Fifty-six infants (21 control, 17 algal DHA, and 18 tuna DHA) were removed from the study by 40 weeks PMA (end of phase 1), mainly for formula intolerance (n=15), unrelated medical complications (n=13), or parental request (n=11); however, there were no significant differences between groups. A further 60 infants (15 control, 23 algal DHA, and 22 tuna DHA) who completed phase 1 did not enter phase 2 for various reasons including lack of fulfilling enrollment criteria (≥80% intake of formula during

hospitalization, 100% intake by end of phase 1, and <1500 g birth weight; n=46), formula intolerance (n=6), elected withdrawal (n=7), and >7 consecutive days off study (n=1).

Two hundred and forty-five infants (83 control, 72 algal DHA, 90 tuna DHA, and 105 breastfed) entered phase 2. Growth (weight, length and head circumference), intake, tolerance, morbidity, and adverse events were assessed on a regular basis throughout the study. Blood samples were collected at 57 weeks PMA for hematology; serum glucose, cholesterol, high-density lipoproteins, triglyceride, mineral and electrolyte levels; and liver and kidney function tests. At 118 weeks PMA, all infants were tested using the Bayley Scales of Infant Development II Mental Development Index and Psychomotor Development Index.

At the start of phase 1, the birth weight and birth head circumference of tuna DHA infants were significantly lower ($p<0.05$) than control or algal DHA infants (Clandinin et al. 2005). Gestational age at birth and age when test formula was first consumed was significantly lower ($p<0.05$) in tuna DHA infants than algal DHA infants. There were no significant differences in weight, length, head circumference, gestational age at birth, age when test formula was first consumed, sex, birth weight category, or racial distribution between the test groups entering the second phase of the study. During hospitalization, there were no differences between test groups in caloric intake or formula tolerance. At 40 weeks PMA, parental reports indicated that the algal DHA group consumed more formula than the tuna DHA group (199.8±8.5 versus 175.4±7.5 ml/kg bw/day; $P<0.01$). Similarly, at 48 weeks, the algal DHA group consumed more formula than controls or the tuna DHA group (214.9±7.7 versus 188.3±7.4 and 189.8±6.9 ml/kg bw/day, respectively; $P<0.01$). By the end of phase 1, the incidence of intraventricular hemorrhage was significantly lower in the algal DHA group than other groups. Other than a greater incidence ($P<0.05$) of "more gas than usual" reported for the algal DHA group compared to controls at 40 and 44 weeks and for the tuna DHA group compared to controls at 48 weeks PMA, no other clinical events were reported. At PMA weeks 66, 79, 92, and 118, the algal DHA group had greater ($P<0.05$) mean weights than controls and at 118 weeks PMA, the algal DHA group had greater ($P<0.05$) mean weights than the tuna DHA group. Breastfed infants had greater ($P<0.05$) weights than all other groups including controls except the algal DHA group at 118 weeks. Length also was greater ($P<0.05$) in breastfed infants compared to other groups at 40, 44, 48, 53, 57, and 66 weeks PMA and was greater ($P<0.05$) than controls and tuna DHA but not algal DHA at 79, 92, and 118 weeks. There were no differences in head circumference between groups up to 66 weeks PMA. Some small, but statistically significant, differences were noted at 79 (algal DHA > tuna DHA; breastfed > controls and tuna DHA) and 92 weeks PMA (breastfed > controls and tuna DHA). By 118 weeks PMA, breastfed infants (48.2±0.29 cm) had a greater head circumference than those of control (46.5±0.30 cm; $P=0.052$) and tuna DHA groups (47.2±0.32 cm; $P=0.004$). The algal DHA group (47.7±0.36 cm) did not differ from any group.

Both DHA groups showed higher mean Bayley MDI ($P=0.056$ and $P<0.05$ for algal and tuna DHA groups, respectively) and PDI ($P<0.05$ for both DHA groups) scores compared to controls at 118 weeks PMA and breastfed term infants had significantly higher scores

than any preterm group. Blood work showed a few significant changes compared to controls: increased mean corpuscular hemoglobin in the tuna DHA group (27.6 versus 27.0 pg/cell; P=0.03), increased total cholesterol in the tuna DHA group (3.85 versus 3.43 mmol/l; P<0.05), and decreased serum potassium in the tuna DHA group (5.0 versus 5.3 mmol/l; P=0.003). The authors concluded that feeding formulas supplemented with DHA and AA to preterm infants enhanced growth and benefitted development. They also observed that, "Despite hypothetical concerns about adding DHA and ARA to formulas for preterm infants such as potential interference with host defense mechanisms or impact on hemostasis, we found no increase in morbidity associated with supplementation. Our analysis of a wide spectrum of clinical data, including serum chemistry and hematology values and incidence and severity of medical conditions related to prematurity, found no safety issues related to the supplemented formulas."

Makrides et al. (1999) conducted a prospective, randomized, double-blind, placebo-controlled, multicenter study with healthy preterm infants born at less than 33 weeks' gestation, with follow-up to 18 months. A total of 657 infants (born to 545 mothers) was enrolled within 5 days of first enteral feeds. Exclusion criteria included major congenital or chromosomal abnormalities , multiple birth with not all live-born infants eligible, or born to lactating mothers for whom tuna oil was contraindicated. The sample was randomized with stratification based on center, birth weight, and sex. Lactating mothers in the high-DHA group (n = 272 with 322 infants) consumed six 500-mg tuna-oil capsules daily (3.0 g/day providing about 900 mg DHA) to achieve a breast-milk DHA concentration of about 1% of total fatty acids; if supplementary formula was needed, a preterm formula providing 1% DHA and 0.6% AA was given. Mothers in the placebo group (n = 273 with 335 infants) received similar capsules containing soy oil; supplementary or replacement formula was standard preterm formula with approximately 0.35% DHA and 0.6% AA. The tuna oil (containing about 30% DHA) was provided by Clover Corp. (Sydney, Australia) and the infant formulas were specially prepared by Mead Johnson Nutritionals (Evansville, IN). Treatment continued until infants reached their expected date of delivery; postterm, mothers were encouraged to continue breastfeeding and those who had weaned to formula were encouraged to use a term formula supplemented with DHA and AA. Feed intake was recorded, as well as growth and infant health; breast milk was analyzed for fatty acid content. The MDI and PDI were administered at 18 months.

In the high-DHA group, 6 infants died during treatment and 3 others died prior to 18 months; in the control group, 4 infants died during treatment and 5 more prior to 18 months (Makrides et al. 1999) . The cause of death was not reported, but were regarded as normal for this cohort and not related to the intervention. Four infants in the high-DHA group and 3 controls withdrew during treatment, and 11 and 7 infants, respectively, were lost to the 18-month follow-up. The median duration of treatment and maternal compliance with capsule ingestion did not differ between the groups. The DHA content of milk in the high-DHA women was 0.85%, significantly higher than the 0.25% of fatty acids in the control-group women's milk, but the AA contents did not differ. There were no differences in maternal reports of diarrhea, constipation, nausea, or vomiting.

MDI scores were significantly higher for girls in the high-DHA group than those in the control group, but the scores of boys did not differ between groups. PDI scores did not differ significantly between groups. There were no differences in weight or head circumference, but infants in the high-DHA group were significantly longer than controls. No differences were seen in infant mortality, days in intensive care, incidence of adverse events, or breastfeeding duration. Post-hoc analysis indicated that the frequency of mild mental delay in smaller infants (<1250 g) was reduced by 45% in the high-DHA group compared with controls. The authors concluded that the dose of DHA used in this study—approximately 0.85% of the total fatty acids in mother's milk—was safe and warrants further study.

Makrides et al. (2009) conducted a prospective, randomized, double-blind, placebo-controlled, multicenter trial in 5 Australian perinatal centers to study long-term neurodevelopmental outcomes of preterm infants fed high-dose DHA. The study included infants with gestational age less than 33 weeks without major congenital or chromosomal abnormalities. Mother-infant pairs including 545 women with 657 infants were enrolled and randomly assigned either the high-DHA group (n = 272 mothers and 322 infants) or the standard-DHA group (n = 273 mothers and 335 infants). The mean birthweights of infants assigned to the high-DHA and standard-DHA groups were 1308 and 1307 g, respectively; mean gestational ages were 30 weeks in both groups. Lactating mothers in the high-DHA group consumed six 500-mg DHA-rich tuna oil capsules per day (total 3 g tuna oil/day) to achieve a breast-milk DHA concentration of approximately 1% of the total fatty acids, with no alteration it the breast milk's AA concentration. If supplementary formula was needed, the infants received high-DHA (~1% total fatty acids) preterm formula which also contained 0.6% AA (specially manufactured by Mead Johnson). In the standard-DHA groups, mothers consumed six 500-mg placebo soy oil capsules and, if needed, infants were fed standard preterm formula with DHA at ~0.35% total fatty acids and AA at 0.6%, also specially manufactured by Mead Johnson. The intervention began within 2-4 days of birth and continued until the infants reached their expected date of delivery. At that time, weight, length, and head circumference were measured and breastfeeding women donated a milk sample. All cases of NEC, sepsis, intraventricular hemorrhage, retinopathy of prematurity, and oxygen treatment were recorded. At 18 months CA, the MDI and PDI of the Bayley Scales of Infant Development, Second Edition, and the Home Screening Questionnaire were administered, and the infants' weight, length, and head circumference were assessed.

In the high-DHA group, 10 infants—including 6 who died--did not complete treatment, while 7 standard-DHA infants failed to complete treatment, including 4 who died. Deaths were not related to treatment and completion rates did not differ significantly between the 2 groups. Fourteen of the infants in the high-DHA group were lost to the 18-month follow-up, 3 due to death, while 12 standard-DHA infants, including 5 who died, did not complete the follow-up. The 2 groups did not differ significantly in the duration of treatment, with means of 9.4 weeks in both groups. The mean DHA level of breast milk in the high-DHA mothers was 0.85% of fatty acids v. 0.25% in the standard-DHA group, while the tested DHA concentrations in the 2 formulas were 1.11% and 0.42% of total fatty acids,

respectively. Mean AA concentrations in breast milk (0.4%) or in formula (0.7%) did not differ significantly between the 2 groups.

MDI scores, the primary outcome, did not differ significantly between the 2 groups, but the interaction between MDI and sex was statistically significant, with girls in the high-DHA group significantly outperforming girls in the standard-DHA group; MDI scores of boys in the 2 groups did not differ significantly from each other. PDI scores showed no significant differences between groups. Post-hoc analyses indicated that fewer infants had significantly delayed mental development with high-DHA diets compared with standard DHA. There were no differences in anthropometric measures except that high-DHA infants were slightly longer than standard-DHA infants at 18 months. There were no significant differences in adverse events except that fewer infants fed high-DHA diets required oxygen treatment than did those given standard-DHA diets. The authors concluded that, given the benefits of the high-DHA diet along with the "apparent safety of the current dose of DHA [~1% of the total fatty acids in breast milk or formula], further studies are warranted."

4.1.1.2. Term Infants

A longitudinal, prospective, randomized study was conducted with healthy term infants (≥37 weeks gestation) fed formulas supplemented with DHA and AA to assess visual acuity, erythrocyte fatty acid composition, and growth (Auestad et al. 1997). The infants were divided into 3 groups: formula with no added LCPUFA (control group; n=45); formula supplemented with 0.43% AA and 0.12% DHA (from egg yolk phospholipid) of total fatty acids (AA+DHA-supplemented group; n=46); and formula supplemented with 0.2% DHA (from tuna oil with a DHA/EPA ratio of ~4:1) of total fatty acids (DHA-only-supplemented group; n=43). Formulas were fed exclusively for a minimum of 4 months. The ready-to-use formulas contained 14.3-15.0 g protein, 72.4-74.8 g carbohydrate, 35.9-37.2 g fat and 670-694 kcal. A reference group of infants (n=63) was breastfed exclusively for a minimum of 3 months and then commercial formula (60% soy oil/40% coconut oil) supplementation was permitted. Formula intake, growth, and tolerance were recorded at various intervals until 12 months of age. Blood samples were collected at 2, 4, 6, and 12 months of age for analysis of erythrocyte lipids. Visual function was assessed at various intervals longitudinally, by the acuity card procedure, and/or using the swept-spatial frequency VEP depending on the geographic location of the infants.

Formula intake, tolerance, growth, and visual function did not differ between the groups. Erythrocyte AA and DHA levels of infants fed the AA+DHA-supplemented formula were within 10% of those of the breastfed infants whereas control infants had 10-40% lower erythrocyte AA and DHA levels than those of the breastfed infants. Infants fed the DHA-only-supplemented formula had erythrocyte DHA levels that were 25-55% higher and erythrocyte AA levels that were 15-40% lower than those of breastfed infants. The authors concluded that "the fact that visual function was not different among any of the groups in this study does not support adding DHA or AA to infant formula. However, with regard to

safety, "the present study showed normal growth and visual acuity development in infants fed [any of the tested formulas]."

Scott et al. (1998) reported additional evaluations conducted on the Auestad et al. (1997) cohort. The Bayley Scales of Infant Development were used to derive MDI and PDI scores at 12 months of age. In addition, the MacArthur Communicative Development Inventories were used to assess language at 14 months of age. MDI and PDI scores did not differ between the groups. For language assessment, vocabulary comprehension was significantly lower in DHA-only-supplemented infants compared to breastfed infants and vocabulary production was slightly (P=0.052) lower in DHA-only-supplemented infants compared to controls. However, the infants receiving both DHA and AA did not differ from either the controls or the human-milk reference group.

This same cohort was evaluated once again when the infants reached the age of 39 weeks (Auestad et al. 2003). A total of 157 of the infants studied at 12 months participated. The groups—including the 3 formula-fed groups as well as the breastfed reference group—did not differ in weight, length, or head circumference at 39 months, nor were differences in IQ, receptive and expressive language, visual-motor function, or visual acuity. There was no evidence of differences in healthy status based on measures such as number of prescriptions for antibiotics or number of hospitalizations. The authors concluded that "The present follow-up evaluation of growth, visual development, and neurodevelopmental outcomes at 39 months found no adverse effects or benefits of infant formula supplemented with DHA or with both DHA and ARA."

A prospective, randomized, double-blind trial was conducted to determine the effect of LCPUFA supplementation of infant formula on growth (Makrides et al. 1999). Term infants were allocated to one of 3 groups: standard unsupplemented formula (Nestec Ltd., Switzerland; control; n=28); standard formula supplemented with 0.35% DHA from tuna oil (DHA-only-supplemented group; n=27); or standard formula supplemented with 0.34% DHA and 0.34% AA from an egg phospholipid fraction (AA+DHA-supplemented group; n=28). The formulas were fed for 12 months. A reference group of 63 infants was breastfed. Weight, length, head circumference, and fatty acid status were evaluated at 6, 16, and 34 weeks and at 1 and 2 years.

At 34 weeks, 21 controls, 23 DHA-only-supplemented, 24 AA+DHA-supplemented, and 46 breastfed infants were assessed. Growth parameters did not differ significantly between groups. At 16 weeks, DHA plasma phospholipid levels were significantly lower in controls than supplemented and breastfed infants. These levels were significantly lower in breastfed infants when compared with supplemented infants. AA plasma phospholipid levels were lowest in the DHA-only-supplemented group followed by the control group and then the AA+DHA-supplemented group with the breastfed group having the highest levels (all differences statistically significant). The authors noted that even though the fatty acid profiles changed in a manner similar to other studies showing effects on growth, LCPUFA supplementation of infant formula in this study did not affect growth parameters. Indeed, they concluded that, "The aim of our trial was to determine if LCPUFA treatment of formula-

fed infants influenced growth. ... [W]e observed no differences in weight, length, or head circumference...Our trial was sufficiently powered to detect clinically relevant changes in weight and length."

In a follow-up to Makrides et al. (1999), the same infants (21 controls, 23 DHA-only-supplemented, 24 AA+DHA-supplemented, and 46 breastfed infants) were assessed using Bayley's I Scales of Childhood Development to determine MDI and PDI at 1 and 2 years of age and were assessed for VEP acuity at 16 and 34 weeks (Makrides et al. 2000). MDI and PDI scores and VEP acuity did not differ between groups at any time point examined. This follow-up report did not include any discussion of growth, safety, or adverse events.

A prospective, randomized, double-blind parallel-group clinical trial was conducted with 239 term infants (37-42 weeks gestational age) fed formulas with or without supplemented DHA and AA starting within 9 days of age until 12 months of age (Auestad et al. 2001). Infants were assigned to 1 of 3 groups: unsupplemented formula (control; n=77); formula supplemented with 0.13% DHA and 0.45% AA from fish oil or fungal oil (n=82); or formula supplemented with 0.13% DHA and 0.45% AA from egg-derived triglyceride (n=80). A reference group of infants (n=165) was breastfed for at least 3 months and then weaned to formulas with (n=83) and without DHA+AA (n=82). All infants were allowed other food after 4 months of age. Blood samples were taken at 4 and 12 months of age for red blood cell fatty acid analysis. Breast milk fatty-acid analysis was conducted on a subset of breastfeeding mothers at 4 months. Growth (weight, length, and head circumference measured at enrollment and at 1, 2, 4, 6, 9, and 12 months), visual acuity (using the Teller Acuity Card Procedure at 2, 4, 6, and 12 months), information processing (using the Fagan Test of Infant Intelligence at 6 and 9 months), general development level (using the Bayley Scales of Infant Development at 6 and 12 months), language (using the MacArthur Communicative Development Inventories at 9 and 14 months), and temperament (using the Infant Behavior Questionnaire at 6 and 12 months) were assessed.

A total of 294 infants completed the study, 76% of the breastfed infants, 75% of the infants receiving formula with egg-derived triglyceride, 70% receiving formula supplemented with fish oil, and 62% of the infants in the control-formula group. Reasons given for infants leaving the study did not differ between the groups. Levels of AA and DHA in red blood cell phospholipids were significantly higher in infants fed supplemented formula compared to controls. Breastfed infants weaned to formula with AA+DHA showed no difference in AA and DHA red blood cell phospholipid levels at 4 months compared to controls, but at 12 months the breastfed infants weaned to supplemented formula had significantly higher DHA red blood cell phospholipid levels than corresponding controls. Growth, visual acuity, information processing, general development level, language, and temperament overall were similar between groups. The results of this study indicated that AA+DHA supplementation neither inhibited nor enhanced growth or development of term infants. However, there was no indication of safety or tolerance issues; "there were no overall or gender specific differences for increases in weight, length, or head circumference

among groups during the 12-month study," and there was no difference in the numbers of infants withdrawn from the study due to reported intolerance for the assigned formula.

Healthy term infants were fed formulas supplemented with LCPUFAs for 2 months in a prospective, randomized, double-blind, placebo-controlled study to assess their general movements (Bouwstra et al. 2003). A group of 167 infants was fed the control formula (commercial formula, Nutrilon Premium; Nutricia, Zoetemeeer, Netherlands) and a second group (n=145) was fed a similar formula supplemented with 0.30% DHA derived from egg yolk and tuna oil low in EPA and 0.45% AA derived from egg yolk and a single-cell oil from the soil fungus *Mortierella alpina* by weight. A reference group of 160 breastfed term infants was also included. At 3 months of age, 397 infants (131 controls, 119 supplemented, and 147 breastfed) were videotaped for 15 minutes to assess spontaneous motility, and weight and length were recorded.

The reduction in the number of infants in the 3-month follow-up was explained as follows: "The major reason that infants were not followed up was simply an overload of work for the research team." Movements were classified as normal-optimal, normal-suboptimal, mildly abnormal, and definitely abnormal. There were no definitely abnormal general movements in any of the infants. Infants supplemented with AA and DHA had a significantly reduced frequency of mildly abnormal general movements compared to controls (19% versus 31%). The frequency did not differ between breastfed and supplemented infants. Breastfed infants tended to have a nonsignificantly higher frequency of normal-optimal general movements compared to supplemented and control infants (34% versus 18 or 21%, respectively). The authors concluded that supplementation of infant formula with DHA and AA for 2 months reduces the occurrence of mildly abnormal general movements. The authors did not indicate any issues regarding tolerance of the formula or the occurrence of any adverse events.

Infants from the Bouwstra et al. (2003) study were assessed at 18 months of age using Bayley Scales of Infant Development to derive MDI and PDI scores and using a neurological technique described by Hempel (1993) which measures motor functions (Bouwstra et al. 2005). Children were classified as neurologically normal, showing signs of minor neurological dysfunction, or as definitely abnormal. The number of children assessed for MDI was 155, 135, and 148 for controls, supplemented group, and breastfed group, respectively, for PDI was 149, 134, and 144 for controls, supplemented group, and breastfed group, respectively, and for Hempel assessment was 157, 135, and 154 for controls, supplemented group, and breastfed group, respectively. The MDI and PDI scores did not differ among any of the groups and the Hempel assessment showed no differences in neurological condition. The results indicated that 2-month supplementation of infant formula with DHA and AA had no effect on neurological condition at 18 months of age; there was no discussion of any other markers of the safety of the supplemented formula such as growth or general health.

Table 3. Summary of Infant Studies Using Fish Oil as the Source of DHA.					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
PRETERM INFANTS					
Randomized study: (1) unsupplemented formula (control; n=10) (2) formula supplemented with 0.5% LCPUFA containing 0.1% DHA and 0.2% AA (n=8) Reference group=breastfed infants (n=11)	LCPUFA from egg lipids and fish oil	Formula fed from age 4 to 21 days.	Growth, tolerance, and other clinical characteristics were similar between groups. Breastfed infants showed no changes in LCPUFA levels in plasma lipids between days 4 and 21; however, these levels decreased significantly in controls. Infants fed the supplemented formula had levels higher than controls but lower than breastfed infants.	No safety concern; altered LCPUFA levels in plasma lipids. The authors reported that "All infants tolerated the feeds well, and no side effects of the LCPUFA-formula were noted."	Koletzko et al. (1989)
Same as Koletzko et al. (1989)	Same as Koletzko et al. (1989)	Same as Koletzko et al. (1989)	8 controls, 9 supplemented infants and 15 breastfed infants were assessed for antioxidant status. Ratio of erythrocyte membrane α-tocopherol/total lipid did not change between day 4 and 21 for controls and breastfed infants, but there was a significant decrease in this ratio for infants fed the supplemented formula. No differences were noted in plasma α-tocopherol/total lipid ratios between the groups. Plasma α-tocopherol concentrations significantly increased (140%) from day 4 to 21 in the breastfed group but not in any other group.	No safety concern but vitamin E status should be considered.	Koletzko et al. (1995)
Randomized, double-blind study: (1) unsupplemented formula (control; n=45) (2) 0.2% DHA-supplemented formula	DHA from fish oil	Supplemented formulas were fed from 7-10 days PMA to 59 weeks PMA.	63 infants (32 controls and 31 supplemented) completed the study. The formulas were well tolerated. Four SIDS deaths occurred in DHA-supplemented infants; the SIDS deaths were independently reviewed and considered unrelated to study participation. No differences between control and DHA-supplemented female infants were noted in any parameter. DHA-supplemented males showed significantly decreased growth over the study period when compared to controls. Plasma phospholipid DHA levels were significantly higher in DHA-supplemented infants compared to controls. In males, there was an inverse correlation between plasma phospholipid DHA levels and recumbent length.	No safety concern but reduced growth in supplemented males should be further assessed.	Ryan et al. (1999)

Table 3. Summary of Infant Studies Using Fish Oil as the Source of DHA.					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized, blinded study: (1) unsupplemented formula (control; n=144); (2) formula supplemented with 0.26% DHA and 0.42% AA (n=140); or (3) formula supplemented with 0.26% DHA and 0.42% AA (n=143). A reference group of 43 infants breastfed exclusively until term corrected age (CA)	From fish/fungal oil (group 2); from egg-derived triglyceride/fish oil (group 3)	Formula and/or human milk fed from first enteral feeding to 12 months CA.	376 infants from the formula and breastfed groups completed the study. 15 deaths were reported over the course of the study—6 each in the control and egg/fish groups and 3 in the fish/fungal group . Investigators concluded that these deaths were unrelated to study feedings. AA and DHA plasma and red blood cell phospholipid levels were significantly higher in supplemented groups than controls except for AA red blood cell phosphatidylethanolamine levels at 4 and 12 months CA. Anthropometric measurements, in-hospital feeding tolerance, clinical problems, serious and/or unexpected adverse events, and behavioral visual acuity of supplemented infants showed no consistent differences from controls. VEP acuity of supplemented infants at 4 months CA was similar to controls but by 6 months CA was significantly greater in supplemented infants than in controls. Infants supplemented with AA+DHA from egg triglyceride/fish source scored higher in the Fagan test of novelty preference at 6 months CA, but not 9 months CA, than infants supplemented with AA+DHA from fish/fungal source or controls. The Bayley Mental Development Index was similar between groups at 12 months CA but was higher for infants weighing ≤1250 and supplemented with AA+DHA from fish/fungal source when compared to corresponding controls. At 14 months CA, control infants had lower vocabulary comprehension than supplemented infants.	No safety concern; some beneficial effect on visual acuity and development. The authors noted that "no difference among study formula groups was found with respect to indicators of feeding tolerance" or serious adverse events.	O'Connor et al. (2001)

Table 3. Summary of Infant Studies Using Fish Oil as the Source of DHA.					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized, double-blind study: (1) LCPUFA-supplemented formula (n=122) (2) unsupplemented formula (control; n=116)	Borage oil (gamma-linolenic acid) and tuna fish oil (DHA)	Supplemented formulas were fed to 9 months after term.	By 9 months 34 infants (25 control and 9 supplemented) ceased consumption of the formula, nearly all for parent-initiated reasons not believed to be treatment-related, and by the 18-month follow-up 93 controls and 106 supplemented infants were assessed. The incidence of adverse clinical events, tolerance, and MDI and PDI were similar between groups. Weight and length gain between birth or first measurement and 9 months of age were significantly greater in supplemented infants but by 18 months of age the differences were insignificant.	No safety concern; benefit to growth and neurodevelopment. The authors stated, "The trial formulas were well tolerated, and there was no evidence for any adverse effect of LCPUFA supplementation on clinical outcome. ... In conclusion, supplementation of infant formula with LCPUFA from tuna oil and borage oil up to 9 months after term proved to be a safe strategy with benefits for growth for the cohort as a whole and for mental development in boys."	Fewtrell et al. (2004)
Randomized, double-blind study conducted in 2 phases: (1) unsupplemented formula (control; n=119) (2) algal DHA + AA supplemented formula (n=112) (3) fish DHA + AA supplemented formula (n=130) Reference group=breastfed infants (n=105)	Algal DHA from algal oil; Fish DHA from tuna fish oil; AA from fungal oil	DHA (17 mg/kcal) +AA (34 mg/kcal) supplementation until 92 weeks PMA and follow-up until 108 weeks PMA	56 infants (21 control, 17 algal DHA, and 18 fish DHA) left the study by 40 weeks PMA (end of phase 1) mainly for formula intolerance (n=15), unrelated medical complications (n=13), and parental request (n=11). 60 infants (15 control, 23 algal DHA, and 22 fish DHA) who completed phase 1 did not enter phase 2. 245 infants (83 control, 72 algal DHA, 90 fish DHA, and 105 breastfed) entered phase 2. No adverse events reported and no increase in morbidity. Body weight was significantly increased from control in algal DHA group (weeks 66-118) and in fish DHA group (week 118). Body length was significantly increased from control in algal DHA group (weeks 48, 79, and 92) and in fish DHA group (weeks 57, 79, and 92 weeks). Body weight and length were similar to breastfed group at 118 weeks and 79-118 weeks, respectively.	No safety concern; improved growth and development The authors concluded that, "Despite hypothetical concerns about adding DHA and ARA to formulas for preterm infants such as potential interference with host defense mechanisms or impact on hemostasis, we found no increase in morbidity associated with supplementation. Our analysis of a wide spectrum of clinical data, including serum chemistry and hematology values and incidence and severity of medical conditions related to prematurity, found no safety issues related to the supplemented formulas.".	Clandinin et al. (2005)

Table 3. Summary of Infant Studies Using Fish Oil as the Source of DHA.					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized, double-blind study: (1) high-DHA diet (n=322) from breast milk with 0.85% DHA or formula with 1.11% DHA (2) standard-DHA diet (n=335) from breast milk with 0.25% DHA or formula with 0.42% DHA	DHA from tuna fish oil	Infants received assigned diet from 2-4 days post-delivery to original due date; follow-up at 18 months	MDI scores did not differ significantly between the 2 groups, but the interaction between MDI and sex was significant, with girls in the high-DHA group significantly outperforming girls in the standard-DHA group; MDI scores of boys in the 2 groups did not differ significantly from each other. Post-hoc analyses indicated that fewer infants had significantly delayed mental development with high-DHA diets compared with standard DHA. There were no differences in anthropometric measures except that high-DHA infants were slightly longer than standard-DHA infants at 18 months. There were no significant differences in adverse events except that fewer infants fed high-DHA diets required oxygen treatment than did those given standard-DHA diets.	High-DHA diet improves mental development of premature girls and overall reduces the risk of delayed mental development. No adverse effect on safety. The authors concluded that, given the benefits of the high-DHA diet along with the "apparent safety of the current dose of DHA [~1% of the total fatty acids in breast milk or formula], further studies are warranted."	Makrides et al. (2009)
TERM INFANTS					
Longitudinal, prospective, randomized study: (1) unsupplemented formula (control; n=45) (2) formula supplemented with0.43% AA and 0.12% DHA (n=46) (3) formula supplemented with 0.2% DHA (n=43) Reference group=breastfed infants (n=63)	0.43% AA and 0.12% DHA from egg yolk phospholipid 0.2% DHA from a high DHA, low eicosapentaenoic acid tuna oil; ratio of ~4:1	Formulas were fed exclusively for a minimum of 4 months. Reference group was breastfed a minimum of 3 months.	Formula intake, tolerance, growth, and visual function did not differ between groups. Erythrocyte AA and DHA levels of infants fed the AA+DHA-supplemented formula were within 10% of those of the breastfed infants whereas control infants had 10-40% lower erythrocyte AA and DHA levels than those of the breastfed infants. Infants fed the DHA-supplemented formula had erythrocyte DHA levels that were 25-55% higher and erythrocyte AA levels that were 15-40% lower than those of breastfed infants.	No safety concern; no effect on growth, increased DHA and AA levels. The authors concluded that "the fact that visual function was not different among any of the groups in this study does not support adding DHA or AA to infant formula. However, with regard to safety, "the present study showed normal growth and visual acuity development in infants fed [any of the tested formulas]."	Auestad et al. (1997)
Same as Auestad et al. (1997)	Same as Auestad et al. (1997)	Same as Auestad et al. (1997)	MDI and PDI scores did not differ between groups at 12 months of age. At 14 months of age, vocabulary comprehension was significantly lower in DHA-supplemented infants compared to breastfed infants and vocabulary production was slightly lower in DHA-supplemented infants compared to controls.	No safety concern; slightly reduced vocabulary skills. However, the infants receiving both DHA and AA did not differ from either the controls or the human-milk reference group.	Scott et al. (1998)

Table 3. Summary of Infant Studies Using Fish Oil as the Source of DHA.					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Same as Auestad et al. (1997)	Same as Auestad et al. (1997)	Same as Auestad et al. (1997)	This same cohort was evaluated once again when the infants reached the age of 39 weeks. A total of 157 of the infants studied at 12 months participated. The groups—including the 3 formula-fed groups as well as the breastfed reference group—did not differ in weight, length, or head circumference at 39 months, nor were differences in IQ, receptive and expressive language, visual-motor function, or visual acuity. There was no evidence of differences in healthy status based on measures such as number of prescriptions for antibiotics or number of hospitalizations.	No safety concern; no differences between groups. The authors concluded that "The present follow-up evaluation of growth, visual development, and neurodevelopmental outcomes at 39 months found no adverse effects or benefits of infant formula supplemented with DHA or with both DHA and ARA."	Auestad et al. (2003)
Randomized, double-blind study: (1) unsupplemented formula (control; n=28) (2) formula supplemented with 0.35% DHA (n=27) (3) formula supplemented with 0.34% AA and 0.34% DHA (n=28) Reference group=breastfed infants (n=63)	0.35% DHA from tuna oil 0.34% DHA and 0.34% AA from an egg phospholipid fraction	Formulas were fed for 12 months.	Growth was similar between groups. At 16 weeks, DHA plasma phospholipid levels were significantly lower in controls than supplemented and breastfed infants. These levels were significantly lower in breastfed infants when compared with supplemented infants. AA plasma phospholipid levels were lowest in the DHA-supplemented group followed by the control group and then the AA+DHA-supplemented group with the breastfed group having the highest levels (all differences statistically significant).	No safety concern; altered fatty acid profile and no effect on growth. The authors concluded that, "The aim of our trial was to determine if LCPUFA treatment of formula-fed infants influenced growth. ... [W]e observed no differences in weight, length, or head circumference...Our trial was sufficiently powered to detect clinically relevant changes in weight and length."	Makrides et al. (1999)
Same as Makrides et al. (1999)	Same as Makrides et al. (1999)	Same as Makrides et al. (1999)	MDI and PDI were similar between groups at 1 and 2 years of age. VEP was similar between groups at 16 and 34 weeks.	No safety concern; no effect on neurodevelopment or visual function.	Makrides et al. (2000)

Table 3. Summary of Infant Studies Using Fish Oil as the Source of DHA.					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized, double-blind, placebo-controlled, multicenter study: Mothers received: (1) 6 capsules providing 3.0 g/day soy oil (2) 6 capsules providing 3.0 g/day tuna oil with ~900 mg DHA	Tuna oil consumed by mothers and increasing the DHA content of their milk from ~0.25% to ~0.85% of the total fatty acids	Mothers consumed capsules from delivery of preterm infants to the originally expected date of delivery	In the high-DHA group, 6 infants diet during treatment and 3 others died prior to 18 months; in the control group, 4 infants died during treatment and 5 more prior to 18 months (Makrides et al. 1999) . The cause of death was not reported. The DHA content of milk in the high-DHA women was 0.85%, significantly higher than the 0.25% of fatty acids in the control-group women's milk, but the AA contents did not differ. MDI scores were significantly higher for girls in the high-DHA group than those in the control group, but the scores of boys did not differ between groups. Post-hoc analysis indicated that the frequency of mild mental delay in smaller infants (<1250 g) was reduced by 45% in the high-DHA group compared with controls.	There were no differences in maternal reports of diarrhea, constipation, nausea, or vomiting. No effects on growth or adverse events for infants. The authors concluded that the dose of DHA used in this study—approximately 0.85% of the total fatty acids in mother's milk—was safe.	Makrides et al. (1999)
Randomized, double-blind study: (1) unsupplemented formula (control; n=77); (2) formula supplemented with 0.13% DHA and 0.45% AA (n=82); or (3) formula supplemented with 0.13% DHA and 0.45% AA (n=80). A reference group of infants (n=165) were breastfed for at least 3 months and then weaned to formulas with (n=83) and without DHA+AA (n=82)	From fish oil or fungal oil (group 2); from egg-derived triglyceride (group 3)	Formula fed from ≤9 days of age to 12 months of age.	294 of the infants from the formula and breastfed groups completed the study. Levels of AA and DHA in red blood cell phospholipids were significantly higher in infants fed supplemented formula compared to controls. Breastfed infants weaned to formula with AA+DHA showed no difference in AA and DHA red blood cell phospholipid levels at 4 months compared to controls, but at 12 months the breastfed infants weaned to supplemented formula had significantly higher DHA red blood cell phospholipid levels than corresponding controls. Growth, visual acuity, information processing, general development level, language, and temperament overall were similar between groups.	No safety concern; no tolerance issues; no effect on growth or development. The authors stated,. "there were no overall or gender specific differences for increases in weight, length, or head circumference among groups during the 12-month study," and there was no difference in the numbers of infants withdrawn from the study due to reported intolerance for the assigned formula.	Auestad et al. (2001)

Table 3. Summary of Infant Studies Using Fish Oil as the Source of DHA.					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized, double-blind study: (1) unsupplemented formula (control; n=167) (2) formula supplemented with 0.45% AA and 0.30% DHA (n=145) Reference group=breastfed infants (n=160)	AA derived from egg yolk and a single-cell oil from the soil fungus, *Mortierella alpina* DHA derived from egg yolk and tuna oil low in eicosapentaenoic acid	Formula fed for 2 months.	At 3 months of age, 131 controls, 119 supplemented infants and 147 breastfed infants were assessed. Supplemented infants had a significantly reduced frequency of mildly abnormal general movements compared to controls (19% versus 31%). The frequency did not differ between breastfed and supplemented infants.	No safety concern; reduction in incidence of mildly abnormal general movements.	Bouwstra et al. (2003)
Same as Bouwstra et al. (2003)	Same as Bouwstra et al. (2003)	Same as Bouwstra et al. (2003)	At 18 months of age, MDI and PDI were similar between groups. Neurological assessment showed no differences between groups.	No safety concern; no effect on neurodevelopment.	Bouwstra et al. (2005)
AA=arachidonic acid; BAEP=brainstem acoustic evoked potentials; DHA=docosahexaenoic acid; ERG=electroretinogram; LCPUFA=long-chain polyunsaturated fatty acids; MDI=Mental Development Index; PDI=Psychomotor Development Index; PMA=postmenstrual age; VEP=visual evoked potential					

4.1.1.3. Characteristics of Fish Oil Supplementation of Infant Formulas

The characteristics of the fish-oil supplementation of infant formulas in the 13 published studies of preterm or term infants are shown in Table 4. Somewhat surprisingly, not a single study reported the DHA and/or EPA concentration of the fish oil, nor how much fish oil was added to the infant formula in order to achieve the reported concentration of DHA. In the two studies by Koletzko et al. (1989 and 1995), the fish oil was not further identified; in all of the remaining studies the oil used was identified as tuna oil or can be determined to have been tuna oil. In Table 4, the DHA:EPA ratio is given only if it was reported in the published article, but this ratio could be calculated in several other studies since the concentrations of both DHA and EPA in the formula were reported. Including these calculated ratios, the range of DHA:EPA ratios in published studies ranged from less than 3:1 up to 5:1.

Levels of DHA supplementation ranged from a low of 0.1% of the total fatty acids to a maximum of 0.35%; five of the published studies were based on DHA supplementation of 0.30% of the fatty acids or higher.

Table 4. Characteristics of Fish Oil Supplementation of Infant Formulas.

Study	Source of DHA	DHA and EPA in Fish Oil	DHA:EPA Ratio in Fish Oil	DHA (% total fatty acids) in Formula	EPA (% total fatty acids) in Formula
Auestad et al. (1997)	Tuna oil	NR[1]	~4:1	0.2	NR
Auestad et al. (2001)	Tuna oil[2]	NR	NR	0.13	≤0.04
Bouwstra et al. (2003)	Tuna oil	NR	NR	0.30	0.07
Bouwstra et al. (2005)	Tuna oil	NR	NR	0.30	0.07
Clandinin et al. (2005)	Tuna oil	NR	NR	0.32	0.1
Fewtrell et al. (2004)	Tuna oil	NR	5:1	NR	NR
Koletzko et al. (1989)	Fish oil	NR	NR	0.1	NR
Koletzko et al. (1995)	Fish oil	NR	NR	0.1	NR
Makrides et al. (1999)	Tuna oil	NR	NR	0.35	0.10
Makrides et al. (2000)	Tuna oil	NR	NR	0.35	0.10
Makrides et al. (2009)	Tuna oil	NR	NR	In breast milk: 0.85 In formula: 1.15	NR
O'Connor et al. (2001)	Tuna oil[2]	NR	NR	In-hospital: 0.24 Discharge: 0.15	In-hospital: NR Discharge: NR
Ryan et al. (1999)	Tuna oil	NR	~5:1	Preterm: 0.2 Term: 0.2	Preterm: 0.04 Term: 0.07
Scott et al. (1998)	Tuna oil	NR	~4:1	0.2	NR

1. NR = not reported
2. Reported only as fish oil, but manufacturer information indicates the test article was tuna oil.

4.1.2. Studies with Other Sources of DHA

Table 5 at the end of the section summarizes infant studies using other, non-fish-oil, sources of DHA.

4.1.2.1. Preterm Infants

Visual function and the fatty acid composition of red blood cell membranes were studied in preterm infants (<33 weeks gestation) fed formulas with or without DHA supplementation (Faldella et al. 1996). Infants were fed either a traditional formula for preterm infants (control; n=26) or a formula for preterm infants supplemented with LCPUFA (Preaptamil with Milupan, Milupa AG, Germany) (LCPUFA-supplemented group; n=23). A reference group of 17 infants was breastfed. Infants receiving formula received <25% of their caloric intake from breast milk. The feeding regime continued until the infants were 52 weeks postconceptional age by which time 58 infants were still in the study (25 controls, 21 supplemented, and 12 breastfed). Growth and food tolerance were recorded throughout the study. During week 52, blood samples were taken for analysis of fatty acid composition of red blood cell membranes. VEP, ERG, and brainstem acoustic evoked potentials (BAEP) were tested at 52 weeks postconceptional age.

Growth, ERG, and BAEP did not differ between groups. VEP was similar between breastfed infants and those receiving the supplemented formula; however, control infants showed longer wave latencies indicating slower maturation of visual acuity. Breastfed infants and infants receiving supplemented formula had significantly increased levels of erythrocyte LCPUFA (particularly DHA) compared to controls. The authors concluded that "a balanced LCPUFA enriched milk formula represents important progress in the early nutrition of preterm infants when mother's milk is not available."

Foreman-Van Drongelen et al. (1996) studied the effect of LCPUFA supplementation of formula for preterm infants (<1800 g) on fatty acid compositions of plasma and erythrocyte phospholipids in a prospective, randomized, double-blind trial. Infants received either a commercially available preterm formula containing no LCPUFA (control; n=16) or the same formula supplemented with DHA and AA from single-cell oils at a 1:2 ratio with levels similar to those found in breast milk (AA+DHA-supplemented group; n=15) during hospitalization and after discharge. When the infants reached a weight of 2000 g, the base formula was changed from preterm to regular formula. Feeding of formulas began when infants were able to tolerate enteral feedings (average=12th day of life) and continued up to 3 months after the initially expected date of delivery. A reference group of infants was breastfed (n=12). Blood samples were taken at birth (cord blood) and at various intervals throughout the study for fatty acid analysis.

In the supplemented group, DHA and AA levels in plasma and erythrocyte phospholipids were significantly higher than those of controls, but were similar to those of breastfed infants for the first 35 days. By 3 months of corrected age, the differences were further increased. The authors concluded that "adding these two major LCPUFA to formulas in balanced ratios, and in amounts comparable with those found in preterm human milk,

successfully raises both the 22:6n-3 and 20:4n-6 status of preterm-formula-fed infants to values found in plasma and RBC PL of preterm infants fed on human milk."

Dietary supplementation with different levels of DHA (0-0.76% of total fatty acids) and AA (0-1.1% of total fatty acids) in infant formula was studied to determine effects on fatty acid composition of erythrocyte membrane lipids (Clandinin et al. 1997). Preterm infants (<2300 g) were divided into groups fed breast milk or formula. The base formula was commercially available (Preemie SMA®, Wyeth Nutritionals International) and served as the control (n=18). Test formulas were supplemented with 0.32% AA and 0.24% DHA (low supplemented group; n=18), 0.49% AA and 0.35% DHA (medium supplemented group; n=18), or 1.1% AA and 0.76% DHA (high supplemented group; n=12). AA and DHA were obtained from single-cell oils (Martek Biosciences Corporation). The breast-milk-fed infants served as a reference control (n=25). The infants were observed up to 6 weeks of age. Blood samples were taken at 2 and 6 weeks of age and analyzed for fatty acid composition of erythrocyte membrane phospholipids, lymphocyte membrane phospholipids, and plasma lipoprotein. Length and head circumference was measured weekly and weight was measured daily.

At 2 weeks, growth was similar in all groups; however, by 6 weeks, formula-fed infants showed greater growth (weight and length) than breastfed infants regardless of supplementation; the growth rates did not differ among the four formula groups. Clinical blood values did not differ between groups and were within the normal range. Infants fed the control formula had reduced levels of AA in erythrocyte phosphatidylcholine and of DHA in phosphatidylethanolamine compared to those of breastfed infants and infants fed the supplemented formulas. A clear dose-response was observed with increasing levels of AA and DHA supplementation and the levels of AA and DHA identified in erythrocyte membrane phospholipids. The authors suggested that approximately 0.6% AA and 0.4% DHA provide sufficient (and perhaps optimum) levels of these fatty acids. They also concluded that "the range of supplementation for AA and DHA used does not result in any adverse effects on growth or clinical parameters normally monitored.

In a continuation of the work conducted by Clandinin et al. (1997), Clandinin et al. (1999), using the same cohort, determined the distribution of essential fatty acids in lipoprotein lipids. Of the AA and DHA identified in lipoprotein fractions, most were found in the high density lipoproteins (HDL) and low density lipoproteins (LDL) phospholipid and cholesterol ester fractions. AA levels in the phospholipid fraction of all lipoproteins and in the HDL and LDL cholesterol ester fraction were reduced in infants fed control formula. DHA levels of control infants also were lower (mostly in the lipoprotein phospholipid fraction) than breastfed infants or supplemented infants. Increasing AA levels in the HDL and LDL phospholipid fraction were directly related to increasing levels of supplementation with AA and DHA. The authors determined that supplementation of infant formula with 0.49% AA and 0.35% DHA of total fatty acids provided a similar fatty acid profile to that of breastfed infants. There was no further discussion of growth or safety.

A prospective, randomized, double-blind clinical trial was conducted to assess potential effects of feeding preterm infants ≤32 weeks gestation and weighing 725-1375 g formulas supplemented with DHA and AA at levels similar to breast milk (Carlson et al. 1998). Infants were divided into 3 groups: infants receiving commercially available preterm formula in the hospital and after discharge (control; n=85); infants receiving commercially available preterm formula in the hospital until discharge and then formula supplemented with 0.13% DHA and 0.41% AA from egg phospholipids; and infants receiving formula supplemented with 0.13% DHA and 0.41% AA from egg phospholipids both in the hospital and after discharge. Growth and energy intake were recorded. Clinical events were reported and infants with feeding intolerance, gastrointestinal bleeding, dysmotility, abnormal bowel sounds, abdominal distention or tenderness, or bilious emesis were evaluated for necrotizing enterocolitis. Plasma phospholipid concentration and fatty acid composition also were determined.

The 2 groups receiving the AA+DHA-supplemented formula were combined in the discussion of results (n=34). No differences in total energy intake and growth were noted between the groups. The incidence of necrotizing enterocolitis was significantly lower in infants receiving the AA+DHA-supplemented formula than controls (2.9% versus 17.6%). The hospital nursery historic incidence of necrotizing enterocolitis in very low birth weight infants was 22.4% (i.e., higher than that of controls in this study). Plasma phosphatidylcholine AA and DHA levels in the supplemented group showed little change in the first 2 weeks of the study; whereas these levels were reduced by about 40% in controls. Conversely, plasma phosphatidylethanolamine AA levels increased by 98% in supplemented infants but remained unchanged in controls. Plasma phosphatidylethanolamine DHA levels were reduced in both supplemented and control groups from the time of enrollment to 2 weeks after commencement of test formula feeding. During the same time frame, total plasma phosphatidylcholine and phosphatidylethanolamine levels increased by 27.7% and 40%, respectively, in supplemented infants but remained unchanged in controls. The authors suggested that one or more of the components of egg phospholipids may have benefitted the immature intestinal functions in order to reduce the incidence of necrotizing enterocolitis. There was no specific discussion of adverse events, but none was reported; the authors noted the significant reduction in NEC and observed that there was no difference in the incidence or severity of other common diseases of hospitalized preterm infants.

Growth, tolerance, and plasma fatty acid concentrations were evaluated in groups of preterm infants weighing 750-2000 g fed formulas with or without LCPUFA supplementation for up to 48 weeks postconceptional age (PCA) (Vanderhoof et al. 1999). Infants were randomly allocated into 2 groups: standard preterm formula (Wyeth Preemie SMA) (control, n=78); or preterm formula supplemented with 0.35% DHA and 0.5% AA from triglycerides derived from microbial fermentation (Martek Biosciences Corporation) (AA+DHA-supplemented group; n=77). A reference group of infants (n=133) was fed breast milk; when indicated, milk was fortified with Enfamil Human Milk Fortifier, Mead-Johnson. Phase I of the study started at enrollment and continued to 40 weeks PCA and Phase II of

the study went from 40 weeks PCA until 48 weeks PCA. Formulas fed during Phase I and II were 24 and 20 kcal/oz, respectively. Infants weaned from breast milk at 40 weeks PCA received the control formula in Phase II. Growth parameters were measured weekly during Phase I and at 40 and 48 weeks. Blood samples were taken at enrollment, first day of full feedings, and at 40 and 48 weeks PCA.

At 40 weeks PCA, 66 control, 60 supplemented, and 66 breastfed infants remained in the study and at 48 weeks PCA, 50 control, 48 supplemented, and 53 breastfed infants remained. Growth parameters showed some statistically significant changes: supplemented infants were longer than breastfed infants at 40 weeks PCA and supplemented infants were heavier and had a larger midarm circumference than breastfed infants at full feedings and 48 weeks PCA. Growth parameters were similar between control and supplemented infants at 40 or 48 weeks PCA. Length and head circumference did not differ between groups at full feedings and 48 weeks PCA. Hematology and urinalysis were similar between groups at 40 and 48 weeks PCA. Except for triacylglycerol and cholesterol, there were no differences in serum chemistry values between control and supplemented infants. Triacylglycerol was significantly different between groups (105±42 mg/dl, 98±38 mg/dl, and 132±57 mg/dl for controls, supplemented, and breastfed infants, respectively) at 40 weeks PCA. Total cholesterol concentrations were significantly higher in supplemented infants than controls or breastfed infants at 40 and 48 weeks PCA.

There were no significant differences in any study events between the groups (Vanderhoof et al. 1999). One infant in the supplemented group died of sudden infant death syndrome after 51 days in the study but this was determined by the attending physician to be unrelated to the study formula. Another infant from the breastfed group died from necrotizing enterocolitis at 19 days of age. At 40 and 48 weeks PCA, plasma AA concentrations were significantly higher in supplemented infants than in controls, but were similar to breastfed infants. In controls, plasma DHA levels decreased over time, but in the other 2 groups, these levels increased over time. Compared to breastfed infants, supplemented infants had slightly, but significantly, higher plasma DHA levels at 40 and 48 weeks PCA. In plasma phosphatidylethanolamine profiles, breastfed infants had significantly greater AA and DHA levels than controls or supplemented infants. The authors concluded that "The results of this study demonstrate the safety and efficacy of a preterm formula supplemented with long-chain polyunsaturated fatty acids from single-cell oils."

Vanderhoof et al. (2000) reported some additional results from the cohort described by Vanderhoof et al. (1999). LCPUFA supplementation ceased at 48 weeks PCA and control formula was fed to the infants from the supplemented group and infants weaned from breast milk from 48 to 92 weeks PCA. At 92 weeks, growth parameters did not differ between groups except for midarm circumference which was significantly smaller in breastfed infants than in controls or supplemented infants. DHA levels in erythrocyte phosphatidylcholine, erythrocyte phosphatidylethanolamine, total phospholipid, and plasma phosphatidylcholine were significantly greater than those of controls, but were similar to breastfed infants. The conclusion of the study authors was: "The incidences and

types of adverse events were similar among the feeding groups, indicating the safety of the experimental formula. The results of this study demonstrate the efficacy and long-term safety of preterm formula supplemented with AA and DHA."

Henriksen et al. (2008) examined the effect of supplementation of human milk with DHA and AA in preterm infants during the early neonatal period. Very low birth weight infants (<1500 g; gestational age of ~27-31 weeks) were randomly allocated to receive either AA+DHA-supplemented human milk (n=68) or non-supplemented human milk (n=73). The infants began to receive human milk on the first or second day after birth and as enteral feeding increased, the milk was fortified with proteins, minerals, vitamins, iron, and folic acid. The infants also received 0.5 ml study oil/100 ml human milk/day. For the AA+DHA-supplemented group, the study oil consisted of 6.9% AA and 6.9% DHA by weight as triacylglycerol (Martek Biosciences) dispersed in a mixture of soy oil and medium-chain triglyceride oil. The non-supplemented (control) group received the mixture of soy oil and medium-chain triglyceride oil only. The AA and DHA content was 31 and 32 mg/0.5 ml study oil/100 ml human milk, respectively, which was more than double the normal content of AA and DHA in unfortified human milk. The study oils were sonicated into human milk and fed to the infants by gavage. Feeding of the study oils began when the infants were ingesting more than 100 ml milk/kg bw/day and stopped at the end of hospitalization. During the last week of hospitalization, the infants were given a fixed dose of 1 ml study oil twice daily. Growth data, nutrient intake, and adverse events were obtained from medical charts. Breast milk samples were taken 4 weeks after birth and analyzed by gas-liquid chromatography with flame ionization detection for fatty acid patterns. At the start and end of the study, blood samples were taken from the infants and plasma was analyzed for fatty acid patterns. Cognitive development was assessed at the corrected age of 6 months. Parents were given an Ages and Stages Questionnaire to complete that is designed to assess mental and motor development (i.e., communication, gross motor, fine motor, problem-solving, and personal-social skills). Electrophysiological recordings related to recognition memory also were performed by a single investigator at approximately the same time. For event-related potentials, standard and novel images were shown to the infant for approximately 10 minutes and the EEG traces were recorded using 6 active electrodes.

Twelve infants did not complete the study (7 possible adverse events, 2 prolonged parenteral feeding, 2 deaths, 1 congenital abnormality, and 1 parents declined) leaving 62 infants in the supplemented group and 67 infants in the control group. Adverse events, energy and nutrient intakes (except for DHA and AA), and growth did not differ between the 2 groups. Mean daily intakes of DHA and AA were 59 and 47 mg/kg bw/day, respectively, for supplemented infants and 32 and 22 mg/kg bw/day, respectively, for control infants. Plasma fatty acid patterns showed that plasma DHA increased in the supplemented group by 12% and decreased in the control group by 9% and that plasma AA decreased in the supplemented group by 6% and decreased in the control group by 24%. AA and DHA-supplemented infants had a significantly higher score than controls on the problem-solving subtest of the Ages and Stages Questionnaire but other subtests of the questionnaire showed no differences between groups. EEG data from 81 infants showed that

supplemented infants had significantly lower (more negative) amplitudes (calculated using mean amplitude in the interval 400-650 milliseconds after presentation of standard or novel images) than controls after presentation of the standard image. There was no difference between groups after presentation of novel images. The results of this study indicated that DHA and AA supplementation of milk during the early neonatal period benefitted infants at 6 months with improved problem-solving skills and better discrimination between familiar and unfamiliar objects. The authors noted that "There was no significant difference in registered adverse events between the 2 groups," and "The study oil was well tolerated and absorbed," and they concluded that "we confirmed earlier studies by not detecting any negative effect of supplementation on weight gain or growth."

4.1.2.2. Term Infants

Healthy term infants of 38.5-41.5 weeks gestation, weighing 2800-4000 g, were fed either conventional formula (Pre-Aptamil, Milupa AG, Germany) (control) or formula supplemented with AA and DHA from egg lipid fractions at levels similar to those found in breast milk (supplemented group) for up to 3 months (Kohn et al. 1994). A reference group of infants was breastfed. Blood samples were taken at 0, 7, 30, and 90 days for analysis of fatty acid composition in plasma and erythrocyte membranes.

Growth parameters did not differ between groups and the formulas were well tolerated. At day 30 and 60, DHA and AA levels in plasma phospholipids of supplemented infants were significantly higher than those of controls but similar to those of breastfed infants. In controls, these levels decreased over time. A similar pattern was seen with AA and DHA levels in erythrocyte phosphatidylcholine. No notable differences were reported in AA and DHA levels in erythrocyte phosphatidylethanolamine. The authors concluded that supplementing infant formula with AA and DHA results in a blood lipid pattern similar to that seen in breastfed infants. The authors reported that "the three groups of formula- or breastfed infants did not differ significantly ... for increase of weight and length during the study period of 3 months. All infants tolerated the feeds well and clinically relevant side effects of the LCPUFA-containing formula were not observed."

Term infants were fed supplemented formulas in a prospective, randomized, double-blind study to evaluate the potential effect of DHA and AA on visual acuity and blood phospholipid fatty acid composition (Carlson et al. 1996). The infants were randomly allocated to one of 2 groups: standard formula (control; n=20) or standard formula with added 0.1% DHA and 0.43% AA from egg yolk lecithin (AA+DHA-supplemented group; n=19). A reference group consisted of infants (n=19) breastfed for ≥3 months. Formulas were fed for up to 4 months. Blood samples were taken at birth (cord blood) and at 2, 4, 6, and 12 months of age for fatty acid analysis. Visual function was assessed using Teller Acuity Cards at approximately 2, 4, 6, and 12 months of age.

For the first 6 months, AA+DHA-supplemented infants had significantly higher AA and DHA levels than controls; however, by 12 months, there was no difference between the groups. At 2 months, AA and DHA levels of the supplemented group were similar to

breastfed infants. Erythrocyte phosphatidylethanolamine DHA was significantly higher in supplemented infants compared to controls during the first 6 months but was similar to breastfed infants. Visual acuity was improved at 2 months in infants that were breastfed or received AA+DHA supplemented formula but the effect was transient and no differences were noted between groups at 4-12 months. There was no discussion of growth or mention of any adverse events.

Infant attention and cognitive behavior were studied in term infants of 37-42 weeks gestation weighing 2500-4000 g fed formulas with or without LCPUFA supplementation for 4 months (Willatts et al. 1996). Infants received either standard formula without supplementation (control; n=24) or standard formula supplemented with LCPUFA consisting of 0.57% n-6 and 0.25% n-3 fatty acids (supplemented group; n=24). A reference group of 27 infants was breastfed. The LCPUFA content of the control formula was 0.04% n-6 and <0.01% n-3. Anthropometric measurements (weight, length, head circumference, etc.) were taken at birth and at 3 months. Infant habituation was assessed using the Infant Control Procedure (Horowitz et al., 1972).

Controls and supplemented infants had similar anthropometric measurements at 3 months. Breastfed infants had a significantly larger head circumference than controls and were significantly longer than either controls or supplemented infants at 3 months. Formula intake was similar between controls and supplemented infants. Even though there were no statistically significant differences between the groups in the habituation test (possibly due to sample size), the data suggested that breastfed infants were more efficient at processing information and those fed control formula were least efficient.

Willatts et al. (1998a) assessed the influence of LCPUFA supplementation of infant formula on cognitive function as a continuation of the study described by Willatts et al. (1996). The formula compositions were described in more detail in this paper. The control group (n=20 at 9 months) received commercially available Aptamil (Milupa Ltd, UK) and the supplemented group (n=20 at 9 months) received Aptamil supplemented with Milupan (Milupa Ltd, UK), a fat blend derived from milk fat, vegetable oils, and egg lipids providing 0.30-0.40% AA and 0.15-0.25% DHA (of total fatty acids). Both groups received the formula for 4 months. Problem solving was assessed and scored at 9 months.

Supplemented infants tended to have non-significantly higher problem solving scores than controls. When problem solving scores were related to early and late peak fixation on the 3-month habituation assessment, there was a significant diet × peak fixation interaction, covaried with gestation and birth weight. The number of intentional solutions was significantly reduced in the late peak-fixation infants from the control group. The late peak-fixation infants receiving supplemented formula had scores similar to early peak-fixation infants fed formula with or without supplementation. These results suggested that LCPUFA supplementation may be beneficial to term infants who have reduced growth parameters at birth and reduced cognitive function. The authors did not discuss any findings regarding formula tolerance or safety.

In a further continuation of the Willatts et al. (1996; 1998a) trial, infants were given a problem solving assessment at 10 months of age. Twenty-three controls and 21 supplemented infants completed the assessment (Willatts et al. 1998b). The number of intentional solutions was significantly increased in supplemented infants compared to those of controls, indicating that LCPUFA supplementation may be beneficial in the development of childhood intelligence. The authors again noted that there were no differences between the formula groups in intake or growth.

Lucas et al. (1999) evaluated the safety and efficacy of supplementing infant formula with LCPUFA for 6 months in a prospective, randomized, double-blind, placebo-controlled trial. Term infants were fed either a non-supplemented formula (Nestec Ltd., Switzerland) (control; n=155) or the same formula supplemented with 0.32% DHA and 0.30% AA of total fatty acids (from purified egg phospholipid and triglyceride fractions; Lipid Teknic, Norway) (supplemented group; n=154). A reference group of 138 infants was breastfed. Participants were followed to 18 months of age, at which time the Bayley Scales of Infant Development II were used to derive MDI and PDI scores. Growth and formula intake were monitored throughout the study.

By 6 months, there were 131 and 117 infants remaining in the supplemented group and controls, respectively, and at 18 months, there were 127 and 115 infants, respectively. The reasons for withdrawal from the study did not differ significantly between the groups. There were no significant differences between groups in any of the parameters tested. The authors noted that the data show that 1) "LCPUFA supplementation can be achieved without growth suppression," 2) "the two formulas in this study seemed to be equally well tolerated," 3) "we did not show a significant disadvantage for the LCPUFA group in terms of infection-related events," and 4) "the present much larger randomized trial has not shown an effect of LCPUFA supplementation on atopy."

Birch et al. (1998) conducted a prospective, randomized, double-blind clinical trial in healthy term infants to study the effects of supplementing infant formula with DHA or AA+DHA until 17 weeks of age. Seventy-nine infants were randomly allocated to one of 3 formulas: Enfamil® (Mead Johnson Nutritional Research) with iron (controls; n=26), Enfamil® with iron supplemented with DHA at 0.35% of total fatty acids (DHA-supplemented group; n=26), or Enfamil® with iron supplemented with DHA at 0.36% and AA at 0.72% of total fatty acids (AA+DHA-supplemented group; n=27). DHA and AA were obtained from single-cell oils (DHASCO® and ARASCO®, Martek Biosciences). The formulas were provided in 32-oz cans and contained 2.2 g protein, 5.6 g of fat, and 10.3 g of carbohydrate per 100 kcal and fed exclusively from birth to 17 weeks of age. A reference group of 29 term infants was exclusively breastfed from birth to 17 weeks of age. Growth, sweep VEP acuity, and forced choice preferential looking acuity were assessed 4 times within a year after birth. Blood samples were taken at 17 and 52 weeks to determine blood lipids.

Sixty-eight infants (23 controls, 22 DHA-supplemented, and 23 AA+DHA supplemented) completed the 17-week treatment. Withdrawals due to intolerance for the

formula did not differ among the three formula groups. Growth parameters were similar between the groups. Visual acuity was significantly better in infants supplemented with DHA or AA+DHA except at 26 weeks when visual acuity was similar between all the groups. Infants supplemented with DHA or AA+DHA showed similar visual acuity maturation as that of breastfed infants. By 17 weeks of age, controls had significantly lower red blood cell DHA levels than those supplemented with DHA or AA+DHA and the DHA-supplemented group had significantly lower AA levels than the group supplemented with AA+DHA or the reference group. At 52 weeks, the reduced DHA levels in controls persisted. Linear regression was used to demonstrate that better visual acuity is associated with higher red blood cell DHA levels. The results indicated that early dietary supplementation with DHA improved development of visual acuity in non-breastfed infants. The authors stated that "With the caveats that the current study was not designed to fully assess safety issues (rare events could not be detected with these sample sizes) and had sufficient power to assess a 0.9 SD difference in growth (approximately 9% weight, 3% length, and 2.5% head circumference), infants in all diet groups had similar rates of growth and tolerated all diets well."

Hoffman et al. (2000) reported a second arm of the Birch et al. (1998) study involving a cohort of 33 infants used for ERG testing and determination of blood lipid profiles (in combination with the VEP cohort described by Birch et al. [1998]). These infants were also divided into the 3 formula groups described by Birch et al. (1998). Nine infants were withdrawn from the ERG cohort due to lactose intolerance within the first 6 weeks. Infants underwent ERG testing at 6 and 17 weeks of age. Blood samples were taken upon enrollment (placental cord blood) and at 6 and 17 weeks of age.

At 6 weeks, the AA+DHA-supplemented group had a significantly more mature ERG response compared to the other groups. By 17 weeks, there was no difference between the groups. While at birth (cord blood), the lipid profiles were similar between groups, by 6 weeks AA and DHA levels in red blood cells were significantly higher in DHA- and AA+DHA-supplemented groups than in controls. Differences at 17 weeks were described previously (Birch et al. 1998). The authors did not discuss tolerance or safety endpoints other than in noting that "current results reinforce the importance of an optimized biochemical fatty acid profile and its association with functional performance in term infants."

In another follow-up to Birch et al. (1998), 56 of the original 79 infants (20 controls, 17 DHA-supplemented, and 19 AA+DHA supplemented) were tested at 18 months of age using the Bayley Scales of Development, 2nd edition (Bayley 1993) to derive MDI and PDI scores (Birch et al. 2000). Cognitive, language, and motor subscales and a behavioral rating scale (BRS) also were used. PDI and BRS scores did not differ between groups. Infants supplemented with AA+DHA had significantly higher MDI scores than those of controls. DHA-supplemented infants had MDI scores similar to controls. Scores for cognitive and motor development were significantly higher in DHA- and AA+DHA-supplemented infants compared to those of controls. Language scores were significantly higher in only the AA+DHA-supplemented group compared to controls. The authors suggested that early

supplementation with DHA or AA+DHA provided a significant developmental age advantage, but there was no additional discussion of safety-related endpoints or any mention of adverse events.

In still another follow-up to Birch et al. (1998), 52 out of the 79 healthy term infants were available for testing of visual acuity and IQ at 4 years of age (Birch et al. 2007). Four groups were tested: control formula group (n=19), DHA-supplemented group (n=16), AA+DHA supplemented group (n=17), and breastfed group (n=32). DHA and AA had been obtained from single-cell oils (DHASCO® and ARASCO®, Martek Biosciences). Visual acuity was assessed by HOTV testing using the Amblyopia Treatment Study protocol (Holmes et al. 2001) and Electronic Visual Acuity system (Moke et al. 2001). IQ was tested using the Wechsler Preschool and Primary Scale of Intelligence.

Children from the DHA-supplemented group had significantly better HOTV acuity in the right eye than controls, but HOTV acuity in the left eye was similar among groups. Performance IQ was higher, but not significantly, in supplemented and breastfed groups compared to controls. Verbal IQ was significantly poorer in controls and DHA-supplemented groups compared to the breastfed group. Full Scale IQ also was higher, but without a significant trend, in supplemented groups compared to controls. The authors concluded that AA and DHA supplementation of infant formula supports visual acuity and IQ maturation similar to that of breastfed infants.

In a randomized controlled clinical trial, 65 healthy term infants were weaned from breastfeeding at 6 weeks of age and placed on either commercial formula (Enfamil with iron, Mead Johnson Nutritional Group) (control; n=33) or commercial formula supplemented with DHA at 0.36% and AA at 0.72% of total fatty acids (supplemented group; n=32) until 52 weeks of age (Birch et al. 2002). DHA and AA were obtained from single-cell oils (DHASCO® and ARASCO®, Martek Biosciences). Both formulas were prepared in 946-ml ready-to-use cans and provided 14.7 g protein, 37.5 g fat, 69.0 g carbohydrate, and 2805 kJ/L. VEP acuity (at 6, 17, 26, and 52 weeks of age), growth (at 6, 17, 26, and 52 weeks of age), stereoacuity (at 17, 26, 39, and 52 weeks of age), and blood lipids (at 17 and 52 weeks of age) were determined.

Fifty-eight infants completed the study (30 controls and 28 supplemented) but stereoacuity testing was completed for only 28 controls and 25 supplemented infants due to problems with a few children wearing the polarized glasses. VEP acuity, as assessed according to the sweep parameter protocol, was significantly better in supplemented infants than control infants at 17, 26, and 52 weeks of age. Both formulas were generally well tolerated by the infants. Growth parameters (weight, length, head circumference, weight-for-length, subscapular fat, and triceps fat deposition) did not differ between the groups. Stereoacuity was significantly better in supplemented infants at 17 weeks of age, but not at 39 or 52 weeks of age. Plasma and red blood cell concentrations of DHA were significantly higher in supplemented infants than in controls at 17 and 52 weeks of age. In supplemented infants at 52 weeks of age, the difference from controls of DHA red blood cell concentration was greater than at 17 weeks of age. Plasma and red blood cell lipid

concentrations of ALA were similar between groups. Concentration of EPA in plasma was similar between groups but EPA concentration in red blood cell lipids was lower in supplemented infants than in controls at 17 and 52 weeks of age. Plasma AA concentration in supplemented infants was significantly higher than that of controls at 17 and 52 weeks of age whereas red blood cell AA concentration in supplemented infants was significantly higher than that of controls at 17, but not 52, weeks of age. Plasma and red blood cell concentrations of LA were significantly higher than those of controls in supplemented infants at 17 but not 52, weeks of age.

Linear regression analysis was used to examine the relationship between sweep VEP acuity (at 17 and 52 weeks of age) or stereoacuity (at 17 weeks of age) and the LCPUFA composition of plasma and red blood cells. Higher concentrations of DHA and AA in both plasma and red blood cells were associated with better sweep VEP acuity at 17 and 52 weeks of age. Higher plasma, but not red blood cell, DHA concentrations were associated with better stereoacuity. Higher red blood cell, but not plasma, LA concentrations were associated with poorer stereoacuity. The results indicated that feeding supplemented formula after weaning from breastfeeding at 6 weeks of age improved functioning of the visual cortex.

Healthy term infants with mean age = 6.5±0.9 weeks were randomly assigned to receive either commercial infant formula (Enfamil with iron, Mead Johnson Nutritionals) or the same formula supplemented with DHA at 0.36% and AA at 0.72% of total fatty acids to assess visual acuity (Hoffman et al. 2003). DHA and AA were obtained from single-cell oils (DHASCO® and ARASCO®, Martek Biosciences). Both formulas were prepared in 946-ml ready-to-use cans and provided 14.7 g protein, 37.5 g fat, 69.0 g carbohydrate, and 2805 kJ/L. Formulas were fed after weaning from breast feeding at 4-6 months until 12 months of age. Growth (at 4, 6, 9, and 12 months of age), VEP acuity (at 4, 6, and 12 months), and stereoacuity (at 4, 6, 9, and 12 months) were determined. Blood samples preweaning (4-6 months) and at 12 months of age were taken to determine blood lipid fatty acid profiles by gas chromatography.

Sixty-one infants completed the trial (31 controls and 30 supplemented). Growth parameters (weight, length and head circumference) did not differ between the groups. The distribution of fatty acids in red blood cells was similar between the groups prior to weaning, but at the end of the study DHA in red blood cells was significantly higher (2.5-fold) in supplemented infants compared to controls. In supplemented infants, AA and DHA levels remained similar to preweaning levels at 12 months whereas control infants had a 50% decrease in DHA and a small but significant increase in LA levels. VEP acuity, as assessed according to the sweep parameter protocol, was significantly better in supplemented infants than control infants at 12 months. Prior to weaning, VEP acuity was similar between groups. Stereoacuity did not differ between groups at any time during the study. Linear regression analysis was used to examine the relationship between sweep VEP acuity and the relative weight percent of DHA in red blood cells at 12 months. Infants with more mature visual cortical function had a higher level of DHA in red blood cells. It was also

reported that infants with high red blood cell levels of LA and oleic acid had poorer VEP acuity (P=0.002). It was concluded that dietary DHA and AA fed to term infants during their first year contributed to improved visual development.

The effect of dietary DHA supplementation on maturation of visual acuity in term infants >37 weeks gestation and weighing >2800 g receiving human milk was evaluated (Hoffman et al. 2004). Fifty-five infants were randomly allocated to 2 groups and fed one 113-g jar of baby food/day either without supplemented DHA (controls) or supplemented with egg yolk providing 115 mg DHA/100 g food (0.115%). Although many of the infants continued to breast feed until a mean age of 9 months, the baby food was started at 6 months of age and was discontinued at 12 months. The daily intake of supplemental DHA was determined by gravimetric measures to be 0 mg for controls and 83 mg for supplemented infants. DHA intake from breast feeding was estimated to be 37 mg/day in controls and 28 mg/day in supplemented infants. Growth, VEP acuity, and stereoacuity were assessed at 6, 9, and 12 months of age and blood samples were taken at 6 and 12 months for determination of hematology, clinical chemistry, blood lipid profiles, and total antioxidant capacity of plasma.

Fifty-one infants (26 controls and 25 supplemented) completed the study. The diet was well tolerated. There were no differences in weight, length, head circumference, or skin-fold thickness or in hematological and clinical chemistry parameters between the groups during the study. Prior to supplementation with baby food diets, the lipid profiles were similar between the groups. Afterwards, the red blood cell DHA content in controls decreased from 3.8% at 6 months to 3.0% at 12 months but increased in supplemented infants from 4.1% at 6 months to 5.5% at 12 months. VEP acuity was significantly improved (equivalent to ~1.5 lines in an eye chart) in supplemented infants (P<0.002) compared to controls at 9 and 12 months. Stereoacuity did not differ between the groups. At 12 months, infants with high red blood cell DHA had better visual acuity. Dietary DHA supplementation within the first year of an infant's life improved visual acuity without any adverse effects.

In a study to assess the efficacy and safety of AA+DHA-supplemented formulas, 245 term infants were randomized into 3 groups within a week of birth: infants fed with Frisolac Advanced formula (n=69), infants fed with Frisolac Advanced formula and breast milk (n=124), and infants fed Frisolac H formula (n=52) (Ben et al. 2004). A reference group (n=26) was breastfed without formula supplementation. Any infants with known congenital abnormalities affecting development were excluded from the study. The formulas were fed for up to 6 months. The Frisolac Advanced formula was supplemented with linoleic acid (LA; 435 mg/L), α-linolenic acid (ALA; 62 mg/L), AA (6.9 mg/L), and DHA (6.9 mg/L), whereas the Frisolac H formula was supplemented only with LA (440 mg/L) and ALA (44 mg/L). The formulas were identical except for the supplementation. After 3 and 6 months, the infants were assessed using the Revised Bayley Scales of Infant Development and MDI and PDI scores were derived. At the same time, growth parameters plus any medical events (e.g., frequency of upper respiratory tract infections, gastroenteritis) were recorded and blood samples were taken from a subgroup of infants and analyzed for fatty acid content.

Body weight, length, and head circumference did not differ among groups at any time throughout the study. The best growth was reported in infants fed with Frisolac Advanced formula and breast milk (statistical significance not stated). Statistically, the groups did not differ in MDI and PDI scores; however, infants receiving AA+DHA-supplemented formula had slightly higher scores at 3 months. At 3 and 6 months, infants fed Frisolac Advanced formula had higher AA and DHA plasma levels (only statistically significant for AA at 6 months) than infants fed Frisolac H formula and the levels were similar to breastfed infants at 3 months but higher at 6 months (not statistically significant). Medical events were similar among all the groups. Hemoglobin concentration was significantly reduced at 6 months in the reference breastfed infant group (133.6±7.4 g/dm^3) compared to formula-fed infants (142.9±9.0 g/dm^3 and 140.7±4.1 g/dm^3 for Frisolac Advanced formula and Frisolac H formula, respectively). The results of this study showed no safety concerns regarding the consumption of AA+DHA-supplemented formula for a period of up to 6 months. The supplementation had no effect on growth parameters. The authors concluded, "The principal targeted safety outcome was evidence of infection determined by history at each follow-up. ... we did not see a significant disadvantage for the LCPUFA group in terms of infection-related events (upper or lower respiratory infection, gastroenteritis, visit to medical practitioner, or antibiotic use). ... With regard to safety, the second targeted outcome was growth decrease ... we did not find any growth decrease in AA + DHA groups supplemented with a balanced AA/DHA."

Visual evoked potential (VEP) was the primary outcome assessed in a prospective, randomized, double-blind, placebo-controlled clinical trial in which term infants were fed formulas containing AA and DHA for up to 52 weeks (Birch et al. 2005). Within 5 days of birth, 103 infants were randomly allocated to 2 feeding groups: formula containing no DHA or AA (n=52, control) or formula supplemented with DHA at 0.36% and AA at 0.72% of total fatty acids (n=51). The base formula was commercially available (Enfamil with iron, Mead Johnson Nutritional Group) in 946-ml ready-to-use cans and provided about 15% LA and 1.5% ALA with 14.7 g protein, 37.5 g fat, 69.0 g carbohydrate, and 2805 kJ/L. DHA and AA were obtained from single-cell oils (DHASCO® and ARASCO®, Martek Biosciences). None of the infants received solid foods before 17 weeks of age. The trial was subdivided into 2 sub-studies. One sub-study focused on VEP (n=71) and the other on electroretinogram (ERG; n=32), although in the ERG sub-study VEP was assessed at all but the first time point (6 weeks). The results of the VEP testing were discussed in this paper. In addition to VEP, growth, tolerance, random dot stereoacuity, and blood lipid profiles were evaluated. Infants were evaluated at 6, 17, 39, and 52 weeks of age and blood samples were taken 17 and 39 weeks of age.

Within the first 6 weeks of the study, 7 infants were removed (3 controls and 4 supplemented) because of recommendations by the pediatrician to switch to a soy-based formula due to lactose or cow milk protein intolerance. One control group infant also was dropped from the study due to inability to contact the parents. Forty-two and 44 infants in the supplemented and control groups, respectively, completed the study. Fatty acid profiles in total red blood cell lipids showed that the mean concentration of DHA in infants fed the

supplemented formula was significantly higher at 6, 17, and 39 weeks by 29, 142, and 215%, respectively, compared to controls. Mean AA concentrations also were significantly increased by 15-18% in infants fed the supplemented formula compared to controls at the same time points, but LA concentrations were significantly lower. There was no difference in length, weight, or head circumference between the groups. VEP acuity was significantly better in infants fed the supplemented formula than that of controls in both the VEP sub-study and the combined ERG and VEP sub-studies. Infants fed the supplemented formula also had significantly better random dot stereoacuity at age 17 weeks, but not at 39 or 52 weeks, than that of controls. The results of this study indicated that supplementation of infant formula with AA+DHA improved visual function and altered total red blood cell lipid composition. The authors concluded that "The growth of infants fed LCPUFA-supplemented and control formulas did not differ significantly, and both diets were well tolerated."

A total of 229 infants participating in 3 previously described randomized clinical trials (Birch et al. 2002, 2005; Hoffman et al. 2003) participated in a study of cognition at 9 months of age (Drover et al. 2009). The infants were assessed for problem-solving abilities by completing a 2-step task that involved successfully retrieving a rattle. Initially, the infants underwent pretesting to determine their ability to retrieve a rattle placed on a cloth out of their reach and to find a rattle covered by a cloth while they were watching. Infants who completed the pretest within 3 attempts were permitted to participate in the test trials. The test trials consisted of placing a rattle on a cloth out of reach and covering it, and the infant was numerically scored based on its ability to obtain the rattle. In addition, the infants were scored in a trial for their intention over 6 component tasks: (1) pulls cloth; (2) looks at cover; (3) grasps cover; (4) removes cover; (5) looks at toy; and (6) picks up toy. Each of the 6 tasks were scored from 0-2, with 2 indicating that the infant appeared visually focused on the task, performed no irrelevant behaviors, and accomplished the step quickly. For each trial, an infant could score from 0-12. Three trials were conducted and an average score was calculated.

Twenty-seven infants did not complete the 2-step test, so a total of 202 infants (98 LCPUFA-supplemented and 104 controls) completed the trials with follow-up. The average intention score was significantly ($P<0.05$) lower in corresponding controls compared to LCPUFA-supplemented infants from the 12-month feeding study (6.9±4.0 versus 8.6±3.7; Birch et al. 2005) and the 6-week weaning study (4.3±3.8 versus 6.8±5.2; Birch et al. 2002). Similar results were reported for the percentage successful on all 3 trials. Infants from the 12-month feeding study had a 51% success rate compared to a 29% success rate in corresponding controls. Infants from the 6-week weaning study had a 46% success rate compared to a 13% success rate in corresponding controls. In addition, the infants from the 6-week weaning study showed a significantly ($P<0.01$) different percentage who obtained a perfect intention score (35% in LCPUFA-supplemented infants versus 7% in controls). No significant differences were noted between LCPUFA-supplemented and control infants in the 4-6-month weaning study (Hoffman et al. 2003), in which the infants had been breastfed from birth to 4-6 months. Supplementation with LCPUFA for 9 and 7.5 months in the 12-month feeding and 6-week weaning studies, respectively, significantly improved

performance in the cognitive testing conducted in this study. Lack of effect in infants from the 4-6-month study was possibly due to the short duration of LCPUFA supplementation (3-5 months), the timing of the supplementation, or the extended breastfeeding period.

A prospective, randomized, double-blind clinical trial was conducted to determine the effect of different levels of DHA supplementation of infant formula on visual acuity, red blood cell fatty acid profiles, tolerance, growth, and adverse events of 12-month-old infants fed the formula (Birch et al. 2010). At 1-9 days of age, 343 term infants (37-42 weeks gestation; weighing 2490-4200 g) from 2 different locations (Dallas and Kansas City) were randomly allocated to one of the following four groups: formula without supplementation (control; n=86; Enfamil with Iron, Mead Johnson Nutrition), formula supplemented with 0.32% DHA (n=84; Enfamil LIPIL, Mead Johnson Nutrition; 17 mg/100 kcal), formula supplemented with 0.64% DHA (n=85; 34 mg/100 kcal), or formula supplemented with 0.96% DHA (n=88; 51 mg/100 kcal). DHA and AA were obtained from single-cell oils (Martek Biosciences). The formulas supplemented with DHA also contained 0.64% AA (34 mg/100 kcal). LA and ALA content was similar among the formulas. The formulas were fed solely until ~4 months of age when other food could be introduced and formula feeding continued until 12 months of age. Infants were assessed at approximately 1.5, 4, 6, 9, and 12 months of age for VEP acuity, growth, and red blood cell lipid profile (4 and 12 months of age only). Parents provided information on formula consumption, tolerance, and any adverse events.

A total of 244 infants (56 controls, 64 supplemented with 0.32% DHA, 59 supplemented with 0.64% DHA, and 65 supplemented with 0.96% DHA) completed the study. In general, formula consumption, tolerance, and incidence of adverse events were similar between the formula groups. VEP acuity, as assessed according to the sweep parameter protocol, was significantly better in supplemented infants than control infants throughout the study but did not differ between the supplemented groups. Growth parameters were not affected by DHA supplementation; however, infants from Dallas tended to weigh less and have shorter length (statistical significance reached at ages above 1.5 months) than corresponding infants from Kansas City. At 4 and 12 months of age, red blood cell DHA concentration increased significantly with increasing DHA intake; whereas red blood cell AA concentration decreased with increasing DHA intake. For all formula groups, Dallas infants had significantly higher red blood cell DHA concentration than Kansas City infants. The authors concluded that formula supplementation with 0.32-0.64% DHA "appears to be sufficient to promote VEP visual acuity maturation during infancy."

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA

STUDY DESIGN	TEST SUBSTANCE SOURCE	TREATMENT	RESULTS	CONCLUSION	REFERENCE
PRETERM INFANTS					
Randomized study: (1) unsupplemented formula (control; n=26) (2) formula supplemented with LCPUFA (n=23) Reference group=breastfed infants (n=17)	LCPUFA was Preaptamil with Milupan (Milupa AG)	Formula was fed until 52 weeks postconceptional age.	25 controls, 21 supplemented infants, and 12 breastfed infants completed the study. Growth, ERG, and BAEP did not differ between groups. VEP was similar between breastfed and supplemented infants but controls showed slower maturation of visual acuity. Breastfed and supplemented infants had significantly increased levels of erythrocyte LCPUFA (particularly DHA) compared to controls.	No safety concern; no effect on growth, improved maturation of visual acuity.	Faldella et al. (1996)
Randomized, double-blind study: (1) unsupplemented formula (control; n=16); (2) formula supplemented with DHA and AA at a 1:2 ratio (n=15)Reference group=breastfed infants (n=12)	DHA and AA from single-cell oils	Formulas fed from ~12 days of age up to 3 months after the initially expected date of delivery.	Plasma DHA and AA levels and erythrocyte phospholipids were significantly higher in supplemented infants than controls, but were similar to those of breastfed infants for the first 35 days. By 3 months of corrected age, the differences were further increased.	No safety concern; altered total red blood cell and plasma lipid composition. The authors concluded that "adding these two major LCPUFA to formulas in balanced ratios, and in amounts comparable with those found in preterm human milk, successfully raises both the 22:6n-3 and 20:4n-6 status of preterm-formula-fed infants to values found in plasma and RBC PL of preterm infants fed on human milk."	Foreman-Van Drongelen et al. (1996)
Randomized study: (1) unsupplemented formula (control; n=18); (2) formula supplemented with 0.32% AA and 0.24% DHA (n=18) (3) formula supplemented with 0.49% AA and 0.35% DHA (n=18) (4) formula supplemented with 1.1% AA and 0.76% DHA (n=12) Reference group=breastfed infants (n=25)	AA and DHA from single-cell oils	Formulas were fed up to 6 weeks of age.	At 2 weeks, growth was similar in all groups. By 6 weeks, formula-fed infants showed greater growth than breastfed infants regardless of supplementation. Clinical blood values did not differ between groups and were within the normal range. Infants fed the control formula had reduced levels of AA in erythrocyte phosphatidylcholine and of DHA in phosphatidylethanolamine compared to those of breastfed infants and infants fed the supplemented formulas.	No safety concern; clear dose-response with increasing levels of AA and DHA supplementation and the erythrocyte levels of AA and DHA. The authors concluded that "the range of supplementation for AA and DHA used does not result in any adverse effects on growth or clinical parameters normally monitored.	Clandinin et al. (1997)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Same as Clandinin et al. (1997)	Same as Clandinin et al. (1997)	Same as Clandinin et al. (1997)	DHA levels of control infants also were lower than breastfed infants or supplemented infants. Increasing AA levels in the phospholipid fraction were directly related to increasing levels of supplementation with AA and DHA. Supplementation of infant formula with 0.49% AA and 0.35% DHA of total fatty acids provides a similar fatty acid profile to that of breastfed infants.	No safety concern; altered fatty acid profile.	Clandinin et al. (1999)
Randomized, double-blind study: (1) unsupplemented formula (control; n=85); (2) formula supplemented with 0.13% DHA and 0.41% AA only after hospital discharge (3) formula supplemented with 0.13% DHA and 0.41% AA before and after hospital discharge	AA and DHA from egg phospholipids		The 2 groups receiving the AA+DHA-supplemented formula were combined in the discussion of results (n=34). Total energy intake and growth were similar between groups. The incidence of necrotizing enterocolitis was significantly lower in infants receiving the AA+DHA-supplemented formula than controls (2.9% versus 17.6%). Plasma phosphatidylcholine AA and DHA levels in the supplemented group showed little change in the first 2 weeks of the study; whereas these levels were reduced (~40%) in controls. Plasma phosphatidylethanolamine AA levels increased in supplemented infants but remained unchanged in controls. Plasma phosphatidylethanolamine DHA levels were reduced in both supplemented and control groups from the time of enrollment to 2 weeks after commencement of test formula feeding. During the same time frame, total plasma phosphatidylcholine and phosphatidylethanolamine levels increased by 27.7% and 40%, respectively, in supplemented infants but remained unchanged in controls.	No safety concern; no effect on growth; altered plasma DHA and AA levels; reduced incidence of necrotizing enterocolitis; no difference in the incidence or severity of other common diseases of hospitalized preterm infants.	Carlson et al. (1998)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized study: (1) unsupplemented formula (control; n=78) (2) formula supplemented with 0.5% AA and 0.35% DHA (n=77) Reference group=breastfed infants (n=133)	AA and DHA from triglycerides derived from microbial fermentation	Formulas were fed up to 48 weeks postconceptional age	50 control, 48 supplemented infants, and 53 breastfed infants completed the study. Growth, incidence of study events, hematology, and urinalysis were similar between control and supplemented infants. Triglycerides were significantly different between groups (105±42 mg/dl, 98±38 mg/dl, and 132±57 mg/dl for breastfed >controls> supplemented) at 40 weeks. Total cholesterol concentrations were significantly higher in supplemented infants than controls or breastfed infants at 40 and 48 weeks. Plasma AA concentrations were significantly higher in supplemented infants than in controls, but were similar to breastfed infants. In controls, plasma DHA levels decreased over time, but in the other 2 groups, these levels increased over time. Compared to breastfed infants, supplemented infants had slightly, but significantly, higher plasma DHA levels.	No safety concern; no effect on growth, increased plasma AA and DHA levels. The authors concluded that "The results of this study demonstrate the safety and efficacy of a preterm formula supplemented with long-chain polyunsaturated fatty acids from single-cell oils."	Vanderhoof et al. (1999)
Same as Vanderhoof et al. (1999)	Same as Vanderhoof et al. (1999)	Same as Vanderhoof et al. (1999)	At 92 weeks, growth parameters did not differ between groups except for midarm circumference which was significantly smaller in breastfed infants. Erythrocyte and plasma DHA levels in supplemented infants were significantly greater than controls, but were similar to breastfed infants.	No safety concern; increased erythrocyte and plasma DHA levels. Authors' conclusions: "The incidences and types of adverse events were similar among the feeding groups, indicating the safety of the experimental formula. The results of this study demonstrate the efficacy and long-term safety of preterm formula supplemented with AA and DHA."	Vanderhoof et al. (2000)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized, double-blind study: (1) AA+DHA-supplemented human milk (n=68) (2) unsupplemented human milk (control; n=73) Infants started to receive supplemented milk when ingestion was >100 ml of milk/kg bw and stopped at the end of hospitalization	6.9% AA and 6.9% DHA by weight as triacylglycerol (Martek Biosciences; assumed to be vegetarian source)	. AA and DHA content was 31 and 32 mg/100 ml milk, respectively. Mean daily intakes of DHA and AA were 59 and 47 mg/kg bw/day, respectively, for supplemented infants and 32 and 22 mg/kg bw/day, respectively, for controls.	By the end of the study there were 62 supplemented infants and 67 controls. Incidence of adverse events, energy, and growth were similar between groups. Plasma DHA increased in the supplemented group by 12% and decreased in the control group by 9% and that plasma AA decreased in the supplemented group and control group by 6 and 24%, respectively. AA and DHA-supplemented infants had a significantly higher score than controls on the problem-solving subtest and showed better discrimination between familiar and unfamiliar objects.	No safety concern; no effect on growth, benefit to neurodevelopment. The authors noted that "There was no significant difference in registered adverse events between the 2 groups," and "The study oil was well tolerated and absorbed," and they concluded that "we confirmed earlier studies by not detecting any negative effect of supplementation on weight gain or growth."	Henriksen et al. (2008)
TERM INFANTS					
Randomized study: (1) unsupplemented formula (control) (2) formula supplemented with AA and DHA Reference group=breastfed infants	AA and DHA from egg lipid fractions	Formula fed for up to 3 months. AA and DHA levels in formula similar to those found in breast milk.	Formula well tolerated. Growth was similar between groups. DHA and AA levels in plasma phospholipids of supplemented infants were significantly higher than controls but similar to those of breastfed infants. A similar pattern was seen with AA and DHA levels in erythrocyte phosphatidylcholine. No notable differences were reported in AA and DHA levels in erythrocyte phosphatidylethanolamine.	No safety concern; no effect on growth; alteration of blood lipid pattern (similar to breastfed infants). The authors reported that "the three groups of formula- or breastfed infants did not differ significantly ... for increase of weight and length during the study period of 3 months. All infants tolerated the feeds well and clinically relevant side effects of the LCPUFA-containing formula were not observed."	Kohn et al. (1994)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized, double-blind study: (1) unsupplemented formula (control; n=20); (2) formula supplemented with 0.1% DHA and 0.43% AA (n=19) Reference group=breastfed infants (n=19)	DHA and AA from egg yolk lecithin	Formulas fed for up to 4 months. Reference group was breastfed for a minimum of 3 months.	AA and DHA blood levels were significantly higher in supplemented infants than controls but were similar by 12 months. AA and DHA levels of the supplemented group were similar to breastfed infants at 2 months. Visual acuity was improved at 2 months in breastfed and supplemented infants but the effect was transient and no differences were noted between groups at 4-12 months.	No safety concern; transient improvement of visual acuity.	Carlson et al. (1996)
Randomized study: (1) unsupplemented formula (control; n=24) (2) formula supplemented with LCPUFA (n=24) Reference group=breastfed infants (n=27)	Not stated	Formula fed for 4 months.	Growth and formula intake were similar in controls and supplemented infants at 3 months whereas breastfed infants had a significantly larger head circumference than controls and were significantly longer than either controls or supplemented infants. The data indicated that breastfed infants were more efficient at processing information and controls were least efficient.	No safety concern; no effect on growth.	Willatts et al. (1996)
Same cohort as Willatts et al. (1996) but described in more detail.	LCPUFA supplied as Milupan (fat blend derived from milk fat, vegetable oils, and egg lipids providing 0.30-0.40% AA and 0.15-0.25% DHA	Same as Willatts et al. (1996)	20 controls and 20 supplemented infants assessed at 9 months. Supplemented infants tended to have higher, but not statistically significant, problem solving scores than controls. Results of habituation assessment indicate that LCPUFA supplementation may be beneficial to term infants who have reduced growth parameters at birth and reduced cognitive function.	No safety concern; possible benefit to infants with reduced growth parameters at birth and reduced cognitive function.	Willatts et al. (1998a)
Same as Willatts et al. (1996; 1998a)	Same as Willatts et al. (1996; 1998a)	Same as Willatts et al. (1996; 1998a)	23 controls and 21 supplemented infants were assessed at 10 months of age. The number of intentional solutions was significantly increased in supplemented infants compared to those of controls.	No safety concern; may be beneficial in development of childhood intelligence.	Willatts et al. (1998b)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized, double-blind study: (1) unsupplemented formula (control; n=155) (2) formula supplemented with 0.3% AA and 0.32% DHA (n=154) Reference group=breastfed infants (n=138)	DHA and AA from purified egg phospholipid and triglyceride fractions	Formulas fed for 6 months.	By 6 months, there were 131 supplemented infants and 117 controls. At 18-month follow-up, there were 127 supplemented infants and 115 controls. There were no significant differences between groups in any of the parameters tested (MDI, PDI, growth, and formula intake.	No safety concern; no effect on growth or development. The authors noted that the data show that "LCPUFA supplementation can be achieved without growth suppression, ... the two formulas in this study seemed to be equally well tolerated, ... we did not show a significant disadvantage for the LCPUFA group in terms of infection-related events, ... the present much larger randomised trial has not shown an effect of LCPUFA supplementation on atopy."	Lucas et al. (1999)
Randomized, double-blind study: (1) unsupplemented formula (control; n=26) (2) formula supplemented with 0.35% DHA (n=26) (3) formula supplemented with 0.72% AA and 0.36% DHA (n=27) Reference group=breastfed infants (n=29)	AA and DHA from single-cell oils	Formulas fed exclusively from birth to 17 weeks of age. Reference group was breastfed for 17 weeks.	Growth parameters were similar between the groups. Visual acuity was significantly better in infants supplemented with DHA or AA+DHA (similar to breastfed infants). By 17 weeks of age, controls had significantly lower erythrocyte DHA than those supplemented with DHA or AA+DHA and the DHA-supplemented group had significantly lower AA levels than the group supplemented with AA+DHA or the reference group. At 52 weeks, the reduced DHA levels in controls persisted.	No safety concern; no effect on growth but visual acuity was improved and DHA and AA levels were increased. The authors concluded that "infants in all diet groups had similar rates of growth and tolerated all diets well."	Birch et al. (1998)
Same test groups as Birch et al. (1998), but including a cohort of 33 infants.	Same as Birch et al. (1998)	Same as Birch et al. (1998)	AA+DHA-supplemented group had a significantly more mature ERG response compared to the other groups at 6 weeks but by 17 weeks no differences were noted. By 6 weeks, AA and DHA levels in red blood cells were significantly higher in DHA- and AA+DHA-supplemented groups than in controls.	No safety concern; more mature ERG response and increased DHA and AA levels.	Hoffman et al. (2000)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Same as Birch et al. (1998)	Same as Birch et al. (1998)	Same as Birch et al. (1998)	In a follow-up at 18 months of age with 20 controls, 17 DHA-supplemented infants, and 19 AA+DHA-supplemented infants, MDI was significantly higher in AA+DHA-supplemented infants but similar between DHA-supplemented and controls. Scores for cognitive and motor development were significantly higher in DHA- and AA+DHA-supplemented infants compared to those of controls. Language scores were significantly higher in only the AA+DHA-supplemented group compared to controls.	No safety concern; improved development.	Birch et al. (2000)
Same as Birch et al. (1998)	Same as Birch et al. (1998)	Same as Birch et al. (1998)	Another follow-up at 4 years of age with 19 controls, 16 DHA-supplemented infants, and 17 AA+DHA-supplemented infants, visual acuity was significantly better in the right eye (but not left eye) of DHA-supplemented infants than controls. Verbal IQ was significantly better in breastfed infants than DHA-supplemented infants or controls.	No safety concern; supports visual acuity and IQ maturation.	Birch et al. (2007)
Randomized study: (1) unsupplemented formula (control; n=33) (2) formula supplemented with 0.36% DHA and 0.72% AA (n=32)	AA and DHA from single-cell oils	Formula fed after weaning at 6 weeks of age until 52 weeks of age.	30 controls and 28 supplemented infants completed the study. Growth parameters did not differ between the groups. Stereoacuity was significantly better in supplemented infants at 17 weeks of age, but not at 39 or 52 weeks. Plasma and red blood cell DHA were significantly higher in supplemented infants at 17 and 52 weeks. Plasma AA concentration in supplemented infants was higher than that of controls at 17 and 52 weeks of age; red blood cell AA concentration in supplemented infants was significantly higher at 17 but not 52 weeks. Higher plasma concentrations of DHA and AA were associated with better sweep VEP acuity at 17 and 52 weeks of age. Higher red blood cell concentrations were associated with better sweep VEP at 17 and 52 weeks of age. Higher plasma, but not red blood cell, DHA concentrations were associated with better stereoacuity.	No safety concern; no effect on growth, improved visual acuity and altered total red blood cell lipid composition.	Birch et al. (2002)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized study: (1) unsupplemented formula (control) (2) formula supplemented with 0.36% DHA and 0.72% AA	AA and DHA from single-cell oils	Formulas fed after weaning at 4-6 months until 12 months of age.	31 controls and 30 supplemented infants completed the study. Growth did not differ between the groups. At the end of the study, DHA in red blood cells was significantly higher (2.5-fold) in supplemented infants compared to controls. VEP acuity was significantly better in supplemented infants than control infants at 12 months. Stereoacuity did not differ between groups.	No safety concern; no effect on growth, improved visual acuity and altered total red blood cell lipid composition.	Hoffman et al. (2003)
Randomized study: (1) unsupplemented baby food (control) (2) baby food supplemented with DHA	DHA from egg yolk	Baby food (113 g jar/day) was started at 6 months of age and discontinued at 12 months of age. Added daily DHA intake was 0 mg for controls and 83 mg for supplemented infants.	26 controls and 25 supplemented infants completed the study. No differences in growth, hematology, or clinical chemistry between groups. Erythrocyte DHA content in controls decreased from 3.8% at 6 months to 3.0% at 12 months but increased in supplemented infants from 4.1% at 6 months to 5.5% at 12 months. VEP acuity was significantly improved in supplemented infants compared to controls. Stereoacuity did not differ between the groups.	No safety concern; no effect on growth, improved visual acuity and altered total red blood cell lipid composition.	Hoffman et al. (2004)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA					
STUDY DESIGN	**TEST SUBSTANCE SOURCE**	**TREATMENT**	**RESULTS**	**CONCLUSION**	**REFERENCE**
Randomized trial: (1) Frisolac Advanced formula (n=69) (2) Frisolac Advanced formula + breast milk (n=124) (3) Frisolac H formula (n=52) Reference group=breastfed infants (n=26)	Not stated	Frisolac Advanced formula supplemented with LA (435 mg/dm^3), ALA (62 mg/dm^3), AA (6.9 mg/dm^3), and DHA (6.9 mg/dm^3). Frisolac H formula supplemented with LA (440 mg/dm^3) and ALA (44 mg/dm^3) Formula was fed for up to 6 months.	Body weight, length, head circumference, MDI, PDI, and incidence of medical events did not differ among groups. At 6 months, infants fed Frisolac Advanced formula had higher AA plasma levels than infants fed Frisolac H formula and the levels were similar to breastfed infants at 3 months. Hemoglobin concentration was significantly lower at 6 months in the reference breastfed infant group than in formula-fed infants.	No safety concern; no effect on growth. The authors concluded, " we did not see a significant disadvantage for the LCPUFA group in terms of infection-related events (upper or lower respiratory infection, gastroenteritis, visit to medical practitioner, or antibiotic use). ... we did not find any growth decrease in AA + DHA groups supplemented with a balanced AA/DHA."	Ben et al. (2004)
Randomized, double-blind study: (1) unsupplemented formula (control; n=52) (2) formula supplemented with 0.36% DHA and 0.72% AA (n=51)	AA and DHA from single-cell oils	Formulas fed for up to 52 weeks.	42 and 44 infants in the supplemented and control groups, respectively, completed the study. Mean concentration of erythrocyte DHA in supplemented infants was significantly higher at 6, 17, and 39 weeks by 29, 142, and 215%, respectively, compared to controls. Mean AA concentrations also were significantly increased by 15-18% in supplemented infants compared to controls, but linoleic acid concentrations were significantly lower. Growth was similar between the groups. VEP acuity was significantly better in supplemented infants than controls.	No safety concern; no effect on length, weight, or head circumference; improved visual function and altered total red blood cell lipid composition. The authors concluded that "The growth of infants fed LCPUFA-supplemented and control formulas did not differ significantly, and both diets were well tolerated."	Birch et al. (2005)

Table 5. Summary of Infant Studies Using Non-Fish Oil Sources of DHA

STUDY DESIGN	TEST SUBSTANCE SOURCE	TREATMENT	RESULTS	CONCLUSION	REFERENCE
Same as Birch et al. (2002; 2005) and Hoffman et al. (2003)	Same as Birch et al. (2002; 2005) and Hoffman et al. (2003)	Same as Birch et al. (2002; 2005) and Hoffman et al. (2003)	Total of 202 infants (98 LCPUFA-supplemented and 104 controls) completed the trial. The average intention score was significantly ($P<0.05$) lower in corresponding controls compared to LCPUFA-supplemented infants from the 12-month feeding study and the 6-week weaning study. Infants from the 12-month feeding study had 51% success rate compared to a 29% success rate in corresponding controls. Infants from the 6-week weaning study had a 46% success rate compared to a 13% success rate in corresponding controls. In addition, the control infants from the 6-week weaning study showed a significantly lower percentage who obtained a perfect intention score than corresponding LCPUFA-supplemented infants. No significant differences were noted between LCPUFA-supplemented and control infants in the 4-6-month weaning study.	No safety concern; improved cognitive function in infants receiving LCPUFA for ≥7.5 months	Drover et al. (2009)
Randomized, double-blind study: (1) unsupplemented formula (control; n=86); (2) formula supplemented with 0.32% DHA (n=84); (3) formula supplemented with 0.64% DHA (n=85); or (4) formula supplemented with 0.96% DHA (n=88).	AA and DHA from single-cell oils	Formulas fed from 1-9 days of age to 12 months of age.	A total of 244 infants (56 controls, 64 supplemented with 0.32% DHA, 59 supplemented with 0.64% DHA, and 65 supplemented with 0.96% DHA) completed the study. Formula consumption, tolerance, and incidence of adverse events were similar between the formula groups. VEP acuity was significantly better in supplemented infants than control infants throughout the study but did not differ between the supplemented groups. Growth parameters were not affected by DHA supplementation. Red blood cell DHA concentration increased significantly with increasing DHA intake; whereas red blood cell AA concentration decreased with increasing DHA intake.	No safety concern; no effect on growth, improved visual function and altered total red blood cell lipid composition.	Birch et al. (2010)

AA=arachidonic acid; BAEP=brainstem acoustic evoked potentials; DHA=docosahexaenoic acid; ERG=electroretinogram; LCPUFA=long-chain polyunsaturated fatty acids; MDI=Mental Development Index; PDI=Psychomotor Development Index; PMA=postmenstrual age; VEP=visual evoked potential

5.1. Purity

The product specifications established by ONC, along with the results of chemical testing of multiple non-consecutive lots of tuna oil, provide assurance that ONC's refined tuna oil is a wholesome food-grade product. This assurance is further supported by a comparison of the specifications and analytical findings of ONC's refined tuna oil as compared with another refined tuna oil already regarded as GRAS for addition to infant formula, i.e., the tuna oil described by Abbott Ross in GRN 000094.

5.1.1. Side-by-Side Comparison of Product Specifications

The product specifications for ONC's refined tuna oil and those of the refined tuna oil described in GRN 000094 are similar but not identical. There are a number of parameters for which specifications are offered for one product but not the other. The GRAS tuna oil described in GRN 000094 includes a single specification for total heavy metals as lead, while the ONC refined tuna oil includes specifications for the individual heavy metals lead, arsenic, cadmium, and mercury. On the other hand, the GRN 000094 tuna specifications lack a number of parameters included in those for ONC's refined tuna oil: Gardner color, acid value, p-anisidine value, totox number[1], moisture content, total n-3 fatty acids, cadmium, benzo(a)pyrene, and several microbiological parameters (aerobic plate count, *Enterbacteriaceae, E. coli, Salmonella* spp., and yeast and mold).

The specification parameters that the two oils have in common are compared side by side in Table 6. While there are some slight differences, it is evident that the specifications for the two oils do not differ significantly. Perhaps of most importance, the specifications for DHA and EPA content and the DHA:EPA ratio are nearly identical.

5.1.2. Side-by-Side Comparison of Analytical Findings

In GRN 000094, Ross provided the results of analytical testing of four batches of refined tuna oil, although no individual test was conducted on more than three batches. ONC has conducted the analyses of 4 batches of refined tuna oil; results are shown in Appendix A. Table 7 shows a side-by-side comparison of the minimum and maximum values for all of the parameters that were tested in both data sets.

Not surprisingly, given that the specifications for the two tuna oils are similar, the analytical results are also similar, both showing extremely low levels of heavy metals or environmental contaminants.

[1] The totox number is a value for the oxidative state of total oxidation. It is the value of 2X the peroxide value + the p-anisidine value. It is a calculation, not an analytical test.

Table 6. ONC and Ross Refined Tuna Oil Specifications.

Parameter	Ross Specification	ONC Specification
Color/clarity	Yellow clear liquid oil	Clear yellow-amber oil
Flavor	Bland, slight (or less) fishy, slight (or less/none) green, no painty flavor	Bland
DHA content (%)	20.0 min (absolute); 22.0% min (relative)	25-30
EPA content (%)	7.2 max (absolute); 8.0 max (relative)	5-8
DHA:EPA ratio	≥3.1	≥3.0
Free fatty acids (%)	0.1 max	0.5 max
Peroxide value (meq/kg)	<2.0	<1.0
Unsaponifiable matter (%)	<1.5	<2.0
Arsenic (mg/kg)	<0.1	<0.1
Lead (mg/kg)	<0.1	<0.1
Mercury (mg/kg)	<0.5	<0.01
PCBs (mg/kg)	ND[1]	<0.09
Dioxins/furans (pg WHO-PCDD/FTEQ/g)	ND[1]	≤1.5
Pesticides (mg/kg)	ND[1]	<0.05

1. GRN 000094 does not indicate the limit of detection associated with the "not detected" specification.

Table 7. ONC and Ross Refined Tuna Oil Analytical Results.

Parameter	Ross Minima & Maxima	ONC Minima & Maxima
Peroxide value (meq/kg)	0.85 – 5.8	0.0 – 0.0
p-Anisidine value	5.35 – 7.9	9 – 11
Free fatty acids (%)	0.025 – 0.03	0.1 – 0.2
Unsaponifiable matter (%)	0.72 – 0.94	1.06 – 1.60
Arsenic (mg/kg)	<0.10 -- <0.10	<0.01 -- <0.02
Cadmium (mg/kg)	<0.04 – <0.04	<0.01 -- <0.01
Mercury (mg/kg)	<0.025 -- <0.025	<0.005 -- <0.005
Lead (mg/kg)	<0.05 -- <0.05	<0.05 -- <0.05
PCBs (mg/kg)	ND[1] -- ND	0.0001 -- <0.001

1. GRN 000094 does not indicate the limit of detection associated with the "not detected" result.

5.1.3. Arsenolipids

Arsenic can have many different oxidative states and chemical forms, making the biology, chemistry, and toxicology for this element very complex. At least 25 different chemical forms of arsenic have been detected. As noted by the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) in 2007 and the European Food Safety Authority (EFSA) in 2010, organic arsenic is generally less toxic than inorganic forms. The general consensus is that more than 85% of the arsenic in the edible parts of marine fish and shellfish is organic arsenic while less than 15% is inorganic (ATSDR 2007). Further, the principal organic arsenic species found in fish is arsenobetaine, which is considered relatively non-toxic even in comparison to other organic arsenic forms and is widely regarded as being of no toxicological concern; other organic forms such as arsenoplipids are present at lower levels (Codex 1999; EFSA 2010).

One reason why inorganic arsenic is more toxic than organic arsenic is that, although most ingested inorganic arsenic is excreted in the urine, it tends to accumulate in the body (ATSDR 2007). Organic arsenic, on the other hand, appears to be completely excreted relatively rapidly with no residual bioaccumulation (ATSDR 2007; EFSA 2010). Animal studies have revealed no accumulation of arsenic in hair after exposure to arsenobetaine (Vahter et al. 1983).

Schmeisser et al. (2006) reported on the human metabolism of organic arsenic present in fish oil (cod liver oil), using samples in which arsenolipids predominated and ones in which arsenobetaine predominated. These investigators demonstrated that organic arsenic does not accumulate: arsenobetaine was excreted unchanged from its original chemical state, indicating that it was not metabolized in any way, while arsenolipids were quickly metabolized to water-soluble compounds and excreted in the urine. Arsenolipids are metabolized into demethylarsinate and four other arsenical fatty acids, but they are rapidly cleared. Excretion of the arsenolipid metabolites peaked at 6 hours post-ingestion and 90% of the ingested arsenic was accounted for in the urine within 48 hours. These findings suggest that arsenolipids are effectively absorbed from the intestines but are rapidly excreted (Schmeisser et al. 2006).

At ONC, analysis of total arsenic is performed on all incoming crude fish oil lots prior to their entering an ONC facility. All refined fish oil is also tested for total arsenic a minimum of 3 times per annum. The specification is listed as not more than 0.1 mg total arsenic/kg oil. ONC's test method for total arsenic quantifies the total organic and inorganic arsenic species but does not provide speciation data. The established specification assures safety even if all measured arsenic were present in inorganic forms; the fact that research indicates that 85% or more is actually in organic form—and most of that as arsenobetaine—provides an additional margin of safety.

6. Safety Evaluation and GRAS Determination

6.1. Introduction

This chapter presents an assessment that demonstrates that the addition of ONC's refined tuna oil to infant formula, when this addition is accompanied by the addition of an appropriate source of arachidonic acid, is safe and is also GRAS under the FDCA. This safety assessment and GRAS determination entail two steps. In step one, the purity of the refined tuna oil and its safety under its intended conditions of use are demonstrated. In the second step, the intended use of refined tuna oil is determined to be GRAS by demonstrating that the methods by which this product is produced and refined as well as the safety of refined tuna oil under its intended conditions of use are generally recognized among qualified scientific experts.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the FDCA is set forth under 21 CFR 170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. This GRAS determination employs scientific procedures established under 21 CFR 170.30(b).

In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components: 1) the data and information relied upon to establish the scientific element of safety must be generally available; and 2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific procedures GRAS determination are applied below in an analysis of whether the addition of ONC's refined tuna oil, in conjunction with an appropriate source of arachidonic acid, to infant formula is safe and is also GRAS.

6.2. Safety of the Intended Use of Refined Tuna Oil

6.2.1. Purity

ONC's refined tuna oil is a food-grade product with very low levels of heavy metals, pesticide residues, or environmental contaminants. The maximum concentrations of these substances found in ONC's refined tuna oil are well within American and internationally accepted safe levels. Additionally, it was shown that the specifications and analytical testing results of ONC's refined tuna oil indicate a product fully equivalent in purity to tuna oil already accepted as GRAS for addition to infant formula.

6.2.2. Safety of the Intended Exposure to DHA from Tuna Oil

The FDA has previously reviewed safety concerns regarding consumption of fish oil containing the two omega-3 fatty acids EPA and DHA in the 1997 final rule affirming menhaden oil as GRAS (FDA 1997b). The primary safety concerns evaluated by the FDA associated with excessive intakes of EPA and DHA included increased bleeding times, reduced glycemic control among diabetics, and increased levels of LDL cholesterol among diabetics and hyperglycemics. Based on this review, the FDA concluded that a combined intake of EPA and DHA of up to 3 g/person/day would not result in any adverse health effects. Newer evidence pertaining to the safety of fish-oil consumption has been considered several times since 1997 with no change in this conclusion. For a 60-kg individual, intake of 3 g of a substance is equivalent to intake of 50 mg/kg bw of that substance. While the primary basis for establishing the safety of the intended use of refined tuna oil is the extensive published record of research in infants, this basis is corroborated by noting that the 90th percentile estimated daily intake of DHA and EPA from the intended use of ONC's refined tuna oil in preterm and term infant formulas does not exceed 53 mg/kg bw/day (40 mg DHA + 13 mg EPA), and thus approximates this level.

Since the GRAS evaluation entitled "GRAS Determination for Docosahexaenoic Acid Rich Oil Derived from Tuna and Arachidonic Acid Rich Oil Derived from *Mortierella alpina*" (GRN No. 000094) was submitted to FDA in 2001, 13 additional clinical studies examining growth and/or development in infants receiving DHA have been published. Ten (10) of these studies (Birch et al. 2002, 2005, 2010; Hoffman et al. 2003, 2004; Bouwstra et al. 2003; Fewtrell et al. 2004; Ben et al. 2004; Clandinin et al. 2005; Henriksen et al. 2008) employed completely new cohorts and examined the effect of DHA from fish oil or other sources on infant development, particularly visual acuity, growth, and cognition, when fed to preterm or term infants for periods up to a year. The remaining 3 studies (Bouwstra et al. 2005; Birch et al. 2007; Drover et al. 2009) presented additional data and follow-up studies from previous trials up to 4 years later.

In all of these studies, as with the previously evaluated infant studies, there were no adverse events or tolerance issues of any significance compared to corresponding controls when DHA was fed (with AA) to infants, typically at concentrations of 0.32% of total fatty acids and as high as 0.96% of total fatty acids for up to one year.

With the GRAS evaluation submitted to FDA, a concern arose regarding an observed increase in incidence of apnea events in the treatment group compared to the control group in the studies by O'Connor et al. (2001). FDA thoroughly reviewed the studies plus additional information provided by the notifier in a February 2, 2004 amendment and the FDA Medical Officer concluded:

> "...the observed increase in incidence of apnea events in the treatment group compared to the control group during Study AG38 [O'Connor et al. 2001] does not raise safety issues. FDA Medical Officers note that the statistical analyses, provided by [the notifier] and evaluated by FDA's Division of Mathematics, show a lack of significant association between the type of formula consumed and the

000070

number of infants experiencing apneic events. Moreover, multiple compounding factors in the infants' clinical histories lessen the clinical significance of the observed differences. FDA Medical Officers concur with [the notifier] that a large number of reported apnea events could be explained by external factors and were unrelated to ingestion of the AA-rich fungal oil and DNA-rich tuna oil that is the subject of [the notifier's] notice" (Luccioli and Hendrickson 2006).

Based on the data presented in GRN No. 000094 as submitted to FDA in 2001 and the 2004 amendment and the conclusions of the FDA Medical Officer, the FDA stated:

"...the agency has no questions at this time regarding [the notifier's] conclusion that AA-rich fungal oil and DHA-rich tuna oil are GRAS under the intended conditions of use (i.e., when added to preterm infant formulas, for use by hospitalized premature infants at the target mean concentration (g/100 g fatty acids) of 0.40 percent AA and 0.25 percent DHA; when added to preterm infant formula, for use by post-discharge infants, and to term infant formula, at target mean concentrations of 0.40 percent AA and 0.15 percent DHA), provided that the ingredients, as produced and analyzed, are in compliance with the specifications and associated analytical limits of detection for potential contaminants, as stated in [the notifier's] notice" (Tarantino 2006).

6.2.3. Safety of the Intended Exposure to EPA from Refined Tuna Oil

This same body of evidence demonstrates that exposure to EPA, present at not more than 33% of the concentration of DHA in ONC's refined tuna oil, is also safe. In the cited studies, the DHA:EPA ratio ranged from about 3:1 to 5:1. ONC's refined tuna oil has an average DHA:EPA ratio of 3.3:1, and is thus similar to the various oils that failed to produce adverse effects in the numerous clinical trials cited.

6.3. General Recognition of Safety

6.3.1. General Recognition of the Processing and Refining Methods Used

Some of the scientific literature gathered in support of the ONC oil refining process is from non-fish-oil refining, including published papers using vegetable oils such as sunflower, corn, canola, etc., commonly referred to as edible oils. The term edible oil refers to all edible oils including fish oils. The chemistry of edible-oil refining applies to all natural triacylglycerol edible oils regardless of the source.

Oil refining techniques used by ONC are consistent with industry standards, which have gained scientific consensus as optimal for refining food-grade oils. The quality of ONC fish oil has been demonstrated through repeated reproducible testing of final fish-oil products over many years.

ONC fish oils are subjected to a multitude of in-process and end-product tests to confirm compliance with external standards and internal specifications. In-process testing of ONC fish oils is crucial to assess and gain a 'real time' snapshot of the processing steps and, if necessary, to make adjustments so that the final product meets all parameters and specifications. All crude semi refined tuna oil undergoes routine testing at the time of

arrival at ONC's oil refining plant. Incoming crude semi refined tuna oil is analyzed for PCBs, dioxins and furans, trace metals and benzo(a)pyrene.

Quality Assurance and Quality Control staff closely monitor critical control points within the refining process. For refined tuna oil, ONC incorporates a routine analysis monitoring program.

Table 8. ONC Tuna Oil Contaminant Testing Frequency

Incoming Crude/Semi Refined Tuna Oil	**Refined Tuna Oil**
Every incoming lot analyzed For: PCBs, dioxins and furans, trace heavy metals, benzo(a)pyrene	*Analyzed three times per year:* PCBs, dioxins and furans, trace heavy metals, benzo(a)pyrene *Analyzed once per year*: Pesticides

Analytical methods developed and validated by recognized organizations such as USP, EP, and AOCS are used. New or adapted methods developed by ONC qualified scientists are subject to in-house validation prior to use.

In the textbook *Marine and Freshwater Products Handbook,* edited by Roy E. Martin, Emily Paine Carter, George J. Flick Jr. and Lynn M. Davis. Anthony P. Bimbo outlines the extraction of crude fish oil and the subsequent refining methods used to produce food grade oil capable of being consumed as a food or dietary supplement (Bimbo 2000). There are also two book chapters in *Fish Oils in Nutrition* edited by Maurice E. Stansby, both by Anthony P. Bimbo. Chapter 6 deals with the production of crude fish oil and Chapter 7 deals with processing of fish oils (refining steps).

6.3.1.1. 21 CFR §184.1472: Menhaden Oil

Menhaden oil, which was codified (21 CFR §184.1472) in an FDA final rule in the *Federal Register* on June 5, 1997 (amended March 23, 2005), outlines the manufacturing steps from crude oil to refined menhaden oil (FDA 2005). The fish are cooked and pressed and the resulting pressed liquor is separated into a water fraction and a crude fish oil fraction. The crude fish oil is then subjected to a series of refining steps: winterization, degumming (optional), neutralization, bleaching and deodorization. 21 CFR §184.1472 does not offer a detailed description of these steps. However, the processing steps for fish-oil refining by ONC are essentially the same as those described for menhaden oil although the sequence of operations is slightly different.

000072

6.3.1.2. Extraction of Crude Oil

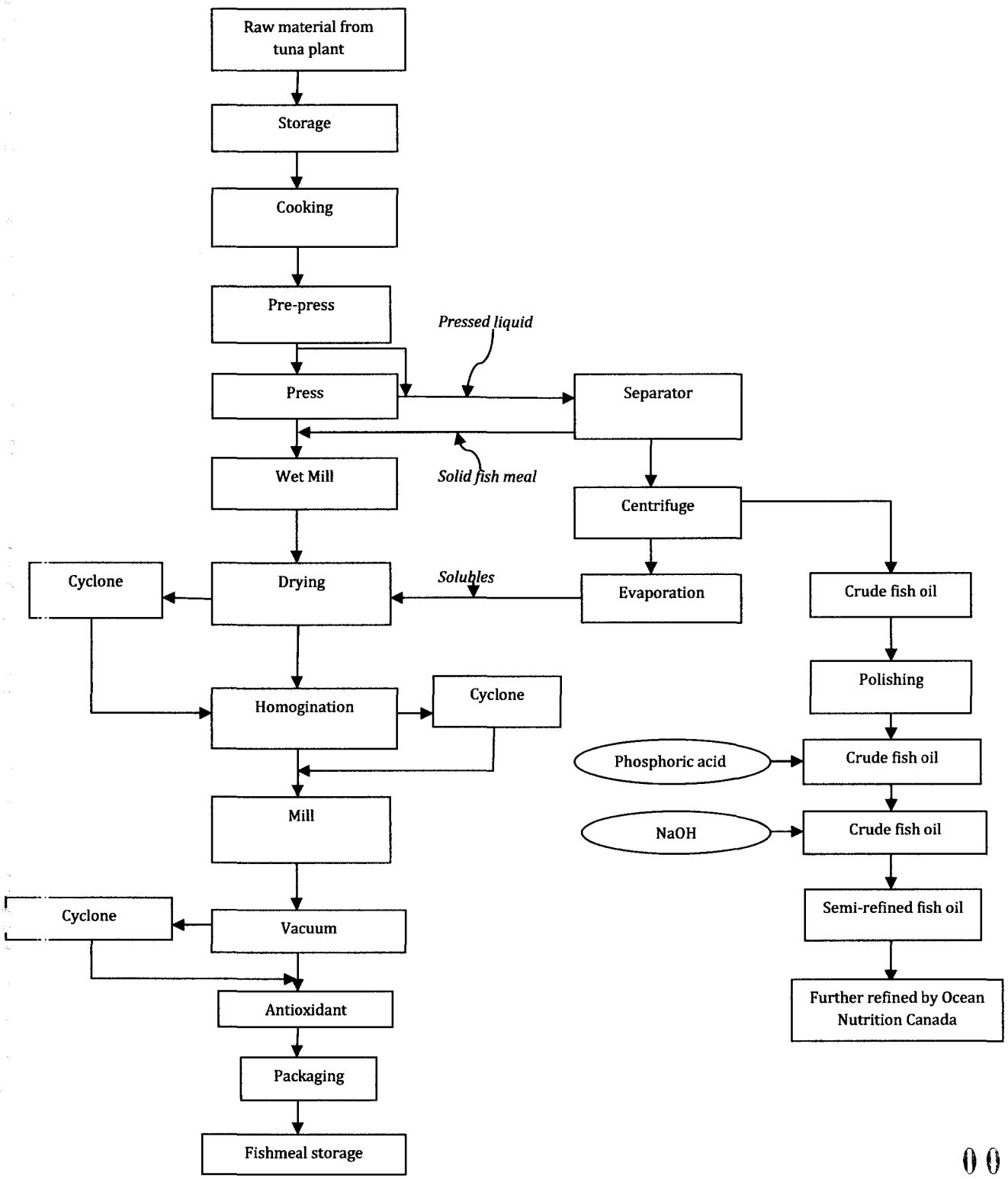
Fish used for the production of fish meal and fish oil can be divided into several categories (Bimbo 1989, 1990, 2000): (1) fish caught for the sole purpose of fish meal and fish oil production (*e.g.*, menhaden and anchovy); (2) by-catches from another fishery (*e.g.*,

shrimp); and (3) fish waste from edible fisheries such as cuttings from a fish cannery (e.g., tuna). Crude tuna oil refined by ONC originates from category 3.

The process of separating fish oil from tuna canning operations begins with trimmings from tuna canneries and then the following steps (Bimbo 1989, 1990, 2000): (1) cooking; (2) pressing; (3) drying; (4) water/oil separation (5) drying (6) antioxidant addition; and (5) storage and shipping, which are described below.

Figure 4: Crude Tuna Oil Extraction Flow Chart

This flow diagram represents the crude oil extraction via fish meal production. Only production steps relevant to crude fish oil extraction are discussed in further detail



6.3.1.2.1. Cooking

The purpose of the cooking process is to denature the protein of the fish so that a mechanical separation of liquid and solid can take place. Cooking temperatures of 90-95ºC are reached and maintained for 20-30 minutes.

6.3.1.2.2. Pressing

The purpose of pressing is to separate the solid and liquid parts of the cooked fish. As described by both Bimbo (1989, 1990, 2000) and Food and Agriculture Organization Fisheries Technical Report 142 (FAO 1986), pressing is done by either a single- or double-screw press. The final result is a liquid/solid phase separation.

6.3.1.2.3. Centrifugation (Water Oil Separation)

This step is described in detail by Bimbo (1989, 1990, 2000) and FAO Fisheries Technical Report 142 (FAO 1986). The purpose of centrifugation is to further separate solid fish meal from liquid fish oil. The fish oil/fish meal mixture is normally maintained at a temperature of 90ºC while entering the centrifuge. Bimbo (1989, 1990, 2000) describes the centrifugation process as essentially a 3 step process. In the first step the pressed liquid is passed through decanter (horizontal bowl) centrifuges which remove fine solids that are expressed from the presses. The second step separates oil from water and the third step polishes or water washes the oil. So the purpose of the centrifugation steps is to essentially separate and wash the oil phase. These processing steps are normally maintained at a temperature of 90°C the optimum temperature for oil water separation (FAO 1986; Bimbo 1990, 2000).

6.3.1.2.4. Drying

This step is specific to the solid press cake used in the fish meal industry, not to the oil, and requires no discussion.

6.3.1.2.5. Addition of Antioxidant

The purpose of adding an antioxidant to fishmeal is to prevent oxidation of the unsaturated fatty acids. The amount of antioxidant added to the fishmeal depends on the amount of unsaturation of the fatty acids. The addition of an antioxidant normally does not take place during the fishmeal extraction step but rather after the drying step (FAO 1986; Bimbo 1989, 1990, 2000).

Antioxidants are traditionally added to the fish oil after the oil is completely refined rather than during the refining process. However, in the processing of ONC tuna oil, antioxidants are added by ONC during the oil-refining stage. A detailed explanation of antioxidant addition can be found in section 2.4.2.4 of this dossier.

6.3.1.3. Fish-Oil Refining

In the early stages of their preparation for food use, oils and fats generally contain minor amounts of non-triglyceride substances. While some of these are considered

beneficial to the stability of the oil, such as tocopherols which protect the oil from oxidation, other impurities are objectionable because they render the oil dark colored, cause it to foam or smoke or are precipitated when the oil is heated in subsequent processing operations (Norris 1982). Other impurities reduce acceptability because of the flavors and odors they produce in the fat or because they reduce stability and shelf life of the foods to which the fats are added.

Hilditch (1949) suggested that some impurities are common to all fats regardless of the source or end use and classified them as follows:

1. Relatively coarse suspended matter.
2. Exceedingly fine suspensions of colloidally dispersed materials.
3. Natural coloring matter.
4. Free fatty acids.
5. Semi-volatile compounds dissolved in the fat or oil.

Of all the operations to which edible oils are subjected during conversion to finished products, the refining process has the most impact on quality. If oils are not adequately refined, subsequent operations such as bleaching, hydrogenation, winterizing, deodorization, etc., will be troublesome and finished products may fail to meet quality standards (Carr 1976).

The following sections demonstrate that the processing steps used by ONC, described earlier, are consistent with what is acknowledged by the scientific community as safe and effective refining steps for edible oils.

6.3.1.3.1. Neutralization (Alkali Refining)

Neutralization is a purifying treatment designed to remove free fatty acids and miscellaneous materials without saponifying neutral oil. The addition of an alkali solution to crude oils results in chemical reactions and physical changes. The alkali combines with the free fatty acids (FFA) present in the oils to form a soap. The soap is then separated from the neutral oil in a centrifuge (continuous process) or by gravity (batch process). From an application standpoint, neutralization (alkali refining to remove FFA) of the oil produces a product which when heated will not darken, foam, or smoke, or become cloudy and form a precipitate. It also allows the later bleaching step to be completed more easily (Bimbo 1989, 1990).

Gunstone et al. (1994) described the process of neutralization in a similar manner. The objective of the neutralization step in refining edible oils is the removal of free fatty acids and the dirt and denatured phosphatides not previously separated, together with pigments and other impurities that are saponifiable. According to Gunstone et al. (1994), the most widely used alkali is sodium hydroxide because of its thorough cleansing action on the oil. ONC uses sodium hydroxide as the alkali solution during neutralization. However, as Gunstone et al. (1994) point out, sodium hydroxide can also be combined with other alkali substances such as sodium carbonate to make an alkali solution. Neutralization is

completed with a water wash to ensure complete removal of soaps and alkali (Gunstone et al. 1994). ONC uses multiple water washes to ensure thorough removal of soaps and any residual sodium hydroxide.

Neutralization is a classical step in edible oil refining (Forster et al. 1983, Carr 1983). Free fatty acids naturally present in the oil are neutralized with caustic soda to form a soapstock that is removed by mechanical separation from the neutral oil. The extent of pre-treatment of the crude oil depends on the particular oil and its quality. The point here is that crude fish oil profiles vary dependent on many factors. Fish species, area of the catch, environmental factors all play a role in the oil profile and thus, may require different degrees of refining to achieve the desired final product.

6.3.1.3.2. Deodorization

Deodorization has the responsibility for removing both the undesirable ingredients occurring in natural fats and oils and those which might be produced by previous processing steps such as caustic refining, bleaching, hydrogenation or even storage conditions. It is this unit process that finally establishes the oil characteristics of "flavor and odor" which are most readily recognized by the consumer (Gavin 1978).

Deodorization of edible oil is designed to remove volatile, odoriferous material present in the oil. Deodorization improves the oil's palatability and oxidative stability by nearly complete removal of free fatty acids and other volatile materials (Dudrow 1983). Thermal treatment is a necessary part of the deodorization process. ONC deodorizes its oil at elevated temperature and reduced pressure. Steam deodorization is a very common process when refining edible oils. Steam deodorizing is also carried out at high temperatures under reduced pressure. The goal is to produce odorless and tasteless oil.

6.3.1.3.3. Decolorization/Adsorbing (Bleaching)

Bleaching is often referred to as decolorization due to the improvements it makes in the color of fish oil through adsorption of colored bodies. However, we will refer to this step from here on out as its common phrase in industry 'bleaching'. Bleaching is used to improve the color, flavor and oxidative stability of the oil, and to remove impurities, such as traces of soap, that interfere with the rest of the refining process steps. While alkali refining removes many color bodies and impurities, some still remain and the bleaching step removes them plus residual soap. There are two methods of bleaching, batch and continuous batch bleaching can be further broken down into atmospheric and vacuum bleaching.

Bleaching is the adsorption of color bodies in the oil by the addition of activated clays. The bleaching step is designed to remove any remaining impurities and soaps that were not removed during previous processing steps. The process involves heating the oil to a desired temperature, adding bleaching clay, and mixing under a vacuum or at atmospheric pressure for the desired period of time. The clay is then removed through filtration. Bleaching is used to improve the color, flavor, and stability of the oil as well as remove impurities such as trace soaps (Bimbo 1989, 1990, 2000).

Gunstone et al. (1994) describes the process of bleaching as contacting the oil with an adsorbent material designed to further remove unwanted impurities such as pigments, soaps, trace metals, phosphotides, and sulfur compounds from the oil. Traditionally, the adsorbent material used is natural bleaching clay but in some cases an acid activated clay is used.

The use of bleaching clays in processing of edible oils involves mixing the clay and oil, applying suitable agitation, elevating the temperature for the proper period of time and filtering to remove the spent clay (Richardson 1978).

ONC bleaches crude fish oil by incorporating bleaching clay to its oil. Bleaching edible oil is typically done with different grades of clay. The percentage of the clay used varies according to the type of oil being bleached. ONC typically bleaches crude fish oil at elevated temperatures and reduced pressure. When using activated bleaching earths, lipid peroxides are destroyed and their breakdown products adsorbed by the clay (Patterson 1976).

6.3.1.3.4. Antioxidant Blending

Oxidation of edible oils results in degradation, often producing off odors and flavors. The degradation process occurs at the unsaturation sites (double bonds) of the glycerin molecule (Sherwin 1978). The more unsaturated sites in the triacylglycerol structure, the more susceptible the edible oil is to oxidative deterioration. Polyunsaturated fatty acids such as DHA and EPA have multiple double bonds, and are very susceptible to oxidation.

The most frequently used antioxidants in edible-oil stabilization are mixed tocopherols. Tocopherols are naturally occurring substances that aid in slowing oxidative reactions in edible oil. Unlike vegetable oils which contain tocopherols, fish oils have relatively little natural antioxidant protection especially after the deodorization process. It is therefore necessary to add antioxidants to the final fish oil product to protect the polyunsaturated fatty acids.

ONC adds a mix of natural tocopherols (beta, gamma, alpha) and/or ascorbyl palmitate to all of its fish oils at a prescribed concentration during the refining process after the bleaching step. More detail on ONCs antioxidant addition is provided in Section 2.4.2.4.

6.3.1.3.5. Filtration, Drumming, Handling and Storage of Finished Oils

Proper handling and storage of the final refined fish oil is critical to maintaining the quality of the final fish oil product. (Wright 1976). ONC filters its oil to remove any trace solid material that was not removed in previous refining steps.

ONC has implemented a preventative maintenance program that accounts for all equipment used in the processing of fish oil. All equipment is installed in accordance with manufacturer's instructions and regular work orders are set up to maintain the equipment in clean and proper working order. The preventative maintenance system is audited both internally and externally to ensure its proper and effective function.

000078

Oxidation of the finished product is considered one of the most serious risks for oil quality. The exclusion of oxygen during finished oil storage is highly desirable. ONC incorporates a nitrogen blanketing system during the drumming step. Oil is stored in a food grade 190-kg steel drum with a nitrogen blanket to prevent oxidation. Oil is stored in a cool dry environment or specific oils can be frozen at or below -18ºC to minimize degradation due to temperature abuse.

6.3.1.4. Conclusions

Refining edible oils is not new science. ONC does not incorporate novel technologies in refining edible fish oil for the global food and dietary supplement markets. All processing steps are well within industry standards and supported by the scientific literature. Fish oil is a natural product and thus shows natural variations dependent on many factors such as the type of fish, where they are caught, environmental factors, fishing season, etc. Crude fish oil can have variations in contaminant levels as well as EPA/DHA levels. To compensate for these variations of the raw material input, the refining steps must also vary in order to produce a consistent finished product. However, ONC does not stray from 'industry norms' when refining its fish oil and ensures compliance to strict internal specifications for all finished product fish oils. The scientific evidence presented here clearly demonstrates that the refining techniques used by ONC are fully supported by published scientific literature.

6.3.2. General Recognition of the Safety of the Intended Use of Tuna Oil

6.3.2.1. Opinions of Authoritative Bodies

A number of authoritative scientific, medical, and regulatory organizations have concluded that the addition of LCPUFA—DHA and arachidonic acid—to infant formula for both preterm and term infants is both safe and beneficial. Importantly, these opinions are based on review of the published research literature and thus demonstrate both general recognition of the safety of the intended use of tuna oil (along with a source of arachidonic acid) and the general availability of the information on which this recognition is based.

6.3.2.1.1. Food and Agriculture Organization of the United Nations and the World Health Organization

In the report of a joint expert consultation to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO, Fats and Oils in Human Nutrition (FAO/WHO 1994), the consultation concluded that supplementation of infant formula with marine oils providing DHA along with a source of arachidonic acid is safe for both preterm and term infants and recommended that preterm infants should receive 40 mg DHA and 60 mg arachidonic acid/kg bw/day (±30%) while term infants should receive 20 mg DHA and 40 mg arachidonic acid/kg bw/day. (Chapter 7: Lipids in early development. "As a guide, formula for preterm babies should provide a mean of 700 mg linoleic acid, 50 mg α-linolenic acid, 60 mg of arachidonic acid and its associated long chain n-6 fatty acids, and 40 mg of DHA per kg body weight." "For term infants, the provision, per kilogramme of body weight should amount to 600 mg of linoleic acid, 50 mg

of α-linolenic acid, 40 mg of arachidonic acid and its associated n-6 fatty acids and 20 mg of docosahexaenoic acid.")

6.3.2.1.2. U.S. Food and Drug Administration

In February 2000, Martek submitted a notice, filed by FDA as GRN No. 000041, which concluded that the addition of DHA from single-cell oil (along with arachidonic acid) to both preterm and term infant formula at a level not exceeding 0.5% of the total fatty acids is both safe and GRAS. The estimated intake of DHA by infants was 30 mg/kg bw/day. In its response (FDA 2001), FDA concluded that the agency had no questions at that time regarding this conclusion.

In December 2001, a GRAS notice was submitted by Ross Laboratories concluding that supplementation of infant formula with arachidonic acid and tuna oil containing DHA, with the DHA addition not exceeding 0.25% of the fatty acids in formula intended for pre-hospital-discharge preterm infants and 0.15% of the fatty acids in formula intended for post-discharge preterm infants and term infants, is safe and GRAS. FDA filed this notice as GRN No. 000094. In April 2006, FDA stated that it had no questions at that time regarding this GRAS determination (FDA 2006).

6.3.2.1.3. National Academies, Institute of Medicine

The report of the Panel on Macronutrients (IOM 2005), noted that DHA is important for the developing brain and retina, and that "the DHA content of the brain may depend more heavily upon the dietary supply of DHA rather than its precursor, α-linolenic acid," estimated the Adequate Intake (AI) of infants aged 0-6 months for n-3 fatty acids at 500 mg/day based on average intake of n-3 fatty acids from human milk.

6.3.2.1.4. European Society for Pediatric Gastroenterology, Hepatology and Nutrition

At the request of the Codex Committee on Nutrition and Foods for Special Dietary Uses, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) formed an international expert group to review the latest scientific information bearing on the composition of infant formulas and provide recommendations to the Codex Committee. The expert group completed its work and published its proposals in 2005 (Koletzko et al. 2005). The group established as a guiding principle that infant formulas should contain only components in such amounts that serve a nutritional purpose or provide other benefits. Based on the beneficial effects of the addition of DHA and arachidonic acid to infant formula reported in a number of published studies, the group supported addition of these components. With regard to safety, the group concluded:

> "A large number of studies in which LC-PUFA were added to infant formulae have not raised major safety concerns and a recent meta-analysis found no indication of adverse effects on growth of the addition of both DHA and AA, and neither were adverse effects reported in analyzing the limited number of studies with addition of only n-3 LC-PUFA. However, adverse growth effects have been reported in single studies with supplementation of fish oils without concomitant n-6 LC-PUFA supply, particularly at high EPA intakes. It is noted that at this time there is no sufficient

documentation of the benefits and safety of the addition of DHA to infant formula at levels >0.5% of total fat content, or of DHA without concomitant addition of AA. Until the benefits and suitability for particular nutritional uses and the safety of other additions have been adequately demonstrated, the optional addition of DHA should not exceed 0.5% of total fat intake, and AA contents should be at least the same concentration as DHA, whereas the content of EPA in infant formula should not exceed the DHA content" (Koletzko et al. 2005).

6.3.2.1.5. European Union

Commission Directive 2006/141/EC on infant formulae and follow-on formulae (EU 2006) provides for the addition of n-3 and n-6 fatty acids to infant formula as follows:

"5.7 Long-chain (20 and 22 carbon atoms) polyunsaturated fatty acids (LCPUFA) may be added. In that case their content shall not exceed:
- 1% of the total fat content for n-3 LCPUFA, and
- 2% of the total fat content for n-6 LCPUFA (1% of the total fat content for arachidonic acid (20:4 n-6))

The eicosapentaenoic acid (20:5 n-3) content shall not exceed that of docosahexaenoic (22:6 n-3) acid content.

The docosahexaenoic acid (22:6 n-3) content shall not exceed that of n-6 LCPUFA."

This assessment was renewed in 2009 when a panel the European Food Safety Authority (EFSA) reviewed an application for a health claim regarding the supplementation of infant formula with DHA and ARA (EFSA 2009). The proposed claim stated that "DHA and ARA contribute to the optimal visual development of infants and young children," and was to be conditional upon the formula containing at least 0.3% of the fatty acids as DHA and the ratio of ARA to DHA lying between 1.4:1 and 2.0:1. Although the specific sources of DHA and ARA proposed in the application were derived from the alga *Crypthecodinium cohnii* and the fungus *Mortierella alpina*, respectively, the EFSA panel determined that DHA and ARA are well characterized fatty acids and that "this evaluation will apply to DHA and ARA from all appropriate sources in the specified amounts."

The EFSA panel concluded that, a cause-and-effect relationship "has been established between the intake of infant and follow-on formula supplemented with DHA and visual function at 12 months in formula-fed infants," and that, in order to bear the claim, a formula should contain at least 0.3% of the total fatty acids as DHA (EFSA 2009).

6.3.2.1.6. American Dietetic Association and Dietitians of Canada

In a position statement regarding dietary fatty acids published in the Journal of the American Dietetic Association in 2007 (ADA 2007), it was noted that "no adverse effects of feeding marketed infant formula containing both ARA and DHA in amounts found in human milk are known." The position of the two organizations is that "all infants who are not breastfed be fed a formula containing both ARA and DHA through at least the first year of corrected age."

6.3.2.1.7. Codex Alimentarius Commission

At the 28th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses in 2006, the Committee agreed to retain its existing proposal to set the upper limit for the addition of DHA to infant formula at 0.5% of fatty acids (Codex 2006). This level was chosen as it was based on current scientific evidence regarding the safety of human milk. The decision to retain the 0.5% level was in response to a proposal from the Delegation of Japan, supported by other delegations, to increase the recommended upper limit to 1% of the fatty acids based on studies carried out in Japan and other Asian countries. While the Committee agreed to retain its guidance upper level, it did agree to add a footnote that "national authorities may deviate from these conditions."

6.3.2.1.8. World Association of Perinatal Medicine

A consensus report representing recommendations and practice guidelines of the World Association of Perinatal Medicine, the Early Nutrition Academy, and Child Health Foundation was published in 2008 (Koletzko et al. 2008). The report noted that DHA is a critical component of cell membranes, especially in the brain and the retina, and that brain accumulation of DHA begins *in utero* and continues after birth. It further noted the insufficiency of the rate of elongation and desaturation conversion of DHA from its precursors. In human milk, DHA content is generally in the range of 0.17 to 1.0% of total fatty acids while arachidonic acid is about 0.35 to 0.7% and the circulating levels of these fatty acids in breastfed infants can only be matched with the addition of both DHA and arachidonic acid to formula. Noting that "safety is of primary importance," the report recommended:

> "Highly refined oils from single cell organisms (specific algal and fungal organisms), eggs, or fish as sources of DHA and/or AA are appropriate for use in infant formulae and weaning foods if the purity and safety of the specific oil used has been documented" (Koletzko et al. 2008).

The report went on to note that "a large database exists concerning not only the safety, but also the efficacy, of infant formula containing both AA and DHA." Finally, the report concluded that "we recommend use of an infant formula providing DHA at levels between 0.2 and 0.5 weight percent of total fat, and with the minimum amount of AA equivalent to the contents of DHA. ... and EPA should not exceed levels of DHA."

6.3.2.2. Recent Experience

Most manufacturers of infant formula in Europe and the U.S. have been offering DHA- and arachidonic-acid-supplement preterm and term formulas for a number of years, with DHA addition in the range of 0.25 to 0.30% of the fatty acids. The intended use of ONC's refined tuna oil is consistent with this addition level, which has not been associated with any adverse effects regarded as related to the DHA, EPA, or arachidonic acid content of the formula, and with the most recent recommendations of authoritative bodies.

6.3.2.3. Conclusion of the Expert Panel

The intended use of ONC's refined tuna oil, in conjunction with a source of arachidonic acid, has been determined to be safe through scientific procedures set forth under 21 CFR 170.30(b). This safety was established by demonstrating that ONC's refined tuna oil is (1) compositionally equivalent both to refined tuna oil already GRAS for addition to infant formula and to the fish oils used in published clinical trials of the effects on term and preterm infants of supplementation of infant formula with fish oil and a source of arachidonic acid, and (2) free of contaminants or residues at levels that would suggest a health concern. The intended use levels of ONC's refined tuna oil and the resulting estimated daily intake of DHA and EPA resulting from these use levels are within limits shown to be safe in published research studies and recommended by numerous authoritative bodies. Because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of ONC's refined tuna oil for addition to infant formula under the intended conditions of use (including addition of a source of arachidonic acid at appropriate levels) has been made through the deliberations of an Expert Panel comprising Anthony P. Bimbo, Joseph F. Borzelleca, Ph.D., Berthold V. Koletzko, M.D., and George H. Pauli, Ph.D. These individuals are qualified by scientific training and experience to evaluate the processing methods employed to extract and refine tuna oil and the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, and have concluded:

> *No evidence exists in the available information on ONC's refined tuna oil, or on EPA and DHA, that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when ONC's refined tuna oil, along with an approved source of arachidonic acid, is added to infant formula intended for consumption by preterm and term infants at the intended levels.*

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, ONC's refined tuna oil is safe and is GRAS for addition to infant formula when this addition is accompanied by addition of an appropriate source of arachidonic acid.

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APPENDIX A

Contaminant Comparison Chart- Crude Semi Refined Fish oil vs' Fully Refined Fish Oil

APPENDIX A

Refined lots 19582 19583 originated from crude lots #1 #2. Refined lots 18950 18951 originated from crude lots #3 #4

Crude Semi Refined Tuna Oil			Refined Tuna Oil		Crude Semi Refined Tuna Oil			Refined Tuna Oil	
Contaminantes	Lot #1	Lot #2	Lot (b) (6)	Lot (b) (6)	Contaminantes	Lot #3	Lot #4	Lot (b) (6)	Lot (b) (6)
PCBs					**PCBs**				
ppt	18023	15506	106	1347	ppt	12232	11091	872	814
ppb	18.023	15.506	0.106	1.347	ppb	12.232	11.091	0.872	0.814
ppm	0.019023	0.015506	0.000106	0.001347	ppm	0.012232	0.011091	0.000872	0.000814
Trace Metals					**Trace Metals**				
Arsenic	4.48	3.76	<0.01	0.02	Arsenic	1.8	1.71	<0.01	0.01
Cadmium	<0.01	<0.01	<0.01	<0.01	Cadmium	<0.01	<0.01	<0.01	<0.01
Lead	<0.05	<0.05	<0.05	<0.05	Lead	<0.05	<0.05	<0.05	<0.05
Mercury	0.009	0.008	<0.005	<0.005	Mercury	<0.005	<0.005	<0.005	<0.005
Strontium	0.5	0.4	<0.2	<0.2	Strontium	0.7	0.6	<0.2	<0.2
Dioxins and Furans (pg/g)					**Dioxins and Furans (pg/g)**				
2,3,7,8-Tetra CDD	<0.05	<0.06	<0.06	<0.06	2,3,7,8-Tetra CDD	<0.06	<0.06	<0.06	<0.06
1,2,3,7,8-PentaCDD	<0.05	<0.05	<0.05	<0.05	1,2,3,7,8-PentaCDD	<0.06	<0.05	<0.05	<0.05
1,2,3,4,7,8-HexaCD	<0.10	<0.10	<0.11	<0.11	1,2,3,4,7,8-HexaCD	<0.10	<0.11	<0.11	<0.12
1,2,3,6,7,8-HexaCDD	<0.19	<0.20	<0.21	<0.21	1,2,3,6,7,8-HexaCDD	<0.20	<0.21	<0.21	<0.22
1,2,3,7,8,9-HexaCDD	<0.10	<0.10	<0.11	<0.11	1,2,3,7,8,9-HexaCDD	<0.10	<0.11	<0.11	<0.12
1,2,3,4,5,6,7,8-HeptaCDD	0.79	0.77	<0.14	<0.14	1,2,3,4,5,6,7,8-HeptaCDD	0.9	0.86	<0.14	<0.15
OctaCDD	13	18.5	<0.80	<0.80	OctaCDD	11.1	10.7	<0.82	<0.85
2,3,7,8-TetraCDF	0.67	0.58	<0.1	<0.1	2,3,7,8-TetraCDF	0.46	0.46	<0.10	<0.10
1,2,3,7,8-PentaCDF	<0.08	0.11	<0.09	<0.09	1,2,3,7,8-PentaCDF	<0.09	<0.09	<0.09	<0.10
2,3,4,7,8-PentaCDF	0.084	1.01	<0.09	<0.09	2,3,4,7,8-PentaCDF	0.83	0.93	<0.09	<0.10
1,2,3,4,7,8-HexaCDF	<0.08	<0.09	<0.09	<0.09	1,2,3,4,7,8-HexaCDF	<0.09	<0.09	<0.09	<0.10
1,2,3,6,7,8-HexaCDF	<0.08	<0.09	<0.09	<0.09	1,2,3,6,7,8-HexaCDF	<0.09	<0.09	<0.09	<0.10
1,2,3,7,8,9-HexaCDF	<0.08	<0.09	<0.09	<0.09	1,2,3,7,8,9-HexaCDF	<0.09	<0.09	<0.09	<0.10
2,3,4,6,7,8-HexaCDF	<0.08	<0.09	<0.09	<0.09	2,3,4,6,7,8-HexaCDF	<0.09	<0.09	<0.09	<0.10
1,2,3,4,6,7,8-HeptaCDF	0.46	0.54	<0.12	<0.12	1,2,3,4,6,7,8-HeptaCDF	0.48	0.47	<0.13	<0.13
1,2,3,4,7,8,9-HeptaCDF	<0.10	<0.10	<0.11	<0.11	1,2,3,4,7,8,9-HeptaCDF	<0.10	<0.11	<0.11	<0.12
OctaCDF	3.72	5.14	<0.23	<0.23	OctaCDF	3.33	3.21	<0.23	<0.24
TEQ (WHO) PCDD/F incl. LOQ	0.681	0.77	0.253	0.253	TEQ (WHO) PCDD/F incl. LOQ	0.678	0.721	0.259	0.271
PAH Indicator					**PAH Indicator**				
Benzo(a)pyrene	0.5	0.5	<0.1	<0.1	Benzo(a)pyrene	0.5	0.5	<0.1	<0.1

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Bulk Oil
Certificate of Analysis

Product Name: DHA Fish Oil (non GMO)
Product Code #: XOTDHA-NG
Expiry Date: May. /2009
O.N.C. Lot #: (b)
Manufacture Date: May. 27/2008
TDS#: (b) (6)

ANALYSIS	SPECIFICATIONS	RESULTS
Free Fatty Acid (as % Oleic)	Max. 0.5%	0.1
Acid Value	Max. 1.0 mg of KOH/g	0.3
p-Anisidine Value	Max. 20	11
Peroxide Value	Max. 5.0 meq/Kg	0
%Moisture	Max 0.1%	0.0
Colour	Max. 7	7
Appearance	Clear yellow-amber oil, characteristic of fish oil, with minimum sediment at room temperature	Pass
Totox Number	Max. 26	11
Unsaponifiable Matter	Report Actual	1.5
Fatty Acid Profile		
EPA (%)	5 - 8%	8
DHA (%)	25 - 30%	26
EPA mg/g (expressed as TG)	Min. 45 mg/g	70
DHA mg/g (expressed as TG)	Min. 220 mg/g	225
EPA mg/g (expressed as FFA)	Min. 40 mg/g	70
DHA mg/g (expressed as FFA)	Min. 210 mg/g	218
Total Omega 3 %	Min. 32 - 40%	37
Total Omega 3 (mg/g as TG)	Min. 280 mg/g	327
Antioxidants		
Non-GMO Antioxidant blend	Min. 8000 ppm	Pass
PCBs, PAHs, Dioxins & Furans, Heavy Metals, Microbial and Pesticides*		
PCBs (IUPAC no. 28,52,101,118,138,153,180 Total)	< 0.09 ppm	Compliant
Total PCB (Canada Only)	Max. 0.1 ppm	Compliant
Benzo(a)pyrene	Max. 2.0 ppb	Compliant
Dioxins & Furans (PCDDs and PCDFs)	Max. 2 pg WHO-PCDD/FTEQ/g	Compliant
Dioxin-Like PCBs	Report Actual	Compliant
Arsenic	< 0.1 ppm	Compliant
Cadmium	< 0.1 ppm	Compliant
Mercury	Max.0.01 ppm	Compliant
Lead	< 0.1 ppm	Compliant
Strontium (Canada Only)	Max. 0.5ppm	Compliant
Standard Aerobic Plate Count	< 100 CFU/g	Compliant
Enterbacteriaceae	< 100 CFU/g	Compliant
E. Coli	Not detected in 1 g	Compliant

(b) (6)

Quality Control Manager (or delegate)

June 20/08
Date

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Product Name: DHA Fish Oil (non GMO)
Product Code #: XOTDHA-NG
Expiry Date: May. /2009

O.N.C. Lot #: (b) (
Manufacture Date: May. 27/2008
TDS#: (b) (6)

PCBs, PAHs, Dioxins & Furans, Heavy Metals, Microbial and Pesticides Continued**

Salmonella spp.	Not detected in 10 g	Compliant
Yeast and Mold	< 100 CFU/g	Compliant
DDT	< 0.05 ppm	Compliant
DDE	< 0.05 ppm	Compliant
HCB	< 0.05 ppm	Compliant
Lindane	< 0.05 ppm	Compliant

*Results for contaminants may be expressed as either compliant/non compliant (based on Master Batch Testing) or as actual results.

(b) (6)
Quality Control Manager (or delegate)

Date Jun 20/08

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Bulk Oil
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Product Name: DHA Fish Oil (non GMO)
Product Code #: XOTDHA-NG
Expiry Date: May. /2009

O.N.C. Lot #: (b) (6)
Manufacture Date: May. 28/2008
TDS#: (b) (6)

ANALYSIS	SPECIFICATIONS	RESULTS
Free Fatty Acid (as % Oleic)	Max. 0.5%	0.1
Acid Value	Max. 1.0 mg of KOH/g	0.3
p-Anisidine Value	Max. 20	10
Peroxide Value	Max. 5.0 meq/Kg	0.0
%Moisture	Max 0.1%	0.0
Colour	Max. 7	6
Appearance	Clear yellow-amber oil, characteristic of fish oil, with minimum sediment at room temperature	Pass
Totox Number	Max. 26	10
Unsaponifiable Matter	Report Actual	1.06
Fatty Acid Profile		
EPA (%)	5 - 8%	8
DHA (%)	25 - 30%	26
EPA mg/g (expressed as TG)	Min. 45 mg/g	69
DHA mg/g (expressed as TG)	Min. 220 mg/g	225
EPA mg/g (expressed as FFA)	Min. 40 mg/g	66
DHA mg/g (expressed as FFA)	Min. 210 mg/g	217
Total Omega 3 %	Min. 32 - 40%	37
Total Omega 3 (mg/g as TG)	Min. 280 mg/g	323
Antioxidants		
Non-GMO Antioxidant blend	Min. 8000 ppm	Pass
PCBs, PAHs, Dioxins & Furans, Heavy Metals, Microbial and Pesticides*		
PCBs (IUPAC no. 28,52,101,118,138,153,180 Total)	< 0.09 ppm	Compliant
Total PCB (Canada Only)	Max. 0.1 ppm	Compliant
Benzo(a)pyrene	Max. 2.0 ppb	Compliant
Dioxins & Furans (PCDDs and PCDFs)	Max. 2 pg WHO-PCDD/FTEQ/g	Compliant
Dioxin-Like PCBs	Report Actual	Compliant
Arsenic	< 0.1 ppm	Compliant
Cadmium	< 0.1 ppm	Compliant
Mercury	Max.0.01 ppm	Compliant
Lead	< 0.1 ppm	Compliant
Strontium (Canada Only)	Max. 0.5ppm	Compliant
Standard Aerobic Plate Count	< 100 CFU/g	Compliant
Enterbacteriaceae	< 100 CFU/g	Compliant
E. Coli	Not detected in 1 g	Compliant

(b) (6)

Quality Control Manager (or delegate)

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Manufacture Date: May. 28/2008
TDS#: (b) (6)

PCBs, PAHs, Dioxins & Furans, Heavy Metals, Microbial and Pesticides Continued**

Salmonella spp.	Not detected in 10 g	Compliant
Yeast and Mold	< 100 CFU/g	Compliant
DDT	< 0.05 ppm	Compliant
DDE	< 0.05 ppm	Compliant
HCB	< 0.05 ppm	Compliant
Lindane	< 0.05 ppm	Compliant

*Results for contaminants may be expressed as either compliant/non compliant (based on Master Batch Testing) or as actual results.

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Bulk Oil
Certificate of Analysis

Product Name: DHA Fish Oil (non GMO)
O.N.C. Lot #: (b) (6)
Product/Document Code #: XOTDHA-NG.03/111000
Manufacture Date: Sept. 17/2008
Expiry Date: Sept. /2009

ANALYSIS	SPECIFICATIONS	RESULTS
Sensory Panel Evaluation	Fail at 2.0	1.5
Free Fatty Acid (as % Oleic)	Max. 0.5%	0.1
Acid Value	Max. 1.0 mg of KOH/g	0.3
p-Anisidine Value	Max. 20	9
Peroxide Value	Max. 1.0 meq/kg	0.0
%Moisture	Max 0.1%	0.0
Colour	Max. 7	6
Appearance	Clear yellow-amber oil, characteristic of fish oil, with minimum sediment at room temperature	Pass
Totox Number	Max. 22	9
Unsaponifiable Matter	Report Actual	1.49
Conjugated Dienes	Report Actual	1.5
Cold Test	Report Actual	Fail
Density	Report Actual	0.9264
Fatty Acid Profile		
EPA (%)	5 - 8%	8
DHA (%)	25 - 30%	26
EPA mg/g (expressed as TG)	Min. 45 mg/g	70
DHA mg/g (expressed as TG)	Min. 220 mg/g	230
EPA mg/g (expressed as FFA)	Min. 40 mg/g	70
DHA mg/g (expressed as FFA)	Min. 210 mg/g	220
Total Omega 3 %	Min. 32 - 40%	38
Total Omega 3 (mg/g as TG)	Min. 280 mg/g	330
Antioxidants		
Non-GMO Antioxidant blend	Min. 8 mg	Pass
Contaminant Data*		
Dioxins and Furans: PCDDs & PCDFs	Max. 1.5 pg WHO-PCDD/F-TEQ/g	0.3
Dioxin Like PCBs**	Max. 3 pg WHO-Dioxin-Like PCBs-TEQ/g	0.05
Sum [Dioxins & Furans + Dioxin Like PCBs]	Max. 4.5 ppm	0.35
PCBs***	< 0.09 ppm	0.0008
Total PCBs (Canada Only)	Max. 0.1 ppm	0.001
PAHs: Benzo(a)pyrene	Max. 2.0 ppb	<0.1
Arsenic	< 0.1 ppm	<0.01
Lead	< 0.1 ppm	<0.05
Cadmium	< 0.1 ppm	<0.01
Mercury	Max.0.01 ppm	<0.005
Strontium (Canada Only)	Max. 0.5ppm	<0.2
Microbiological		
Standard Aerobic Plate Count	< 100 CFU/g	Compliant
Enterbacteriaceae	< 100 CFU/g	Compliant
E. Coli	Not detected in 1 g	Compliant
Salmonella spp.	Not detected in 10 g	Compliant
Yeast and Mold	< 100 CFU/g	Compliant

(b) (6)
Quality Control Manager (or delegate)

Oct 16/08
Date

101 Research Drive, Dartmouth, N.S., Canada, B2Y 4T6
Telephone (902) 480-3200 Fax (902) 480-3199

000097

OCEAN NUTRITION CANADA
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Bulk Oil
Certificate of Analysis

Product Name: DHA Fish Oil (non GMO)
O.N.C. Lot #: (b) (6)
Product/Document Code #: XOTDHA-NG.03/111000
Manufacture Date: Sept. 17/2008
Expiry Date: Sept. /2009

Pesticides		
DDT	< 0.05 ppm	<0.005
DDE	< 0.05 ppm	<0.005
HCB	< 0.05 ppm	<0.001
Lindane	< 0.05 ppm	<0.001

*Results for contaminants may be expressed as either compliant/non compliant (based on Master Batch Testing) or as actual results.
**Sum of IUPAC No. 81, 77, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189
***Sum of IUPAC No. 28, 52, 101, 118, 138, 153, 180

(b) (6)
Quality Control Manager (or delegate)

Oct 16/08
Date

101 Research Drive, Dartmouth, N.S., Canada, B2Y 4T6
Telephone (902) 480-3200 Fax (902) 480-3199

000098

OCEAN NUTRITION CANADA
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Bulk Oil
Certificate of Analysis

Product Name: DHA Fish Oil (non GMO)
O.N.C. Lot #: (b) (6)
Product/Document Code #: XOTDHA-NG.03/111000
Manufacture Date: Sept. 17/2008
Expiry Date: Sept. /2009

ANALYSIS	SPECIFICATIONS	RESULTS
Sensory Panel Evaluation	Fail at 2.0	1.5
Free Fatty Acid (as % Oleic)	Max. 0.5%	0.2
Acid Value	Max. 1.0 mg of KOH/g	0.4
p-Anisidine Value	Max. 20	11
Peroxide Value	Max. 1.0 meq/kg	0.0
%Moisture	Max 0.1%	0.0
Colour	Max. 7	6
Appearance	Clear yellow-amber oil, characteristic of fish oil, with minimum sediment at room temperature	Pass
Totox Number	Max. 22	11
Unsaponifiable Matter	Report Actual	1.60
Conjugated Dienes	Report Actual	1.56
Cold Test	Report Actual	Fail
Density	Report Actual	0.9302
Fatty Acid Profile		
EPA (%)	5 - 8%	8
DHA (%)	25 - 30%	28
EPA mg/g (expressed as TG)	Min. 45 mg/g	70
DHA mg/g (expressed as TG)	Min. 220 mg/g	240
EPA mg/g (expressed as FFA)	Min. 40 mg/g	60
DHA mg/g (expressed as FFA)	Min. 210 mg/g	230
Total Omega 3 %	Min. 32 - 40%	36
Total Omega 3 (mg/g as TG)	Min. 280 mg/g	330
Antioxidants		
Non-GMO Antioxidant blend	Min. 8 mg	Pass
Contaminant Data*		
Dioxins and Furans: PCDDs & PCDFs	Max. 1.5 pg WHO-PCDD/F-TEQ/g	0.3
Dioxin Like PCBs**	Max. 3 pg WHO-Dioxin-Like PCBs-TEQ/g	0.06
Sum [Dioxins & Furans + Dioxin Like PCBs]	Max. 4.5 ppm	0.36
PCBs***	< 0.09 ppm	0.0008
Total PCBs (Canada Only)	Max. 0.1 ppm	0.001
PAHs: Benzo(a)pyrene	Max. 2.0 ppb	<0.1
Arsenic	< 0.1 ppm	0.02
Lead	< 0.1 ppm	<0.05
Cadmium	< 0.1 ppm	<0.01
Mercury	Max.0.01 ppm	<0.005
Strontium (Canada Only)	Max. 0.5ppm	<0.2
Microbiological		
Standard Aerobic Plate Count	< 100 CFU/g	Compliant
Enterbacteriaceae	< 100 CFU/g	Compliant
E. Coli	Not detected in 1 g	Compliant
Salmonella spp.	Not detected in 10 g	Compliant
Yeast and Mold	< 100 CFU/g	Compliant

(b) (6)

Quality Control Manager (or delegate)

Oct 16/08
Date

101 Research Drive, Dartmouth, N.S., Canada, B2Y 4T6
Telephone (902) 460-3200 Fax (902) 460-3199

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OCEAN NUTRITION CANADA
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Bulk Oil
Certificate of Analysis

Product Name: DHA Fish Oil (non GMO)
O.N.C. Lot #: (b) (6)
Product/Document Code #: XOTDHA-NG.03/111000
Manufacture Date: Sept. 17/2008
Expiry Date: Sept. /2009

Pesticides		
DDT	< 0.05 ppm	<0.005
DDE	< 0.05 ppm	<0.005
HCB	< 0.05 ppm	<0.001
Lindane	< 0.05 ppm	<0.001

*Results for contaminants may be expressed as either compliant/non compliant (based on Master Batch Testing) or as actual results.
**Sum of IUPAC No. 81, 77, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189
***Sum of IUPAC No. 28, 52, 101, 118, 138, 153, 180

(b) (6)
Quality Control Manager (or delegate)

Oct 16/08
Date

101 Research Drive, Dartmouth, N.S., Canada, B2Y 4T6
Telephone (902) 480-3200 Fax (902) 480-3199

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APPENDIX B

(b) (6)

(b) (6)

APPENDIX B

(b) (6)

Ocean Nutrition Canada
attn. [redacted]
39 England Drive
Mulgrave, NS B0E 2G0
KANADA

Person in charge Dr. N. Lohmann - 707
Client support Dr. N. Lohmann - 707

Report date 28.09.2010

Analytical report: AR-10-JC-073651-01

Sample Code 706-2010-00775009

Reference	Fish Oil XOTDHA-NG
Client Sample Code	19843
Purchase Order Code	P.O.#28436
Number	1
Amount	75 g
Reception temperature	room temperature
Ordered by	[redacted]
Submitted by	[redacted]
Sender	UPS421276210791Z1R56860494372617
Received on	03.09.2010
Packaging	glass with screw closure
Start/end of analyses	03.09.2010 / 20.09.2010

TEST RESULTS

Physical-chemical Analysis

CYR21 Polybrominated diphenylethers (PBDEs) (LR)
Method: SOP QMA504-333, LRMS
Analysed by partner laboratory Eurofins GfA Gmbh Hamburg

2,2',4-TriBDE (BDE-17)	< 0.03	*	ng/g
2,4,4'-TriBDE (BDE-28)	< 0.025	*	ng/g
Total TriBDE	ND		ng/g
2,2',4,4'-TetraBDE (BDE-47)	0.078		ng/g
2,2',4,5'-TetraBDE (BDE-49)	< 0.049	*	ng/g
2,3',4,4'-TetraBDE (BDE-66)	< 0.054	*	ng/g
2,3',4',6-TetraBDE (BDE-71)	< 0.054	*	ng/g
3,3',4,4'-TetraBDE (BDE-77)	< 0.048	*	ng/g
Total TetraBDE	0.078		ng/g
2,2',3,4,4'-PentaBDE (BDE-85)	< 0.048	*	ng/g
2,2',4,4',5-PentaBDE (BDE-99)	< 0.048	*	ng/g
2,2',4,4',6-PentaBDE (BDE-100)	< 0.048	*	ng/g
2,3',4,4',6-PentaBDE (BDE-119)	< 0.048	*	ng/g
3,3',4,4',5-PentaBDE (BDE-126)	< 0.048	*	ng/g
Total PentaBDE	ND		ng/g
2,2',3,4,4',5'-HexaBDE (BDE-138)	< 0.077	*	ng/g
2,2',4,4',5,5'-HexaBDE (BDE-153)	< 0.077	*	ng/g
2,2',4,4',5,6'-HexaBDE (BDE-154)	< 0.077	*	ng/g
2,3,3',4,4',5-HexaBDE (BDE-156)	< 0.077	*	ng/g
Total HexaBDE	ND		ng/g

(b) (6)

Analytical report: AR-10-JC-073651-01
Sample Code 706-2010-00775009

2,2',3',4,4',5,6'-HeptaBDE (BDE-183)	< 0.097	*	ng/g
2,2',3,4,4',6,6'-HeptaBDE (BDE-184)	< 0.097	*	ng/g
2,3,3',4,4',5',6-HeptaBDE (BDE-191)	< 0.097	*	ng/g
Total HeptaBDE	ND		ng/g
2,2',3,4,4',5,5',6-OctaBDE (BDE-196)	< 0.242	*	ng/g
2,2',3,3',4,4',6,6'-OctaBDE (BDE-197)	< 0.242	*	ng/g
Total OctaBDE	ND		ng/g
2,2',3,3',4,4',5,5',6-NonaBDE (BDE-206)	< 0.483	*	ng/g
2,2',3,3'4,4',5,6,6'-NonaBDE (BDE-207)	< 0.483	*	ng/g
Total NonaBDE	ND		ng/g
DecaBDE (BDE-209)	< 1.93	*	ng/g

* = Below indicated quantification level

Signature

Dr. R. Gatermann (General manager)

Dr. K. Hoenicke / Dr. S. Biselli / Dr. C. Schulz (Registered representatives - Prokuristen)

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APPENDIX B

Eurofins WEJ Contaminants GmbH
Neuländer Kamp 1
D-21079 Hamburg
GERMANY

Tel: +49 40 492 94 0
Fax: +49 40 492 94 111

wej-contaminants@eurofins.de
www.eurofins.de

(b) (6)
Ocean Nutrition Canada
attn. [redacted]
39 England Drive
Mulgrave, NS B0E 2G0
KANADA

Person in charge Dr. N. Lohmann - 707
Client support Dr. N. Lohmann - 707

Report date 28.09.2010

Analytical report: AR-10-JC-073653-01

Sample Code (b) (6)

Reference	Fish Oil XOTDHA-NG
Client Sample Code	21653
Purchase Order Code	P.O.#28436
Number	1
Amount	62 g
Reception temperature	room temperature
Ordered by	[redacted]
Submitted by	[redacted]
Sender	UPS421276210791Z1R56860494372617
Received on	03.09.2010
Packaging	glass with screw closure
Start/end of analyses	03.09.2010 / 20.09.2010

TEST RESULTS

Physical-chemical Analysis

CYR21 Polybrominated diphenylethers (PBDEs) (LR)
Method: SOP QMA504-333, LRMS
Analysed by partner laboratory Eurofins GfA Gmbh Hamburg

2,2',4-TriBDE (BDE-17)	< 0.03	*	ng/g
2,4,4'-TriBDE (BDE-28)	< 0.025	*	ng/g
Total TriBDE	ND		ng/g
2,2',4,4'-TetraBDE (BDE-47)	0.115		ng/g
2,2',4,5'-TetraBDE (BDE-49)	< 0.050	*	ng/g
2,3',4,4'-TetraBDE (BDE-66)	< 0.050	*	ng/g
2,3',4',6-TetraBDE (BDE-71)	< 0.050	*	ng/g
3,3',4,4'-TetraBDE (BDE-77)	< 0.050	*	ng/g
Total TetraBDE	0.115		ng/g
2,2',3,4,4'-PentaBDE (BDE-85)	< 0.054	*	ng/g
2,2',4,4',5-PentaBDE (BDE-99)	0.055		ng/g
2,2',4,4',6-PentaBDE (BDE-100)	< 0.050	*	ng/g
2,3',4,4',6-PentaBDE (BDE-119)	< 0.054	*	ng/g
3,3',4,4',5-PentaBDE (BDE-126)	< 0.050	*	ng/g
Total PentaBDE	0.055		ng/g
2,2',3,4,4',5'-HexaBDE (BDE-138)	< 0.087	*	ng/g
2,2',4,4',5,5'-HexaBDE (BDE-153)	< 0.091	*	ng/g
2,2',4,4',5,6'-HexaBDE (BDE-154)	< 0.080	*	ng/g
2,3,3',4,4',5-HexaBDE (BDE-156)	< 0.122	*	ng/g
Total HexaBDE	ND		ng/g

2,2',3',4,4',5,6'-HeptaBDE (BDE-183)	< 0.10	*	ng/g
2,2',3,4,4',6,6'-HeptaBDE (BDE-184)	< 0.10	*	ng/g
2,3,3',4,4',5',6-HeptaBDE (BDE-191)	< 0.10	*	ng/g
Total HeptaBDE	ND		ng/g
2,2',3,4,4',5,5',6-OctaBDE (BDE-196)	< 0.249	*	ng/g
2,2',3,3',4,4',6,6'-OctaBDE (BDE-197)	< 0.249	*	ng/g
Total OctaBDE	ND		ng/g
2,2',3,3',4,4',5,5',6-NonaBDE (BDE-206)	< 0.499	*	ng/g
2,2',3,3'4,4',5,6,6'-NonaBDE (BDE-207)	< 0.499	*	ng/g
Total NonaBDE	ND		ng/g
DecaBDE (BDE-209)	< 1.99	*	ng/g

* = Below indicated quantification level

Signature

Dr. R. Gatermann (General manager)

Dr. K. Hoenicke / Dr. S. Biselli / Dr. C. Schulz (Registered representatives - Prokuristen)

(b) (6)

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APPENDIX B

(b) (6)

Ocean Nutrition Canada
attn. [redacted]
39 England Drive
Mulgrave, NS B0E 2G0
KANADA

Person in charge Dr. N. Lohmann - 707
Client support Dr. N. Lohmann - 707

Report date 28.09.2010

Analytical report: AR-10-JC-073652-01

Sample Code 706-2010-00775010

Reference	Fish Oil XOTDHA-NG
Client Sample Code	20954
Purchase Order Code	P.O.#28436
Number	1
Amount	75 g
Reception temperature	room temperature
Ordered by	[redacted]
Submitted by	[redacted]
Sender	UPS421276210791Z1R56860494372617
Received on	03.09.2010
Packaging	glass with screw closure
Start/end of analyses	03.09.2010 / 20.09.2010

TEST RESULTS

Physical-chemical Analysis

CYR21 Polybrominated diphenylethers (PBDEs) (LR)
Method: SOP QMA504-333, LRMS
Analysed by partner laboratory Eurofins GfA Gmbh Hamburg

2,2',4-TriBDE (BDE-17)	< 0.02	*	ng/g
2,4,4'-TriBDE (BDE-28)	< 0.019	*	ng/g
Total TriBDE	ND		ng/g
2,2',4,4'-TetraBDE (BDE-47)	0.083		ng/g
2,2',4,5'-TetraBDE (BDE-49)	< 0.049	*	ng/g
2,3',4,4'-TetraBDE (BDE-66)	< 0.049	*	ng/g
2,3',4',6-TetraBDE (BDE-71)	< 0.049	*	ng/g
3,3',4,4'-TetraBDE (BDE-77)	< 0.049	*	ng/g
Total TetraBDE	0.083		ng/g
2,2',3,4,4'-PentaBDE (BDE-85)	< 0.052	*	ng/g
2,2',4,4',5-PentaBDE (BDE-99)	< 0.049	*	ng/g
2,2',4,4',6-PentaBDE (BDE-100)	< 0.049	*	ng/g
2,3',4,4',6-PentaBDE (BDE-119)	< 0.053	*	ng/g
3,3',4,4',5-PentaBDE (BDE-126)	< 0.049	*	ng/g
Total PentaBDE	ND		ng/g
2,2',3,4,4',5'-HexaBDE (BDE-138)	< 0.080	*	ng/g
2,2',4,4',5,5'-HexaBDE (BDE-153)	< 0.078	*	ng/g
2,2',4,4',5,6'-HexaBDE (BDE-154)	< 0.078	*	ng/g
2,3,3',4,4',5-HexaBDE (BDE-156)	< 0.112	*	ng/g
Total HexaBDE	ND		ng/g

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2,2',3',4,4',5,6'-HeptaBDE (BDE-183)	< 0.097	*	ng/g
2,2',3,4,4',6,6'-HeptaBDE (BDE-184)	< 0.097	*	ng/g
2,3,3',4,4',5',6-HeptaBDE (BDE-191)	< 0.097	*	ng/g
Total HeptaBDE	ND		ng/g
2,2',3,4,4',5,5',6-OctaBDE (BDE-196)	< 0.243	*	ng/g
2,2',3,3',4,4',6,6'-OctaBDE (BDE-197)	< 0.243	*	ng/g
Total OctaBDE	ND		ng/g
2,2',3,3',4,4',5,5',6-NonaBDE (BDE-206)	< 0.487	*	ng/g
2,2',3,3'4,4',5,6,6'-NonaBDE (BDE-207)	< 0.487	*	ng/g
Total NonaBDE	ND		ng/g
DecaBDE (BDE-209)	< 4.07	*	ng/g

* = Below indicated quantification level

Signature

Dr. R. Gatermann (General manager)

Dr. K. Hoenicke / Dr. S. Biselli / Dr. C. Schulz (Registered representatives - Prokuristen)

(b) (6)

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APPENDIX B

(b) (6)

Ocean Nutrition Canada
attn. [redacted]
39 England Drive
Mulgrave, NS B0E 2G0
KANADA

Person in charge Dr. N. Lohmann - 707
Client support Dr. N. Lohmann - 707

Report date 28.09.2010
Page 1/2

Analytical report: AR-10-JC-073654-01

Sample Code (b) (6)

Reference	Fish Oil XOTDHA-NG
Client Sample Code	22310
Purchase Order Code	P.O.#28436
Number	1
Amount	77 g
Reception temperature	room temperature
Ordered by	[redacted]
Submitted by	[redacted]
Sender	UPS421276210791Z1R56860494372617
Received on	03.09.2010
Packaging	glass with screw closure
Start/end of analyses	03.09.2010 / 20.09.2010

TEST RESULTS

Physical-chemical Analysis

CYR21 Polybrominated diphenylethers (PBDEs) (LR)
Method: SOP QMA504-333, LRMS
Analysed by partner laboratory Eurofins GfA Gmbh Hamburg

2,2',4-TriBDE (BDE-17)	< 0.03	*	ng/g
2,4,4'-TriBDE (BDE-28)	< 0.022	*	ng/g
Total TriBDE	ND		ng/g
2,2',4,4'-TetraBDE (BDE-47)	0.079		ng/g
2,2',4,5'-TetraBDE (BDE-49)	< 0.049	*	ng/g
2,3',4,4'-TetraBDE (BDE-66)	< 0.049	*	ng/g
2,3',4',6-TetraBDE (BDE-71)	< 0.049	*	ng/g
3,3',4,4'-TetraBDE (BDE-77)	< 0.049	*	ng/g
Total TetraBDE	0.079		ng/g
2,2',3,4,4'-PentaBDE (BDE-85)	< 0.049	*	ng/g
2,2',4,4',5-PentaBDE (BDE-99)	< 0.049	*	ng/g
2,2',4,4',6-PentaBDE (BDE-100)	< 0.049	*	ng/g
2,3',4,4',6-PentaBDE (BDE-119)	< 0.049	*	ng/g
3,3',4,4',5-PentaBDE (BDE-126)	< 0.049	*	ng/g
Total PentaBDE	ND		ng/g
2,2',3,4,4',5'-HexaBDE (BDE-138)	< 0.078	*	ng/g
2,2',4,4',5,5'-HexaBDE (BDE-153)	< 0.078	*	ng/g
2,2',4,4',5,6'-HexaBDE (BDE-154)	< 0.078	*	ng/g
2,3,3',4,4',5-HexaBDE (BDE-156)	< 0.078	*	ng/g
Total HexaBDE	ND		ng/g

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2,2',3',4,4',5,6'-HeptaBDE (BDE-183)	< 0.097	*	ng/g
2,2',3,4,4',6,6'-HeptaBDE (BDE-184)	< 0.097	*	ng/g
2,3,3',4,4',5',6-HeptaBDE (BDE-191)	< 0.097	*	ng/g
Total HeptaBDE	ND		ng/g
2,2',3,4,4',5,5',6-OctaBDE (BDE-196)	< 0.243	*	ng/g
2,2',3,3',4,4',6,6'-OctaBDE (BDE-197)	< 0.243	*	ng/g
Total OctaBDE	ND		ng/g
2,2',3,3',4,4',5,5',6-NonaBDE (BDE-206)	< 0.486	*	ng/g
2,2',3,3'4,4',5,6,6'-NonaBDE (BDE-207)	< 0.486	*	ng/g
Total NonaBDE	ND		ng/g
DecaBDE (BDE-209)	< 1.94	*	ng/g

* = Below indicated quantification level

Signature

Dr. R. Gatermann (General manager)

Dr. K. Hoenicke / Dr. S. Biselli / Dr. C. Schulz (Registered representatives - Prokuristen)

(b) (6)

APPENDIX C

CHEMICAL LABORATORY „Dr. A. VERWEY"
Analytical Chemists — Assayers & Samplers

32 COOLHAVEN
POSTBOX 6003
3002 AA ROTTERDAM

TELEPHONE: 010 - 476 10 55
E-MAIL: info@drverwey.nl
TELEFAX: 010 - 476 16 42

APPENDIX C

DATE, 12th April, 2010

Ocean Nutrition Canada Limited
39, England Drive
MULGRAVE - NS.B0E 2GO
Canada
Attn. [redacted]

Certificate of Analysis No. 11050625

The analysis of the sample said to be: Fish Oil.

Sample received: February 26th, 2010. Instructions received: March 2nd, 2010.

Packed: Glass (Abt. 25 ml).

Marked: Product name : XOTDHA-NG Fish Oil.
ONC code : XOTDHA-NG.
Lot no. : (b) (6)

Sealed: /.

The sample as detailed has been analysed and showed following results:

Di-isobutyl phthalate	not detectable, less than	0,1 mg/kg
Di-n-butyl phthalate	not detectable, less than	0,1 mg/kg
Di-(2-ethylexyl)-phthalate	not detectable, less than	5,0 mg/kg
Alpha-BHC	not detectable, less than	0,001 mg/kg
PCB 1254 (Arochlor 1254)	not detectable, less than	0,01 mg/kg
Beta-BHC	not detectable, less than	0,001 mg/kg
Chlorothalonil	not detectable, less than	0,005 mg/kg
DCNA	not detectable, less than	0,01 mg/kg
DCPA	not detectable, less than	0,01 mg/kg
Delta-BHC	not detectable, less than	0,001 mg/kg
Dichlorofenthion	not detectable, less than	0,01 mg/kg
Dicofol	not detectable, less than	0,01 mg/kg
EPN	not detectable, less than	0,01 mg/kg
Folpet	not detectable, less than	0,01 mg/kg
Fonofos	not detectable, less than	0,01 mg/kg
Gamma-BHC	not detectable, less than	0,001 mg/kg
Oxadiazon	not detectable, less than	0,01 mg/kg
PCNB	not detectable, less than	0,005 mg/kg

Phosalone ..... not detectable, less than 0,05 mg/kg
Phosmet ..... not detectable, less than 0,05 mg/kg
Propetamphos ..... not detectable, less than 0,05 mg/kg
Propyzamide ..... not detectable, less than 0,05 mg/kg
Prothiophos ..... not detectable, less than 0,05 mg/kg
Ronnel ..... not detectable, less than 0,005 mg/kg
Technical chlordane ..... not detectable, less than 0,005 mg/kg
Tecnazene ..... not detectable, less than 0,001 mg/kg
Tetradifon ..... not detectable, less than 0,05 mg/kg
Thimet ..... not detectable, less than 0,005 mg/kg
Trithion ..... not detectable, less than 0,05 mg/kg
Vapona ..... not detectable, less than 0,01 mg/kg

Pesticides - List C :

Azinphos-methyl ..... not detectable (less than 0,01 mg/kg)
Bromophos-ethyl ..... not detectable (less than 0,01 mg/kg)
Bromophos-methyl ..... not detectable (less than 0,01 mg/kg)
Chlorfenvinphos ..... not detectable (less than 0,01 mg/kg)
Chlorpyriphos ..... not detectable (less than 0,01 mg/kg)
Coumaphos ..... not detectable (less than 0,01 mg/kg)
Demeton-S ..... not detectable (less than 0,01 mg/kg)
Diazinon ..... not detectable (less than 0,01 mg/kg)
Dibrom ..... not detectable (less than 0,01 mg/kg)
Dichlorvos ..... not detectable (less than 0,01 mg/kg)
Disulfoton ..... not detectable (less than 0,01 mg/kg)
Ethion ..... not detectable (less than 0,01 mg/kg)
Fenchlorphos ..... not detectable (less than 0,01 mg/kg)
Fenitrothion ..... not detectable (less than 0,01 mg/kg)
Fensulphothion ..... not detectable (less than 0,01 mg/kg)
Fenthion ..... not detectable (less than 0,005 mg/kg)
Malathion ..... not detectable (less than 0,01 mg/kg)
Methidathion ..... not detectable (less than 0,01 mg/kg)
Mevinphos ..... not detectable (less than 0,01 mg/kg)
Naled ..... not detectable (less than 0,01 mg/kg)
Parathion-ethyl ..... not detectable (less than 0,01 mg/kg)
Parathion-methyl ..... not detectable (less than 0,005 mg/kg)
Phosphamidon ..... not detectable (less than 0,01 mg/kg)
Phorate ..... not detectable (less than 0,01 mg/kg)
Pirimiphos-ethyl ..... not detectable (less than 0,01 mg/kg)
Pirimiphos-methyl ..... not detectable (less than 0,01 mg/kg)
Prophos ..... not detectable (less than 0,01 mg/kg)
Sulfotep ..... not detectable (less than 0,002 mg/kg)
Tetrachlorvinphos ..... not detectable (less than 0,01 mg/kg)
Tokuthion ..... not detectable (less than 0,01 mg/kg)
Tributyl phosphorotrioite .... not detectable (less than 0,01 mg/kg)
Trichloronat ..... not detectable (less than 0,01 mg/kg)
Trichlorphon ..... not detectable (less than 0,01 mg/kg)
Dichlorbenil ..... not detectable (less than 0,05 mg/kg)
Diclofop-methyl ..... not detectable (less than 0,05 mg/kg)
Captafol ..... not detectable (less than 0,05 mg/kg)
Captan ..... not detectable (less than 0,05 mg/kg)
Procymidon ..... not detectable (less than 0,05 mg/kg)
Vinclozolin ..... not detectable (less than 0,05 mg/kg)
Propoxur ..... not detectable (less than 0,05 mg/kg)
Amitraz ..... not detectable (less than 0,05 mg/kg)

Aldrin	not detectable (less than 0,005 mg/kg)
Chlordane	not detectable (less than 0,005 mg/kg)
Dieldrin	not detectable (less than 0,01 mg/kg)
Endosulfan 1	not detectable (less than 0,01 mg/kg)
Endosulfan 2	not detectable (less than 0,01 mg/kg)
Endosulfan sulphate	not detectable (less than 0,005 mg/kg)
Endrin	not detectable (less than 0,01 mg/kg)
Endrin aldehyde	not detectable (less than 0,01 mg/kg)
PCB	not detectable (less than 0,01 mg/kg)
HCH alpha	not detectable (less than 0,001 mg/kg)
HCH beta	not detectable (less than 0,001 mg/kg)
HCH delta	not detectable (less than 0,001 mg/kg)
HCH gamma (lindane)	not detectable (less than 0,001 mg/kg)
Heptachlor	not detectable (less than 0,005 mg/kg)
Heptachlorepoxide	not detectable (less than 0,005 mg/kg)
Methoxychlor	not detectable (less than 0,01 mg/kg)
op DDD	not detectable (less than 0,005 mg/kg)
op DDE	not detectable (less than 0,005 mg/kg)
op DDT	not detectable (less than 0,005 mg/kg)
pp DDD	not detectable (less than 0,005 mg/kg)
pp DDE	not detectable (less than 0,005 mg/kg)
pp DDT	not detectable (less than 0,005 mg/kg)
Toxaphene	not detectable (less than 0,01 mg/kg)
Mirex	not detectable (less than 0,01 mg/kg)

Chemical Laboratory
"Dr.A.Verwey"

R. Mostert
Chief Chemist

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Analytical Chemists — Assayers & Samplers

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APPENDIX C

DATE, 12th April, 2010

Ocean Nutrition Canada Limited
39, England Drive
MULGRAVE - NS.B0E 2GO
Canada
Attn. [redacted]

Certificate of Analysis No. 11050624

The analysis of the sample said to be: Fish Oil.

Sample received: February 26th, 2010. Instructions received: March 2nd, 2010.

Packed: Glass (Abt. 25 ml).

Marked: Product name : XOTDHA-NG Fish Oil.
ONC code : XOTDHA-NG.
Lot no. : (b) (6).

Sealed: /.

The sample as detailed has been analysed and showed following results:

Di-isobutyl phthalate	not detectable, less than 0,1 mg/kg
Di-n-butyl phthalate	not detectable, less than 0,1 mg/kg
Di-(2-ethylexyl)-phthalate	not detectable, less than 5,0 mg/kg
Alpha-BHC	not detectable, less than 0,001 mg/kg
PCB 1254 (Arochlor 1254)	not detectable, less than 0,01 mg/kg
Beta-BHC	not detectable, less than 0,001 mg/kg
Chlorothalonil	not detectable, less than 0,005 mg/kg
DCNA	not detectable, less than 0,01 mg/kg
DCPA	not detectable, less than 0,01 mg/kg
Delta-BHC	not detectable, less than 0,001 mg/kg
Dichlorofenthion	not detectable, less than 0,01 mg/kg
Dicofol	not detectable, less than 0,01 mg/kg
EPN	not detectable, less than 0,01 mg/kg
Folpet	not detectable, less than 0,01 mg/kg
Fonofos	not detectable, less than 0,01 mg/kg
Gamma-BHC	not detectable, less than 0,001 mg/kg
Oxadiazon	not detectable, less than 0,01 mg/kg
PCNB	not detectable, less than 0,005 mg/kg

Phosalone	not detectable, less than 0,05 mg/kg
Phosmet	not detectable, less than 0,05 mg/kg
Propetamphos	not detectable, less than 0,05 mg/kg
Propyzamide	not detectable, less than 0,05 mg/kg
Prothiophos	not detectable, less than 0,05 mg/kg
Ronnel	not detectable, less than 0,005 mg/kg
Technical chlordane	not detectable, less than 0,005 mg/kg
Tecnazene	not detectable, less than 0,001 mg/kg
Tetradifon	not detectable, less than 0,05 mg/kg
Thimet	not detectable, less than 0,005 mg/kg
Trithion	not detectable, less than 0,05 mg/kg
Vapona	not detectable, less than 0,01 mg/kg

Pesticides - List C :

Azinphos-methyl	not detectable (less than 0,01 mg/kg)
Bromophos-ethyl	not detectable (less than 0,01 mg/kg)
Bromophos-methyl	not detectable (less than 0,01 mg/kg)
Chlorfenvinphos	not detectable (less than 0,01 mg/kg)
Chlorpyriphos	not detectable (less than 0,01 mg/kg)
Coumaphos	not detectable (less than 0,01 mg/kg)
Demeton-S	not detectable (less than 0,01 mg/kg)
Diazinon	not detectable (less than 0,01 mg/kg)
Dibrom	not detectable (less than 0,01 mg/kg)
Dichlorvos	not detectable (less than 0,01 mg/kg)
Disulfoton	not detectable (less than 0,01 mg/kg)
Ethion	not detectable (less than 0,01 mg/kg)
Fenchlorphos	not detectable (less than 0,01 mg/kg)
Fenitrothion	not detectable (less than 0,01 mg/kg)
Fensulphothion	not detectable (less than 0,01 mg/kg)
Fenthion	not detectable (less than 0,005 mg/kg)
Malathion	not detectable (less than 0,01 mg/kg)
Methidathion	not detectable (less than 0,01 mg/kg)
Mevinphos	not detectable (less than 0,01 mg/kg)
Naled	not detectable (less than 0,01 mg/kg)
Parathion-ethyl	not detectable (less than 0,01 mg/kg)
Parathion-methyl	not detectable (less than 0,005 mg/kg)
Phosphamidon	not detectable (less than 0,01 mg/kg)
Phorate	not detectable (less than 0,01 mg/kg)
Pirimiphos-ethyl	not detectable (less than 0,01 mg/kg)
Pirimiphos-methyl	not detectable (less than 0,01 mg/kg)
Prophos	not detectable (less than 0,01 mg/kg)
Sulfotep	not detectable (less than 0,002 mg/kg)
Tetrachlorvinphos	not detectable (less than 0,01 mg/kg)
Tokuthion	not detectable (less than 0,01 mg/kg)
Tributyl phosphorotrioite	not detectable (less than 0,01 mg/kg)
Trichloronat	not detectable (less than 0,01 mg/kg)
Trichlorphon	not detectable (less than 0,01 mg/kg)
Dichlorbenil	not detectable (less than 0,05 mg/kg)
Diclofop-methyl	not detectable (less than 0,05 mg/kg)
Captafol	not detectable (less than 0,05 mg/kg)
Captan	not detectable (less than 0,05 mg/kg)
Procymidon	not detectable (less than 0,05 mg/kg)
Vinclozolin	not detectable (less than 0,05 mg/kg)
Propoxur	not detectable (less than 0,05 mg/kg)
Amitraz	not detectable (less than 0,05 mg/kg)
Aldrin	not detectable (less than 0,005 mg/kg)

Chlordane	not detectable (less than 0,005 mg/kg)
Dieldrin	not detectable (less than 0,01 mg/kg)
Endosulfan 1	not detectable (less than 0,01 mg/kg)
Endosulfan 2	not detectable (less than 0,01 mg/kg)
Endosulfan sulphate	not detectable (less than 0,005 mg/kg)
Endrin	not detectable (less than 0,01 mg/kg)
Endrin aldehyde	not detectable (less than 0,01 mg/kg)
PCB	not detectable (less than 0,01 mg/kg)
HCH alpha	not detectable (less than 0,001 mg/kg)
HCH beta	not detectable (less than 0,001 mg/kg)
HCH delta	not detectable (less than 0,001 mg/kg)
HCH gamma (lindane)	not detectable (less than 0,001 mg/kg)
Heptachlor	not detectable (less than 0,005 mg/kg)
Heptachlorepoxide	not detectable (less than 0,005 mg/kg)
Methoxychlor	not detectable (less than 0,01 mg/kg)
op DDD	not detectable (less than 0,005 mg/kg)
op DDE	not detectable (less than 0,005 mg/kg)
op DDT	not detectable (less than 0,005 mg/kg)
pp DDD	not detectable (less than 0,005 mg/kg)
pp DDE	not detectable (less than 0,005 mg/kg)
pp DDT	not detectable (less than 0,005 mg/kg)
Toxaphene	not detectable (less than 0,01 mg/kg)
Mirex	not detectable (less than 0,01 mg/kg)

Chemical Laboratory
"Dr.A.Verwey"

R. Mostert
Chief Chemist

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APPENDIX C

DATE, 12th April, 2010

Ocean Nutrition Canada Limited
39, England Drive
MULGRAVE - NS.B0E 2GO
Canada
Attn. [redacted]

Certificate of Analysis No. 11050623

The analysis of the sample said to be: Fish Oil.

Sample received: February 26th, 2010. Instructions received: March 2nd, 2010.

Packed: Glass (Abt. 25 ml).

Marked:
Product name : XOTDHA-NG Fish Oil.
ONC code : XOTDHA-NG.
Lot no. : 20954.

Sealed: /.

The sample as detailed has been analysed and showed following results:

Di-isobutyl phthalate	not detectable, less than 0,1 mg/kg
Di-n-butyl phthalate	not detectable, less than 0,1 mg/kg
Di-(2-ethylexyl)-phthalate	not detectable, less than 5,0 mg/kg
Alpha-BHC	not detectable, less than 0,001 mg/kg
PCB 1254 (Arochlor 1254)	not detectable, less than 0,01 mg/kg
Beta-BHC	not detectable, less than 0,001 mg/kg
Chlorothalonil	not detectable, less than 0,005 mg/kg
DCNA	not detectable, less than 0,01 mg/kg
DCPA	not detectable, less than 0,01 mg/kg
Delta-BHC	not detectable, less than 0,001 mg/kg
Dichlorofenthion	not detectable, less than 0,01 mg/kg
Dicofol	not detectable, less than 0,01 mg/kg
EPN	not detectable, less than 0,01 mg/kg
Folpet	not detectable, less than 0,01 mg/kg
Fonofos	not detectable, less than 0,01 mg/kg
Gamma-BHC	not detectable, less than 0,001 mg/kg
Oxadiazon	not detectable, less than 0,01 mg/kg
PCNB	not detectable, less than 0,005 mg/kg

000117

Phosalone	not detectable, less than 0,05 mg/kg
Phosmet	not detectable, less than 0,05 mg/kg
Propetamphos	not detectable, less than 0,05 mg/kg
Propyzamide	not detectable, less than 0,05 mg/kg
Prothiophos	not detectable, less than 0,05 mg/kg
Ronnel	not detectable, less than 0,005 mg/kg
Technical chlordane	not detectable, less than 0,005 mg/kg
Tecnazene	not detectable, less than 0,001 mg/kg
Tetradifon	not detectable, less than 0,05 mg/kg
Thimet	not detectable, less than 0,005 mg/kg
Trithion	not detectable, less than 0,05 mg/kg
Vapona	not detectable, less than 0,01 mg/kg

Pesticides - List C :

Azinphos-methyl	not detectable (less than 0,01 mg/kg)
Bromophos-ethyl	not detectable (less than 0,01 mg/kg)
Bromophos-methyl	not detectable (less than 0,01 mg/kg)
Chlorfenvinphos	not detectable (less than 0,01 mg/kg)
Chlorpyriphos	not detectable (less than 0,01 mg/kg)
Coumaphos	not detectable (less than 0,01 mg/kg)
Demeton-S	not detectable (less than 0,01 mg/kg)
Diazinon	not detectable (less than 0,01 mg/kg)
Dibrom	not detectable (less than 0,01 mg/kg)
Dichlorvos	not detectable (less than 0,01 mg/kg)
Disulfoton	not detectable (less than 0,01 mg/kg)
Ethion	not detectable (less than 0,01 mg/kg)
Fenchlorphos	not detectable (less than 0,01 mg/kg)
Fenitrothion	not detectable (less than 0,01 mg/kg)
Fensulphothion	not detectable (less than 0,01 mg/kg)
Fenthion	not detectable (less than 0,005 mg/kg)
Malathion	not detectable (less than 0,01 mg/kg)
Methidathion	not detectable (less than 0,01 mg/kg)
Mevinphos	not detectable (less than 0,01 mg/kg)
Naled	not detectable (less than 0,01 mg/kg)
Parathion-ethyl	not detectable (less than 0,01 mg/kg)
Parathion-methyl	not detectable (less than 0,005 mg/kg)
Phosphamidon	not detectable (less than 0,01 mg/kg)
Phorate	not detectable (less than 0,01 mg/kg)
Pirimiphos-ethyl	not detectable (less than 0,01 mg/kg)
Pirimiphos-methyl	not detectable (less than 0,01 mg/kg)
Prophos	not detectable (less than 0,01 mg/kg)
Sulfotep	not detectable (less than 0,002 mg/kg)
Tetrachlorvinphos	not detectable (less than 0,01 mg/kg)
Tokuthion	not detectable (less than 0,01 mg/kg)
Tributyl phosphorotrioite	not detectable (less than 0,01 mg/kg)
Trichloronat	not detectable (less than 0,01 mg/kg)
Trichlorphon	not detectable (less than 0,01 mg/kg)
Dichlorbenil	not detectable (less than 0,05 mg/kg)
Diclofop-methyl	not detectable (less than 0,05 mg/kg)
Captafol	not detectable (less than 0,05 mg/kg)
Captan	not detectable (less than 0,05 mg/kg)
Procymidon	not detectable (less than 0,05 mg/kg)
Vinclozolin	not detectable (less than 0,05 mg/kg)
Propoxur	not detectable (less than 0,05 mg/kg)
Amitraz	not detectable (less than 0,05 mg/kg)

Aldrin	not detectable (less than 0,005 mg/kg)
Chlordane	not detectable (less than 0,005 mg/kg)
Dieldrin	not detectable (less than 0,01 mg/kg)
Endosulfan 1	not detectable (less than 0,01 mg/kg)
Endosulfan 2	not detectable (less than 0,01 mg/kg)
Endosulfan sulphate	not detectable (less than 0,005 mg/kg)
Endrin	not detectable (less than 0,01 mg/kg)
Endrin aldehyde	not detectable (less than 0,01 mg/kg)
PCB	not detectable (less than 0,01 mg/kg)
HCH alpha	not detectable (less than 0,001 mg/kg)
HCH beta	not detectable (less than 0,001 mg/kg)
HCH delta	not detectable (less than 0,001 mg/kg)
HCH gamma (lindane)	not detectable (less than 0,001 mg/kg)
Heptachlor	not detectable (less than 0,005 mg/kg)
Heptachlorepoxide	not detectable (less than 0,005 mg/kg)
Methoxychlor	not detectable (less than 0,01 mg/kg)
op DDD	not detectable (less than 0,005 mg/kg)
op DDE	not detectable (less than 0,005 mg/kg)
op DDT	not detectable (less than 0,005 mg/kg)
pp DDD	not detectable (less than 0,005 mg/kg)
pp DDE	not detectable (less than 0,005 mg/kg)
pp DDT	not detectable (less than 0,005 mg/kg)
Toxaphene	not detectable (less than 0,01 mg/kg)
Mirex	not detectable (less than 0,01 mg/kg)

Chemical Laboratory
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R. Mostert
Chief Chemist

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APPENDIX C

DATE. 12th April, 2010

Ocean Nutrition Canada Limited
39, England Drive
MULGRAVE - NS.B0E 2GO
Canada
Attn. [redacted]

Certificate of Analysis No. 11050622

The analysis of the sample said to be: Fish Oil.

Sample received: February 26th, 2010. Instructions received: March 2nd, 2010.

Packed: Glass (Abt. 25 ml).

Marked: Product name : XOTDHA-NG Fish Oil.
ONC code : XOTDHA-NG.
Lot no. : [redacted] (b) (6).

Sealed: /.

The sample as detailed has been analysed and showed following results:

Di-isobutyl phthalate	not detectable, less than	0,1 mg/kg
Di-n-butyl phthalate	not detectable, less than	0,1 mg/kg
Di-(2-ethylexyl)-phthalate	not detectable, less than	5,0 mg/kg
Alpha-BHC	not detectable, less than	0,001 mg/kg
PCB 1254 (Arochlor 1254)	not detectable, less than	0,01 mg/kg
Beta-BHC	not detectable, less than	0,001 mg/kg
Chlorothalonil	not detectable, less than	0,005 mg/kg
DCNA	not detectable, less than	0,01 mg/kg
DCPA	not detectable, less than	0,01 mg/kg
Delta-BHC	not detectable, less than	0,001 mg/kg
Dichlorofenthion	not detectable, less than	0,01 mg/kg
Dicofol	not detectable, less than	0,01 mg/kg
EPN	not detectable, less than	0,01 mg/kg
Folpet	not detectable, less than	0,01 mg/kg
Fonofos	not detectable, less than	0,01 mg/kg
Gamma-BHC	not detectable, less than	0,001 mg/kg
Oxadiazon	not detectable, less than	0,01 mg/kg
PCNB	not detectable, less than	0,005 mg/kg

000120

Phosalone	not detectable, less than 0,05 mg/kg
Phosmet	not detectable, less than 0,05 mg/kg
Propetamphos	not detectable, less than 0,05 mg/kg
Propyzamide	not detectable, less than 0,05 mg/kg
Prothiophos	not detectable, less than 0,05 mg/kg
Ronnel	not detectable, less than 0,005 mg/kg
Technical chlordane	not detectable, less than 0,005 mg/kg
Tecnazene	not detectable, less than 0,001 mg/kg
Tetradifon	not detectable, less than 0,05 mg/kg
Thimet	not detectable, less than 0,005 mg/kg
Trithion	not detectable, less than 0,05 mg/kg
Vapona	not detectable, less than 0,01 mg/kg

Pesticides - List C :

Azinphos-methyl	not detectable (less than 0,01 mg/kg)
Bromophos-ethyl	not detectable (less than 0,01 mg/kg)
Bromophos-methyl	not detectable (less than 0,01 mg/kg)
Chlorfenvinphos	not detectable (less than 0,01 mg/kg)
Chlorpyriphos	not detectable (less than 0,01 mg/kg)
Coumaphos	not detectable (less than 0,01 mg/kg)
Demeton-S	not detectable (less than 0,01 mg/kg)
Diazinon	not detectable (less than 0,01 mg/kg)
Dibrom	not detectable (less than 0,01 mg/kg)
Dichlorvos	not detectable (less than 0,01 mg/kg)
Disulfoton	not detectable (less than 0,01 mg/kg)
Ethion	not detectable (less than 0,01 mg/kg)
Fenchlorphos	not detectable (less than 0,01 mg/kg)
Fenitrothion	not detectable (less than 0,01 mg/kg)
Fensulphothion	not detectable (less than 0,01 mg/kg)
Fenthion	not detectable (less than 0,005 mg/kg)
Malathion	not detectable (less than 0,01 mg/kg)
Methidathion	not detectable (less than 0,01 mg/kg)
Mevinphos	not detectable (less than 0,01 mg/kg)
Naled	not detectable (less than 0,01 mg/kg)
Parathion-ethyl	not detectable (less than 0,01 mg/kg)
Parathion-methyl	not detectable (less than 0,005 mg/kg)
Phosphamidon	not detectable (less than 0,01 mg/kg)
Phorate	not detectable (less than 0,01 mg/kg)
Pirimiphos-ethyl	not detectable (less than 0,01 mg/kg)
Pirimiphos-methyl	not detectable (less than 0,01 mg/kg)
Prophos	not detectable (less than 0,01 mg/kg)
Sulfotep	not detectable (less than 0,002 mg/kg)
Tetrachlorvinphos	not detectable (less than 0,01 mg/kg)
Tokuthion	not detectable (less than 0,01 mg/kg)
Tributyl phosphorotrioite	not detectable (less than 0,01 mg/kg)
Trichloronat	not detectable (less than 0,01 mg/kg)
Trichlorphon	not detectable (less than 0,01 mg/kg)
Dichlorbenil	not detectable (less than 0,05 mg/kg)
Diclofop-methyl	not detectable (less than 0,05 mg/kg)
Captafol	not detectable (less than 0,05 mg/kg)
Captan	not detectable (less than 0,05 mg/kg)
Procymidon	not detectable (less than 0,05 mg/kg)
Vinclozolin	not detectable (less than 0,05 mg/kg)
Propoxur	not detectable (less than 0,05 mg/kg)
Amitraz	not detectable (less than 0,05 mg/kg)

Aldrin	not detectable (less than 0,005 mg/kg)
Chlordane	not detectable (less than 0,005 mg/kg)
Dieldrin	not detectable (less than 0,01 mg/kg)
Endosulfan 1	not detectable (less than 0,01 mg/kg)
Endosulfan 2	not detectable (less than 0,01 mg/kg)
Endosulfan sulphate	not detectable (less than 0,005 mg/kg)
Endrin	not detectable (less than 0,01 mg/kg)
Endrin aldehyde	not detectable (less than 0,01 mg/kg)
PCB	not detectable (less than 0,01 mg/kg)
HCH alpha	not detectable (less than 0,001 mg/kg)
HCH beta	not detectable (less than 0,001 mg/kg)
HCH delta	not detectable (less than 0,001 mg/kg)
HCH gamma (lindane)	not detectable (less than 0,001 mg/kg)
Heptachlor	not detectable (less than 0,005 mg/kg)
Heptachlorepoxide	not detectable (less than 0,005 mg/kg)
Methoxychlor	not detectable (less than 0,01 mg/kg)
op DDD	not detectable (less than 0,005 mg/kg)
op DDE	not detectable (less than 0,005 mg/kg)
op DDT	not detectable (less than 0,005 mg/kg)
pp DDD	not detectable (less than 0,005 mg/kg)
pp DDE	not detectable (less than 0,005 mg/kg)
pp DDT	not detectable (less than 0,005 mg/kg)
Toxaphene	not detectable (less than 0,01 mg/kg)
Mirex	not detectable (less than 0,01 mg/kg)

Chemical Laboratory
"Dr.A.Verwey"

R. Mostert
Chief Chemist

CHEMICAL LABORATORY „Dr. A. VERWEY"
Analytical Chemists — Assayers & Samplers

32 COOLHAVEN
POSTBOX 6003
3002 AA ROTTERDAM

TELEPHONE: 010 - 476 10 55
E-MAIL: info@drverwey.nl
TELEFAX: 010 - 476 16 42

APPENDIX C

DATE, 12th April, 2010

Ocean Nutrition Canada Limited
39, England Drive
MULGRAVE - NS.B0E 2GO
Canada
Attn. [redacted]

Certificate of Analysis No. 11050626

The analysis of the sample said to be: Fish Oil.

Sample received: February 26th, 2010. Instructions received: March 2nd, 2010.

Packed: Glass (Abt. 25 ml).

Marked: Product name : XOTDHA-NG Fish Oil.
ONC code : XOTDHA-NG.
Lot no. : (b) (6).

Sealed: /.

The sample as detailed has been analysed and showed following results:

Di-isobutyl phthalate	not detectable, less than 0,1 mg/kg
Di-n-butyl phthalate	not detectable, less than 0,1 mg/kg
Di-(2-ethylexyl)-phthalate	not detectable, less than 5,0 mg/kg
Alpha-BHC	not detectable, less than 0,001 mg/kg
PCB 1254 (Arochlor 1254)	not detectable, less than 0,01 mg/kg
Beta-BHC	not detectable, less than 0,001 mg/kg
Chlorothalonil	not detectable, less than 0,005 mg/kg
DCNA	not detectable, less than 0,01 mg/kg
DCPA	not detectable, less than 0,01 mg/kg
Delta-BHC	not detectable, less than 0,001 mg/kg
Dichlorofenthion	not detectable, less than 0,01 mg/kg
Dicofol	not detectable, less than 0,01 mg/kg
EPN	not detectable, less than 0,01 mg/kg
Folpet	not detectable, less than 0,01 mg/kg
Fonofos	not detectable, less than 0,01 mg/kg
Gamma-BHC	not detectable, less than 0,001 mg/kg
Oxadiazon	not detectable, less than 0,01 mg/kg
PCNB	not detectable, less than 0,005 mg/kg

000123

Phosalone	not detectable, less than 0,05 mg/kg
Phosmet	not detectable, less than 0,05 mg/kg
Propetamphos	not detectable, less than 0,05 mg/kg
Propyzamide	not detectable, less than 0,05 mg/kg
Prothiophos	not detectable, less than 0,05 mg/kg
Ronnel	not detectable, less than 0,005 mg/kg
Technical chlordane	not detectable, less than 0,005 mg/kg
Tecnazene	not detectable, less than 0,001 mg/kg
Tetradifon	not detectable, less than 0,05 mg/kg
Thimet	not detectable, less than 0,005 mg/kg
Trithion	not detectable, less than 0,05 mg/kg
Vapona	not detectable, less than 0,01 mg/kg

Pesticides - List C :

Azinphos-methyl	not detectable (less than 0,01 mg/kg)
Bromophos-ethyl	not detectable (less than 0,01 mg/kg)
Bromophos-methyl	not detectable (less than 0,01 mg/kg)
Chlorfenvinphos	not detectable (less than 0,01 mg/kg)
Chlorpyriphos	not detectable (less than 0,01 mg/kg)
Coumaphos	not detectable (less than 0,01 mg/kg)
Demeton-S	not detectable (less than 0,01 mg/kg)
Diazinon	not detectable (less than 0,01 mg/kg)
Dibrom	not detectable (less than 0,01 mg/kg)
Dichlorvos	not detectable (less than 0,01 mg/kg)
Disulfoton	not detectable (less than 0,01 mg/kg)
Ethion	not detectable (less than 0,01 mg/kg)
Fenchlorphos	not detectable (less than 0,01 mg/kg)
Fenitrothion	not detectable (less than 0,01 mg/kg)
Fensulphothion	not detectable (less than 0,01 mg/kg)
Fenthion	not detectable (less than 0,005 mg/kg)
Malathion	not detectable (less than 0,01 mg/kg)
Methidathion	not detectable (less than 0,01 mg/kg)
Mevinphos	not detectable (less than 0,01 mg/kg)
Naled	not detectable (less than 0,01 mg/kg)
Parathion-ethyl	not detectable (less than 0,01 mg/kg)
Parathion-methyl	not detectable (less than 0,005 mg/kg)
Phosphamidon	not detectable (less than 0,01 mg/kg)
Phorate	not detectable (less than 0,01 mg/kg)
Pirimiphos-ethyl	not detectable (less than 0,01 mg/kg)
Pirimiphos-methyl	not detectable (less than 0,01 mg/kg)
Prophos	not detectable (less than 0,01 mg/kg)
Sulfotep	not detectable (less than 0,002 mg/kg)
Tetrachlorvinphos	not detectable (less than 0,01 mg/kg)
Tokuthion	not detectable (less than 0,01 mg/kg)
Tributyl phosphorotrioite	not detectable (less than 0,01 mg/kg)
Trichloronat	not detectable (less than 0,01 mg/kg)
Trichlorphon	not detectable (less than 0,01 mg/kg)
Dichlorbenil	not detectable (less than 0,05 mg/kg)
Diclofop-methyl	not detectable (less than 0,05 mg/kg)
Captafol	not detectable (less than 0,05 mg/kg)
Captan	not detectable (less than 0,05 mg/kg)
Procymidon	not detectable (less than 0,05 mg/kg)
Vinclozolin	not detectable (less than 0,05 mg/kg)
Propoxur	not detectable (less than 0,05 mg/kg)
Amitraz	not detectable (less than 0,05 mg/kg)

Aldrin	not detectable (less than 0,005 mg/kg)
Chlordane	not detectable (less than 0,005 mg/kg)
Dieldrin	not detectable (less than 0,01 mg/kg)
Endosulfan 1	not detectable (less than 0,01 mg/kg)
Endosulfan 2	not detectable (less than 0,01 mg/kg)
Endosulfan sulphate	not detectable (less than 0,005 mg/kg)
Endrin	not detectable (less than 0,01 mg/kg)
Endrin aldehyde	not detectable (less than 0,01 mg/kg)
PCB	not detectable (less than 0,01 mg/kg)
HCH alpha	not detectable (less than 0,001 mg/kg)
HCH beta	not detectable (less than 0,001 mg/kg)
HCH delta	not detectable (less than 0,001 mg/kg)
HCH gamma (lindane)	not detectable (less than 0,001 mg/kg)
Heptachlor	not detectable (less than 0,005 mg/kg)
Heptachlorepoxide	not detectable (less than 0,005 mg/kg)
Methoxychlor	not detectable (less than 0,01 mg/kg)
op DDD	not detectable (less than 0,005 mg/kg)
op DDE	not detectable (less than 0,005 mg/kg)
op DDT	not detectable (less than 0,005 mg/kg)
pp DDD	not detectable (less than 0,005 mg/kg)
pp DDE	not detectable (less than 0,005 mg/kg)
pp DDT	not detectable (less than 0,005 mg/kg)
Toxaphene	not detectable (less than 0,01 mg/kg)
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Chemical Laboratory
"Dr.A.Verwey"

R. Mostert
Chief Chemist

CONCLUSION OF THE EXPERT PANEL: DETERMINATION OF THE GRAS STATUS OF REFINED TUNA OIL AS A SOURCE OF DOCOSAHEXAENOIC ACID IN INFANT FORMULA WHEN ACCOMPANIED BY A SOURCE OF ARACHIDONIC ACID

Prepared for:

Ocean Nutrition Canada Ltd.
Dartmouth, Nova Scotia
Canada

March 2011

CONCLUSION OF THE EXPERT PANEL: DETERMINATION OF THE GRAS STATUS OF REFINED TUNA OIL AS A SOURCE OF DOCOSAHEXAENOIC ACID IN INFANT FORMULA WHEN ACCOMPANIED BY A SOURCE OF ARACHIDONIC ACID

We, the members of the expert panel, are qualified by scientific training and experience to evaluate the processing methods employed to extract and refine tuna oil and the safety of food ingredients. We have individually and collectively critically evaluated the publicly available information on the methods employed by Ocean Nutrition Canada to extract and refine its tuna oil and on the safety of the addition of fish oils and other sources of n-3 fatty acids to infant formula, as summarized in supporting documentation prepared by JHeimbach LLC, as well as other material deemed appropriate or necessary. Our evaluation included review of the starting materials and production methods of refined tuna oil; the physiological effects of fish oil and its primary n-3 polyunsaturated fatty acids; and the safety of adding refined tuna oil to infant formula as a source of docosahexaenoic acid, to be accompanied by a source of arachidonic acid. Our summary and conclusion resulting from this critical evaluation are presented below.

Summary

- The substance that is the subject of this generally recognized as safe (GRAS) determination is refined tuna oil produced by Ocean Nutrition Canada, Ltd. (ONC). Refined tuna oil is a mixture of fatty acids, primarily in the form of triacylglycerols, with the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) predominating. The content of DHA is between 25 and 30 percent and the concentration of EPA is between 5 and 8 percent of the oil; the DHA:EPA ratio is between 3.3 and 3.7.

- ONC's refined tuna oil is extracted and refined using methods generally recognized as appropriate for producing food-grade oil with a high degree of purity. The efficacy of these methods is confirmed by the results of numerous chemical analyses of the refined product.

- Refined tuna oil is intended for addition to both term and preterm infant formula to provide DHA at up to 0.5% of the fatty acid content. The estimated daily intake of DHA at the 90th percentile of formula consumption is 40 mg/kg bw/day; EPA intake will not exceed 13 mg/kg bw/day. The estimated daily intake of the refined tuna oil itself will not exceed 160 mg/kg bw/day.

000127

- The safety of consumption of refined tuna oil in infant formula was determined by evaluating the safety of ingestion of the whole product, as well as safety of ingestion of the major constituents, the n-3 polyunsaturated fatty acids EPA and DHA. Safety of consumption of the whole product, refined tuna oil, was determined by evaluating the source of the materials, production processes, nature and quantity of impurities, and product specifications. Appropriate end-product specifications have been established to ensure that the final product

is food grade, and compositional analysis of the product supports the conclusion that there is no toxicological concern from impurities.

- The safety of the addition of the intended level of refined tuna oil to infant formula as a source of DHA, when accompanied by a source of arachidonic acid, has been shown by extensive research with both term and preterm infants. Twenty-eight randomized clinical trials have been published investigating the effects of supplementing infant formula with sources of DHA, both from fish oils and from non-fish sources. Eighteen of these studies were reviewed prior to submission of an earlier GRAS notice to FDA (GRN 000094) while ten were published subsequent to that time. In none of these studies were significant adverse events or tolerance issues observed compared to corresponding controls when DHA was fed (with arachidonic acid) to infants, typically at concentrations of 0.32% of total fatty acids and as high as 0.96% of total fatty acids for up to one year.

- While increased incidence of apnea was observed in the treatment group as compared to controls in studies by one investigator, the FDA Medical Officer concluded that the finding "does not raise safety issues" in that there was "a lack of significant association between the type of formula consumed and the number of infants experiencing apneic events."

- A number of authoritative scientific, medical, and regulatory organizations have supported the addition of DHA and arachidonic acid to infant formula for preterm and/or term infants. These organizations include the Food and Agriculture Organization of the United Nations and the World Health Organization, the U.S. Food and Drug Administration, the Institute of Medicine of the National Academies, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition, the European Union and its European Food Safety Authority, the American Dietetic Association and Dietitians of Canada, the Codex Alimentarius Commission, and the World Association of Perinatal Medicine

Conclusion

We, the undersigned expert panel members, have individually and collectively critically evaluated the materials summarized above and conclude that ONC's refined tuna oil, produced and used in accordance with cGMP and complying with the specifications described in the GRAS supporting documentation, is safe for use in term and preterm infant formula to provide DHA at up to 0.5% of the fatty acid content when this use is accompanied by the addition of a source of arachidonic acid.

We further conclude that the intended use of refined tuna oil in term and preterm infant formula as described is generally recognized as safe (GRAS) based on scientific procedures.

It is our opinion that other individuals qualified by scientific training and experience reviewing the same publicly available information would reach the same conclusion

Anthony P. Bimbo
Consultant
Kilmarnock, Virginia

Signature: (b) (6) Date: 3/22/2011

Joseph F. Borzelleca, Ph.D.
Emeritus Professor of Toxicology and Pharmacology
Virginia Commonwealth University School of Medicine

Signature: _____ Date: _____

Berthold V. Koletzko, Dr med Dr med habil (M.D. Ph.D.)
Professor of Pediatrics
University of Munich

Signature: _____ Date: _____

George H. Pauli, Ph.D.
Consultant
Silver Spring, MD

Signature: _____ Date: _____

Conclusion

We, the undersigned expert panel members, have individually and collectively critically evaluated the materials summarized above and conclude that ONC's refined tuna oil, produced and used in accordance with cGMP and complying with the specifications described in the GRAS supporting documentation, is safe for use in term and preterm infant formula to provide DHA at up to 0.5% of the fatty acid content when this use is accompanied by the addition of a source of arachidonic acid.

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Anthony P. Bimbo
Consultant
Kilmarnock, Virginia

Signature: _____ Date: _____

Joseph F. Borzelleca, Ph.D.
Emeritus Professor of Toxicology and Pharmacology
Virginia Commonwealth University School of Medicine

Signatur (b) (6) Date: 23 March 2011

Berthold V. Koletzko, Dr med Dr med habil (M.D. Ph.D.)
Professor of Pediatrics
University of Munich

Signature: _____ Date: _____

George H. Pauli, Ph.D.
Consultant
Silver Spring, MD

Signature: _____ Date: _____

Conclusion

We, the undersigned expert panel members, have individually and collectively critically evaluated the materials summarized above and conclude that ONC's refined tuna oil, produced and used in accordance with cGMP and complying with the specifications described in the GRAS supporting documentation, is safe for use in term and preterm infant formula to provide DHA at up to 0.5% of the fatty acid content when this use is accompanied by the addition of a source of arachidonic acid.

We further conclude that the intended use of refined tuna oil in term and preterm infant formula as described is generally recognized as safe (GRAS) based on scientific procedures.

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Anthony P. Bimbo
Consultant
Kilmarnock, Virginia

Signature: _____ Date: _____

Joseph F. Borzelleca, Ph.D.
Emeritus Professor of Toxicology and Pharmacology
Virginia Commonwealth University School of Medicine

Signature: _____ Date: _____

Berthold V. Koletzko, Dr med Dr med habil (M.D. Ph.D.)
Professor of Pediatrics
University of Munich (b) (6)

Signature: _____ Date: 23 March 2011

George H. Pauli, Ph.D.
Consultant
Silver Spring, MD

Signature: _____ Date: _____

Conclusion

We, the undersigned expert panel members, have individually and collectively critically evaluated the materials summarized above and conclude that ONC's refined tuna oil, produced and used in accordance with cGMP and complying with the specifications described in the GRAS supporting documentation, is safe for use in term and preterm infant formula to provide DHA at up to 0.5% of the fatty acid content when this use is accompanied by the addition of a source of arachidonic acid.

We further conclude that the intended use of refined tuna oil in term and preterm infant formula as described is generally recognized as safe (GRAS) based on scientific procedures.

It is our opinion that other individuals qualified by scientific training and experience reviewing the same publicly available information would reach the same conclusion

Anthony P. Bimbo
Consultant
Kilmarnock, Virginia

Signature: _____ Date: _____

Joseph F. Borzelleca, Ph.D.
Emeritus Professor of Toxicology and Pharmacology
Virginia Commonwealth University School of Medicine

Signature: _____ Date: _____

Berthold V. Koletzko, Dr med Dr med habil (M.D. Ph.D.)
Professor of Pediatrics
University of Munich

Signature: _____ Date: _____

George H. Pauli, Ph.D.
Consultant
Silver Spring, MD

Signature: (b) (6) Date: March 23, 2011

SUBMISSION END

Edwards, Alison

From: Ramos-Valle, Moraima
Sent: Tuesday, June 28, 2011 4:58 PM
To: Edwards, Alison; Twaroski, Timothy P
Subject: FW: Clarifications needed for GRN 379

Please see #2.

Thanks,
Moraima

From: Jim Heimbach [mailto:jh@jheimbach.com]
Sent: Monday, June 27, 2011 8:52 AM
To: Ramos-Valle, Moraima
Subject: Re: Clarifications needed for GRN 379

Moraima--

I hope you enjoyed your week away from the office!

I'm writing with two questions, totally unrelated:
1. Can you send me a list of the anticipated FDA attendees at the meeting on oligofructose on Wednesday?

2. With regard to the questions the FDA reviewers raised concerning the refined tuna oil (below), we are addressing them. One question about your first issue: If this can best be addressed by our meeting with the appropriate FDA people and describing confidential processing methods, including presenting relevant flow-charts, specifications, etc., but not submitting these documents and thus making them FOIA-able, is this still acceptable? (We have done this in the past--notably with Benecol phytostannol esters--but FDA policies sometimes change.)

Thanks--
Jim

James T. Heimbach, Ph.D., F.A.C.N.
JHeimbach LLC
923 Water Street, P.O. Box 66
Port Royal VA 22535
tel (+1) 804-742-5548
fax (+1) 202-478-0986
cell (+1) 202-320-3063
e-mail jh@jheimbach.com

> ----- Original Message -----
> **From:** Ramos-Valle, Moraima
> **To:** Jim Heimbach
> **Cc:** Ramos-Valle, Moraima
> **Sent:** Friday, June 17, 2011 4:30 PM
> **Subject:** Clarifications needed for GRN 379
>
> Dear Dr. Heimbach,

As we move forward with the review of GRN 379 we seek clarification for the following:

1. FDA notes that certain details of the method of manufacture are not included in the notice. While the notifier refers to the method of manufacture of tuna oil as standard methodology, we note that certain contaminants (e.g., dioxin-like PCBs and heavy PAHs) are less readily removed than others by standard oil processing methods and, in the past 5-10 years, there have been refinements to standard methods (e.g., higher temperature/vacuum conditions, use of short-path distillation, activated carbon treatment) for fish oils that have been described in the literature for successful removal of persistent contaminants.

- Please describe deodorization and steam deodorization conditions (e.g., temperature, vacuum), providing citation to published data supporting this methodology, where applicable. If certain details of the method of manufacture are considered confidential by the notifier, these details may be omitted with citation of publicly-available reference(s) (that address removal of contaminants that may be of health concern) and a statement that the conditions employed by ONC meet or exceed those conditions described in those references.
- Please provide a brief statement describing the bleaching material (and its suitability for food use, including citation to applicable regulations for its use) and whether activated carbon may be used in addition to bleaching clay for removal of persistent organic pollutants.

2. ONC provides results of analyses of PBDEs in four lots of tuna oil, but does not provide a narrative accompanying this data. Further, the notice does not present the PBDE levels in crude oil or discuss the ability of the method of manufacture to remove PBDEs. (FDA notes that removal of PBDEs by short-path distillation techniques has been reported in the published literature; the success of other standard oil purification processes has been questioned).

- Please provide a discussion of the presence of PBDEs in tuna oil and the removal of PBDEs by the method of manufacture (with reference to published studies where applicable)
- Please briefly summarize the significance of this information in relation to the intended use of tuna oil in formulas intended for term and preterm infants.

3. In the Expert Panel conclusion summary (First page of the expert panel section), the first bullet contains a statement that the DHA:EPA ratio is between 3.3 and 3.7. However, the specification for the DHA:EPA ratio on p. 11 is "NLT 3:1" and the compositional analysis given on p. 12 is 3.3-3.5. Please clarify the DHA:EPA ratio considered by ONC and the Panel and discuss the general recognition of safety of the tuna oil ingredient for its intended use, in comparison to published clinical studies and publicly-available recommendations such as that of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) which suggest the ratio of DHA:EPA exceed 3.5:1 for all infant formulas.

4. In the table of specifications on page 11, please clarify the analytical methods used where N/A or NA is indicated.

5. Please provide clarification regarding use of tuna oil with an appropriate source of arachidonic acid (ARA). In accordance with GRN 326 and GRN 80, in the statement of intended use, please indicate the ratio of DHA:ARA consistent with published data supporting the safe use of tuna oil in infant formula.

6. FDA noted that throughout the document other GRNs are mentioned but that no official statement incorporates them by reference. (from a safety perspective this is in regards to the animal studies).

7. FDA notes that under the "Review of Safety Data" section only human studies are discussed. Were there any relevant animal studies published since we received the prior notices, and if so do they continue to support safety?

If you have any questions please feel free to contact me.

Thanks,
Moraima

Moraima J. Ramos Valle, M.S.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Food and Drug Administration

Phone: 240-402-1248

Email: Moraima.Ramos-Valle@fda.hhs.gov

Edwards, Alison

From: Ramos-Valle, Moraima
Sent: Thursday, July 14, 2011 1:48 PM
To: Twaroski, Timothy P; Edwards, Alison
Cc: Chanderbhan, Ronald F; Dinovi, Michael J
Subject: FW: Clarifications needed for GRN 379
Attachments: ONC Responses to Q3-5-6-7.docx

Response to a few of the questions.

From: Jim Heimbach [mailto:jheimbach@va.metrocast.net]
Sent: Thursday, July 14, 2011 1:38 PM
To: Ramos-Valle, Moraima
Cc: Carlson, Susan
Subject: Re: Clarifications needed for GRN 379

Dear Moraima--

As we discussed this morning, we'll send you responses as we have them prepared. Here are our responses to your questions 3, 5, 6, and 7.

Regards,
Jim

James T. Heimbach, Ph.D., F.A.C.N.
JHeimbach LLC
923 Water Street, Box 66
Port Royal VA 22535
tel 804-742-5548
fax 202-478-0986
cell 202-320-3063
email jh@jheimbach.com

From: Ramos-Valle, Moraima
Sent: Tuesday, July 12, 2011 4:34 PM
To: Jim Heimbach
Cc: Carlson, Susan
Subject: RE: Clarifications needed for GRN 379

> Hi Dr. Heimbach,
>
> Just following up with the questions below. Can you give us and idea of when we should be getting back the responses?
>
> Thanks,
>
> Moraima J. Ramos Valle
> Consumer Safety Officer
> FDA/CFSAN/OFAS/DBGNR
> 240-402-1248

----- Original Message -----
From: Ramos-Valle, Moraima
To: Jim Heimbach
Cc: Ramos-Valle, Moraima
Sent: Friday, June 17, 2011 4:30 PM
Subject: Clarifications needed for GRN 379

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- Please describe deodorization and steam deodorization conditions (e.g., temperature, vacuum), providing citation to published data supporting this methodology, where applicable. If certain details of the method of manufacture are considered confidential by the notifier, these details may be omitted with citation of publicly-available reference(s) (that address removal of contaminants that may be of health concern) and a statement that the conditions employed by ONC meet or exceed those conditions described in those references.
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and discuss the general recognition of safety of the tuna oil ingredient for its intended use, in comparison to published clinical studies and publicly-available recommendations such as that of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) which suggest the ratio of DHA:EPA exceed 3.5:1 for all infant formulas.

4. In the table of specifications on page 11, please clarify the analytical methods used where N/A or NA is indicated.

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6. FDA noted that throughout the document other GRNs are mentioned but that no official statement incorporates them by reference. (from a safety perspective this is in regards to the animal studies).

7. FDA notes that under the "Review of Safety Data" section only human studies are discussed. Were there any relevant animal studies published since we received the prior notices, and if so do they continue to support safety?

If you have any questions please feel free to contact me.

Thanks,
Moraima

Moraima J. Ramos Valle, M.S.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Food and Drug Administration

Phone: 240-402-1248

Email: Moraima.Ramos-Valle@fda.hhs.gov

3. In the Expert Panel conclusion summary (First page of the expert panel section), the first bullet contains a statement that the DHA:EPA ratio is between 3.3 and 3.7. However, the specification for the DHA:EPA ratio on p. 11 is "NLT 3:1" and the compositional analysis given on p. 12 is 3.3-3.5. Please clarify the DHA:EPA ratio considered by ONC and the Panel and discuss the general recognition of safety of the tuna oil ingredient for its intended use, in comparison to published clinical studies and publicly-available recommendations such as that of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) which suggest the ratio of DHA:EPA exceed 3.5:1 for all infant formulas.

This question includes 3 parts:

1. Why is the specification for the DHA:EPA ratio only 3:1 while the compositional analyses are higher?
2. What is the correct analytical range of DHA:EPA ratios?
3. How does the analytical range compare with generally available recommendations?

3.1. The specification of 3:1 for the DHA:EPA ratio defines the lowest ratio that is regarded as acceptable in any single lot of the product; it is not expected to reflect the normal range of DHA:EPA ratios found in refined tuna oil. A lot failing to meet that specification would not be regarded as GRAS for addition to infant formula. (For comparison, it might be noted that in GRN 94 the DHA:EPA specification was 3.1:1, while the five analytical values were in the range of 3.1:1 to 3.8:1, with a mean of 3.4:1.

3.2. The compositional summary of DHA and EPA levels and their ratio found on page 12 of the GRAS notice was in error and resulted from omitting several analyses in the calculation. The range of DHA:EPA ratios given in the Expert Panel Conclusion, 3.3:1 to 3.7:1, is correct. This range is based on analyses of 9 lots of refined tuna oil, and the information given on page 12 should have appeared as follows:

DHA content: mean = 26.4% (±0.7); minimum and maximum = 26% and 28%

EPA content: mean = 7.7% (±0.5); minimum and maximum = 7% and 8%

DHA:EPA ratio: mean = 3.5:1 (±0.2); minimum and maximum = 3.3:1 and 3.7:1

The data presented in the table below represent the EPA and DHA content of nine non-consecutive individual lots of refined tuna oil processed by Ocean Nutrition Canada (ONC). EPA and DHA content are tabulated in both area percent and mg/g.

Parameter	Spec	Lot								
		18950	18951	19582	19583	19843	20438	20954	21653	22310
EPA (%)	5-8	8	8	8	8	8	7	7	8	7
DHA (%)	25-30	26	26	26	28	27	26	26	27	26
EPA (mg/g)	≥ 45	70	69	70	70	70	60	60	70	60
DHA (mg/g)	≥ 225	225	225	230	240	240	230	220	230	230

The data from the above table are summarized in the following table.

Parameter	Minimum	Maximum	Mean
DHA Content (%)	26	28	26.4 (±0.7)
EPA Content (%)	7	8	7.7 (±0.5)
DHA:EPA Ratio	3.3:1	3.7:1	3.5:1 (±0.2)

3.3. Since the correct mean DHA:EPA ratio for refined tuna oil is actually 3.5, which is consistent with FDA's suggested generally available guideline, this question is moot. However, we do note that there is no consensus that a 3.5 ratio is either necessary or optimal. Indeed, we have been unable to locate any recommendation regarding DHA:EPA ratio in infant formula from the International Society for the Study of Fatty Acids and Lipids (ISSFAL). We have found other generally available recommendations, which were cited in the GRAS notice:

European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN; Koletzko et al. 2005)

- EPA ≤ DHA (DHA:EPA ≥ 1:1)

European Union, Commission Directive 2006/141/EC on infant formulae and follow-on formulae (EU 2006)

- EPA ≤ DHA (DHA:EPA ≥ 1:1)

Food Standards Australia New Zealand (FSANZ) Standard 2.9.1 (2007)

- DHA ≥ EPA

World Association of Perinatal Medicine, the Early Nutrition Academy, and Child Health Foundation was published in 2008 (Koletzko et al. 2008)

- EPA ≤ DHA (DHA:EPA ≥ 1:1)

ESPGHAN guidelines for preterm infants (Agostoni et al. 2010)

- EPA ≤ 30% of DHA (DHA;EPA ≥ 3.3:1)

Codex standard for infant formula and formula for special medical purposes intended for infants (CODEX.STAN 72 1981)

- EPA≤DHA (DHA:EPA ≥ 1:1)

People's Republic of China National Infant Formula Standard (PRC GB 10765-2010)

- EPA≤DHA (DHA:EPA ≥ 1:1)

Only one of these guidelines (Agostoni et al. 2010) calls for a DHA:EPA ratio greater than 1:1, and that single guideline—intended for the enteral nutrition of preterm infants—calls for EPA addition not to exceed 30% of the DHA, a ratio of 3.3:1 or greater. Every single lot of refined tuna oil tested met this guideline. ONC and the Expert Panel thus concluded that refined tuna oil is consistent with generally available guidelines regarding DHA and EPA content.

References

Agostoni C, G Buonocore, VP Carnielli, M DeCurtis, D Darmaun, T Decsi, M Domellof, ND Embleton, C Fusch, O Genzel-Boroviczeny, O Goulet, SC Kalhan, S Kolacek, B Koletzko, A Lapillonne, W Mihatsch, L Moreno, J Neu, B Poindexter, J Puntis, G Putet, J Rigo, A Riskin, B Salle, P Sauer, R Shamir, H Szajewska, P Thureen, D Turck, JB van Goudoever, EE Ziegler. 2010. Enteral nutrient supply for preterm infants: commentary from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 50:1-9.

Codex Alimentarius. 1981. Standard for infant formula and formula for special medical purposes intended for infants; CODEX.STAN 72.

European Union (EU). 2006. *Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC*. Official J Eur Union 401:1-33.

Food Standards Australia New Zealand (FSANZ). 2007. *Standard 2.9.1—Infant Formula Products*. Available on-line at http://www.foodstandards.gov.au/_srcfiles/Standard_2_9_1_Infant_Formula_Products_v109.pdf

Koletzko, B., Baker, S., Cleghorn, G., Neto, U.F., Gopalan, S., Hernell, O., Hock, Q.S., Jirapinyo, P., Lonnerdal, B., Pencharz, P., Pzyrembel, H., Ramirez-Mayans, J., Shamir, R., Turck, D., Yamashiro, Y., and Zong-Yi, D. 2005. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *J Pediatr Gastroenterol Nutr* 41:584-599.

Koletzko, B., Lien, E., Agostoni, C, Bohles, H, Campoy, C., Cetin, I., Decsi, T., Dudenhausen, J.W., Dupont, C., Forsyth, S., Hoesli, I., Holzgreve, W., Lapillonne, A., Putet, G., Secher, N.J., Symonds, M., Szajewska, H., Willatts, P., and Uauy, R. 2008. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med* 36:5-14.

Peoples Republic of China, Ministry of Health (PRC). 2010. National Standards of the People's Republic of China: National Infant Formula Standard, GB 10765.

5. Please provide clarification regarding use of tuna oil with an appropriate source of arachidonic acid (ARA). In accordance with GRN 326 and GRN 80, in the statement of intended use, please indicate the ratio of DHA:ARA consistent with published data supporting the safe use of tuna oil in infant formula.

We did not indicate the appropriate ratio of DHA:ARA partly because we did not feel it is necessary since we did not determine a specific ratio to be GRAS, but more because we recognize that the science of infant lipid metabolism is still evolving and the ratio that we might recommend today might not be regarded as optimal several years from now. We have surveyed the literature on this subject, and a brief summary of generally available guidelines based on published research follows in the chronological order in which the guideline was published:

Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO, Fats and Oils in Human Nutrition (FAO/WHO 1994)

- preterm infants: 40 mg DHA and 60 mg ARA/kg bw/day (ratio = 1:1.5)
- term infants: 20 mg DHA and 40 mg ARA/kg bw/day (ratio = 1:2)

European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN; Koletzko et al. 2005)

- ARA ≥ DHA

European Union, Commission Directive 2006/141/EC on infant formulae and follow-on formulae (EU 2006)

- DHA ≤ ARA

Food Standards Australia New Zealand (FSANZ) Standard 2.9.1 (2007)

- n-6 LCPUFA:n-3 LCPUFA ≥ 1

International Society for the Study of Fatty Acids and Lipids (ISSFAL) Statement on dietary fats in infant nutrition (2008)

- ARA:n-3 LCPUFA ≥ 1

World Association of Perinatal Medicine, the Early Nutrition Academy, and Child Health Foundation was published in 2008 (Koletzko et al. 2008)

- ARA ≥ DHA

ESPGHAN guidelines for preterm infants (Agostoni et al. 2010)

- ARA:DHA 1.0 – 2.0:1)

As is apparent, the majority view—and that in the more recent guidelines—is that ARA should be present in at least the same concentration as DHA. It was only the early guidance from FAO/WHO that recommended higher levels of ARA. An interesting complication seen in the FSANZ and ISSFAL guidelines is consideration of all n-3 LC-PUFA rather than only DHA, or even consideration of all n-6 LC-PUFA and not only ARA. The most recent guideline, that developed for preterm infants by ESPGHAN, is the only generally available guidance that suggests a range of ARA:DHA addition, i.e. that ARA should be between 1 and 2 times the level of DHA addition.

With the caveat that further research in the future may alter what is currently regarded as the appropriate ratio of ARA to DHA, we conclude that the ratio of DHA:ARA most consistent with the totality of published data supporting the addition of DHA to infant formula is 1 to 1-2. Consequently we propose that the ratio of DHA from refined tuna oil to arachidonic acid from an appropriate source will be in the range of 1:1 to 1:2. (We also note that this was the ratio proposed in GRN 41 and GRN 80, while the proposed ratios in GRN 94 and GRN 326 were slightly higher: 1:1.6-2.7 and 1:1.0-2.7, respectively.)

References

Agostoni C, G Buonocore, VP Carnielli, M DeCurtis, D Darmaun, T Decsi, M Domellof, ND Embleton, C Fusch, O Genzel-Boroviczeny, O Goulet, SC Kalhan, S Kolacek, B Koletzko, A Lapillonne, W Mihatsch, L Moreno, J Neu, B Poindexter, J Puntis, G Putet, J Rigo, A Riskin, B Salle, P Sauer, R Shamir, H Szajewska, P Thureen, D Turck, JB van Goudoever, EE Ziegler. 2010. Enteral nutrient supply for preterm infants: commentary from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 50:1-9.

European Union (EU). 2006. *Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC*. Official J Eur Union 401:1-33.

Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO). 1994. *Fats and oils in human nutrition*. Report of a joint expert consultation 19-26 October 1993. FAO. Via delle Terme di Caracalla, Rome, Italy.

Food Standards Australia New Zealand (FSANZ). 2007. *Standard 2.9.1—Infant Formula Products*. Available on-line at http://www.foodstandards.gov.au/_srcfiles/Standard_2_9_1_Infant_Formula_Products_v109.pdf

Gibson R, M Makrides, B Koletzko, T Brenna, M Craig-Schmidt. 2008. *International Society for the Study of Fatty Acids and Lipids (ISSFAL) Statement on dietary fats in infant nutrition*. Available on-line at http://www.issfal.org/index.php/lipid-matters-mainmenu-8/6-issfal-policy-statements

Koletzko, B., Baker, S., Cleghorn, G., Neto, U.F., Gopalan, S., Hernell, O., Hock, Q.S., Jirapinyo, P., Lonnerdal, B., Pencharz, P., Pzyrembel, H., Ramirez-Mayans, J., Shamir, R., Turck, D., Yamashiro, Y., and Zong-Yi, D. 2005. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. *J Pediatr Gastroenterol Nutr* 41:584-599.

Koletzko, B., Lien, E., Agostoni, C, Bohles, H, Campoy, C., Cetin, I., Decsi, T., Dudenhausen, J.W., Dupont, C., Forsyth, S., Hoesli, I., Holzgreve, W., Lapillonne, A., Putet, G., Secher, N.J., Symonds, M., Szajewska, H., Willatts, P., and Uauy, R. 2008. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med* 36:5-14.

6. FDA noted that throughout the document other GRNs are mentioned but that no official statement incorporates them by reference. (from a safety perspective this is in regards to the animal studies).

We wish to incorporate by reference the animal study discussions from GRN 94 and those from the somewhat more recent GRN 138.

7. FDA notes that under the "Review of Safety Data" section only human studies are discussed. Were there any relevant animal studies published since we received the prior notices, and if so do they continue to support safety?

No recent animals studies of fish oil bear on the determination of the safety of the intended use of refined tuna oil in infant formula. Indeed, most research into the safety of ingestion of fish oils has been conducted in humans rather than animals, and this is especially true recently due to the large body of human data. An extensive review of preclinical and clinical research on DHA from both fish-oil and single-cell sources concluded that DHA is safe in both animals and humans at the intake levels tested (Lien 2009).

The few recent studies in which fish oil was given to animals most often used it as a control to which the effects of other (e.g., algal) oils could be compared. The most recent examples of this are MacKenzie et al. 2010, Belcher et al. 2011, and Fedorova-Dahms et al. 2011. In none of these studies was any adverse effect observed attributable to the fish-oil control (menhaden or sardine/anchovy).

References

Belcher LA, SA MacKenzie, M Donner, GP Sykes, SR Frame, PJ Gillies. 2011. Safety assessment of EPA-rich triglyceride oil produced from yeast: genotoxicity and 28-day oral toxicity in rats. *Regul Toxicol Pharmacol* 59:53-63.

Fedorova-Dahms I, PA Marone, E Bailey-Hall, AS Ryan. 2011. Safety evaluation of algal oil from *Schizochytrium* sp. *Food Chem Toxicol* 49:70-77.

Lien EL. 2009. Toxicology and safety of DHA. *Prostaglandins Leukot Essent Fatty Acids* 81:125-132.

MacKenzie SA, LA Belcher, GP Sykes, SR Frame, P Mukerji, PJ Gillies. 2010. Safety assessment of EPA-rich oil produced from yeast: results of a 90-day subchronic toxicity study. *Regul Toxicol Pharmacol* 58:490-500.

Edwards, Alison

From: Ramos-Valle, Moraima
Sent: Friday, July 15, 2011 11:32 AM
To: Edwards, Alison; Twaroski, Timothy P
Cc: Chanderbhan, Ronald F; Dinovi, Michael J
Subject: FW: Clarifications needed for GRN 379
Attachments: Response to Q4.docx

See below

From: Jim Heimbach [mailto:jheimbach@va.metrocast.net]
Sent: Friday, July 15, 2011 11:18 AM
To: Ramos-Valle, Moraima
Cc: Carlson, Susan
Subject: Re: Clarifications needed for GRN 379

Here is the response to Question 4. Responses to Q1 and Q2 will be coming next week.

Have a good weekend!
Jim

James T. Heimbach, Ph.D., F.A.C.N.
JHeimbach LLC
923 Water Street, Box 66
Port Royal VA 22535
tel 804-742-5548
fax 202-478-0986
cell 202-320-3063
email jh@jheimbach.com

From: Ramos-Valle, Moraima
Sent: Tuesday, July 12, 2011 4:34 PM
To: Jim Heimbach
Cc: Carlson, Susan
Subject: RE: Clarifications needed for GRN 379

> Hi Dr. Heimbach,
>
> Just following up with the questions below. Can you give us and idea of when we should be getting back the responses?
>
> Thanks,
>
> Moraima J. Ramos Valle
> Consumer Safety Officer
> FDA/CFSAN/OFAS/DBGNR
> 240-402-1248
>
> ----- Original Message -----
> **From:** Ramos-Valle, Moraima

To: Jim Heimbach
Cc: Ramos-Valle, Moraima
Sent: Friday, June 17, 2011 4:30 PM
Subject: Clarifications needed for GRN 379

Dear Dr. Heimbach,

As we move forward with the review of GRN 379 we seek clarification for the following:

1. FDA notes that certain details of the method of manufacture are not included in the notice. While the notifier refers to the method of manufacture of tuna oil as standard methodology, we note that certain contaminants (e.g., dioxin-like PCBs and heavy PAHs) are less readily removed than others by standard oil processing methods and, in the past 5-10 years, there have been refinements to standard methods (e.g., higher temperature/vacuum conditions, use of short-path distillation, activated carbon treatment) for fish oils that have been described in the literature for successful removal of persistent contaminants.

- Please describe deodorization and steam deodorization conditions (e.g., temperature, vacuum), providing citation to published data supporting this methodology, where applicable. If certain details of the method of manufacture are considered confidential by the notifier, these details may be omitted with citation of publicly-available reference(s) (that address removal of contaminants that may be of health concern) and a statement that the conditions employed by ONC meet or exceed those conditions described in those references.
- Please provide a brief statement describing the bleaching material (and its suitability for food use, including citation to applicable regulations for its use) and whether activated carbon may be used in addition to bleaching clay for removal of persistent organic pollutants.

2. ONC provides results of analyses of PBDEs in four lots of tuna oil, but does not provide a narrative accompanying this data. Further, the notice does not present the PBDE levels in crude oil or discuss the ability of the method of manufacture to remove PBDEs. (FDA notes that removal of PBDEs by short-path distillation techniques has been reported in the published literature; the success of other standard oil purification processes has been questioned).

- Please provide a discussion of the presence of PBDEs in tuna oil and the removal of PBDEs by the method of manufacture (with reference to published studies where applicable)
- Please briefly summarize the significance of this information in relation to the intended use of tuna oil in formulas intended for term and preterm infants.

3. In the Expert Panel conclusion summary (First page of the expert panel section), the first bullet contains a statement that the DHA:EPA ratio is between 3.3 and 3.7. However, the specification for the DHA:EPA ratio on p. 11 is "NLT 3:1" and the compositional analysis given on p. 12 is 3.3-3.5. Please clarify the DHA:EPA ratio considered by ONC and the Panel and discuss the general recognition of safety of the tuna oil ingredient for its intended use, in comparison to published clinical studies and publicly-available recommendations such as that

of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) which suggest the ratio of DHA:EPA exceed 3.5:1 for all infant formulas.

4. In the table of specifications on page 11, please clarify the analytical methods used where N/A or NA is indicated.

5. Please provide clarification regarding use of tuna oil with an appropriate source of arachidonic acid (ARA). In accordance with GRN 326 and GRN 80, in the statement of intended use, please indicate the ratio of DHA:ARA consistent with published data supporting the safe use of tuna oil in infant formula.

6. FDA noted that throughout the document other GRNs are mentioned but that no official statement incorporates them by reference. (from a safety perspective this is in regards to the animal studies).

7. FDA notes that under the "Review of Safety Data" section only human studies are discussed. Were there any relevant animal studies published since we received the prior notices, and if so do they continue to support safety?

If you have any questions please feel free to contact me.

Thanks,
Moraima

Moraima J. Ramos Valle, M.S.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Food and Drug Administration

Phone: 240-402-1248

Email: Moraima.Ramos-Valle@fda.hhs.gov

4. In the table of specifications on page 11, please clarify the analytical methods used where N/A or NA is indicated.

Analytical methods for the parameters where NA was used have been added to the specifications and highlighted in yellow. Please see page two below for the full specification.

- Appearance, flavor and odor are quality measures. They are assessed by way of an in-house standard operating method. Vision, taste and smell are used to confirm tuna oil acceptance or failure to our specification.
- Totox number is a measure of primary (peroxide) and secondary (p-anisidine) oxidation values obtained by a calculation. The calculation is (2 X peroxide value + p-anisidine value).
- Dioxin/furans, pesticides, *E.coli, Salmonella, Enterbacteriaceae,* yeast and mold, and total aerobic bacteria are all analyzed by a 3rd party lab. The analytical methods obtained from the 3rd party lab have been included.

See Page two for full specification.

↓

Parameter	Specification	Analytical Method
ANALYSIS		
Color and clarity (Gardner)	NMT[1] 7	AOCS Td 1a-64 (09)
Appearance	Clear yellow-amber	In house SOP 80/05/911
Flavor and odor	Bland	In house SOP 80/05/911
Free fatty acids (as % oleic)	NMT 0.5%	AOCS CD 3D-63 modified
Acid value (mg KOH/g)	NMT 1.0	AOCS CD 3D-63 modified
p-Anisidine value (pAV)*	NMT 20	AOCS CD 18-90
Peroxide value (PV) (meq/kg)	NMT 1.0	AOCS CD 8-53
Totox number	NMT 22	Calculation (2x PV + pAV)
Moisture (%)	NMT 0.1	AOCS CA 2E-84 modified
FATTY ACID PROFILE		
EPA (area[3] %)	NLT 5 and NMT 8	EP 2003:1352, 2.4.29 modified
DHA (area %)	NLT 25 and NMT 30	EP 2003:1352, 2.4.29 modified
EPA (mg/g as TG[4])	NLT 45	EP 2003:1352, 2.4.29 modified
DHA (mg/g as TG)	NLT 220	EP 2003:1352, 2.4.29 modified
EPA (mg/g as FFA[5])	NLT 40	EP 2003:1352, 2.4.29 modified
DHA (mg/g as FFA)	NLT 210	EP 2003:1352, 2.4.29 modified
DHA:EPA ratio	NLT 3:1	EP 2003:1352, 2.4.29 modified
Total n-3 fatty acids (area %)	NLT 32 and NMT 40	EP 2003:1352, 2.4.29 modified
Total n-3 fatty acids (mg/g as TG)	NLT 280	EP 2003:1352, 2.4.29 modified
RESIDUES AND CONTAMINANTS		
Cadmium (mg/kg)	NMT 0.1	US EPA 200.7 & 200.8 modified
Arsenic (mg/kg)	NMT 0.1	US EPA 200.7 & 200.8 modified
Lead (mg/kg)	NMT 0.1	US EPA 200.7 & 200.8 modified
Mercury (mg/kg)	NMT 0.01	US EPA 245.6
PCB[6] (mg/kg)	NMT 0.09	US EPA 1668 modified
Benzo(a)pyrene (µg/kg)***	NMT 2.0	In accord with NEN-ISO-15302
Dioxin and furans[7] (pg WHO-PCDD/FTEQ/g)	NMT 1.5	QMA 504-171 3rd party lab
Pesticides[8] (mg/kg)**	<0.05 ppm	NEN-EN 1528 3rd party lab
MICROBIOLOGICAL		
Standard aerobic plate count (cfu[9]/g)	NMT 100	USP32 NF27 2009 61 3rd party lab
Enterbacteriaceae (cfu/g)	NMT 100	MFLP 09 3rd party lab
E. coli (in 1 g)	Not detected	USP32 NF 27 2009 62 3rd party lab
Salmonella spp. (in 10 g)	Not detected	USP32 NF 27 2009 62 3rd party lab
Yeast and mold (cfu/g)	NMT 100	USP32 NF 27 2009 61 3rd party lab

Edwards, Alison

From: Ramos-Valle, Moraima
Sent: Thursday, July 21, 2011 11:10 AM
To: Twaroski, Timothy P; Edwards, Alison; Robert Baldo, Gillian L
Cc: Dinovi, Michael J; Chanderbhan, Ronald F
Subject: FW: Clarifications needed for GRN 379
Attachments: Response to Q1b.docx; Response to Q2.docx

A little bit more!

From: Jim Heimbach [mailto:jheimbach@va.metrocast.net]
Sent: Thursday, July 21, 2011 10:10 AM
To: Ramos-Valle, Moraima
Cc: Carlson, Susan
Subject: Re: Clarifications needed for GRN 379

Dear Moraima--

We have previously provided responses to questions 3, 4, 5, 6, and 7. Here I am attaching responses to the second part of question 1 (designated Q1b) and to question 2. I will be traveling tomorrow, but will respond to the first part of question 1 (Q1a) early next week. This is the only response not yet provided.

Regards,
Jim

James T. Heimbach, Ph.D., F.A.C.N.
JHeimbach LLC
923 Water Street, Box 66
Port Royal VA 22535
tel 804-742-5548
fax 202-478-0986
cell 202-320-3063
email jh@jheimbach.com

1. FDA notes that certain details of the method of manufacture are not included in the notice. While the notifier refers to the method of manufacture of tuna oil as standard methodology, we note that certain contaminants (e.g., dioxin-like PCBs and heavy PAHs) are less readily removed than others by standard oil processing methods and, in the past 5-10 years, there have been refinements to standard methods (e.g., higher temperature/vacuum conditions, use of short-path distillation, activated carbon treatment) for fish oils that have been described in the literature for successful removal of persistent contaminants.

- Please describe deodorization and steam deodorization conditions (e.g., temperature, vacuum), providing citation to published data supporting this methodology, where applicable. If certain details of the method of manufacture are considered confidential by the notifier, these details may be omitted with citation of publicly-available reference(s) (that address removal of contaminants

that may be of health concern) and a statement that the conditions employed by ONC meet or exceed those conditions described in those references.

- Please provide a brief statement describing the bleaching material (and its suitability for food use, including citation to applicable regulations for its use) and whether activated carbon may be used in addition to bleaching clay for removal of persistent organic pollutants.

2. ONC provides results of analyses of PBDEs in four lots of tuna oil, but does not provide a narrative accompanying this data. Further, the notice does not present the PBDE levels in crude oil or discuss the ability of the method of manufacture to remove PBDEs. (FDA notes that removal of PBDEs by short-path distillation techniques has been reported in the published literature; the success of other standard oil purification processes has been questioned).

- Please provide a discussion of the presence of PBDEs in tuna oil and the removal of PBDEs by the method of manufacture (with reference to published studies where applicable)
- Please briefly summarize the significance of this information in relation to the intended use of tuna oil in formulas intended for term and preterm infants.

3. In the Expert Panel conclusion summary (First page of the expert panel section), the first bullet contains a statement that the DHA:EPA ratio is between 3.3 and 3.7. However, the specification for the DHA:EPA ratio on p. 11 is "NLT 3:1" and the compositional analysis given on p. 12 is 3.3-3.5. Please clarify the DHA:EPA ratio considered by ONC and the Panel and discuss the general recognition of safety of the tuna oil ingredient for its intended use, in comparison to published clinical studies and publicly-available recommendations such as that of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) which suggest the ratio of DHA:EPA exceed 3.5:1 for all infant formulas.

4. In the table of specifications on page 11, please clarify the analytical methods used where N/A or NA is indicated.

5. Please provide clarification regarding use of tuna oil with an appropriate source of arachidonic acid (ARA). In accordance with GRN 326 and GRN 80, in the statement of intended use, please indicate the ratio of DHA:ARA consistent with published data supporting the safe use of tuna oil in infant formula.

6. FDA noted that throughout the document other GRNs are mentioned but that no official statement incorporates them by reference. (from a safety perspective this is in regards to the animal studies).

7. FDA notes that under the "Review of Safety Data" section only human studies are discussed. Were there any relevant animal studies published since we received the prior notices, and if so do they continue to support safety?

If you have any questions please feel free to contact me.

Thanks,
Moraima

Moraima J. Ramos Valle, M.S.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Food and Drug Administration

Phone: 240-402-1248

Email: Moraima.Ramos-Valle@fda.hhs.gov

Q1b. Please provide a brief statement describing the bleaching material (and its suitability for food use, including citation to applicable regulations for its use) and whether activated carbon may be used in addition to bleaching clay for removal of persistent organic pollutants.

The bleaching material used in processing ONC refined tuna oil is composed primarily of bentonite. It is an adsorbent specially designed to be used on fatty oils. It is particularly effective for bleaching fatty oils destined for physical refining. ONC's supplier has confirmed that the bleaching material manufactured by them is safe and widely used as a processing aid in food production.

Bentonite is affirmed as generally recognized as safe under the conditions of use described in 21 CFR §184.1155. ONC confirms that the bleaching material complies with the specifications for food-grade material listed in the *Food Chemicals Codex* and is used in accordance with the provisions of §184.1155(c), which call for its use as a processing aid as defined in §170.3(o)(24) at levels not exceeding cGMP, and leaving no significant residue once refining is complete.

Activated carbon used during the refining process of ONC tuna oil is manufactured entirely from vegetable sources. It is specifically developed to be used as an adsorbent in conjunction with bleaching material. Our supplier has confirmed that activated carbon has not been sourced from genetically modified vegetables, and therefore no genetically modified material is present. Like bleaching material, it is used as a processing aid and is removed from the final tuna oil once physical refining is complete. The activated carbon used by ONC complies with the specifications for food-grade material listed in the *Food Chemicals Codex*. ONC's supplier also confirmed that the FDA has provided in writing a letter stating that activated carbon meeting FCC specifications is considered GRAS for use in foods.

Regarding the question of whether activated carbon may be used in addition to bleaching clay for removal of persistent organic pollutants, activated carbon has shown to remove PAHs, PCBs, dioxins, and unwanted organic material in edible oils, particularly fish oils. Using activated carbon as well as bleaching material has proven to be optimal in removing persistent organic material. Using both in combination does not create a safety hazard for the final tuna oil or infant formula to which the oil will be added. Activated carbon is well known as an unlisted GRAS substance, one that is widely used and is accepted as GRAS by both industry and FDA. As illustration, ONC would like to refer to the agency response letter to GRN 138 (APRIL 20, 2004). Paragraphs 7 and 8 refer to the refining steps for ONC fish oil as well as a statement that activated carbon is required for use in fish oil refining. This, coupled with the fact that the use of bleaching material was and still is part of ONC fish oil refining, indicates the agency's acceptance of using both activated carbon and bleaching material in combination in refining fish oil.

Agency Response Letter GRN 138
http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153965.htm

2. ONC provides results of analyses of PBDEs in four lots of tuna oil, but does not provide a narrative accompanying this data. Further, the notice does not present the PBDE levels in crude oil or discuss the ability of the method of manufacture to remove PBDEs. (FDA notes that removal of PBDEs by short-path distillation techniques has been reported in the published literature; the success of other standard oil purification processes has been questioned).

2a. Please provide a discussion of the presence of PBDEs in tuna oil and the removal of PBDEs by the method of manufacture (with reference to published studies where applicable)

The information about PBDEs was provided for completeness with regard to the purity of the refined tuna oil, not because there is any significant degree of hazard associated with PBDEs in ONC's oil. As explained in response to the second part of the FDA question (2b, below), levels of PBDEs in ONC's refined tuna oil are several orders of magnitude below concentrations regarded as potentially presenting a hazard.

In response to the first question regarding the presence of PBDEs in tuna oil, it is important to recognize that, although tuna are predator fish that may bioaccumulate contaminants existing in the food chain, the tuna from which refined tuna oil is derived are pelagic Pacific Ocean fish. As noted by Zennegg and Schmid (2006), "Fish oil collected from species caught in contaminated waters or from farmed fish fed with contaminated feed may contain markedly higher amounts of [persistent organic pollutants] than fish originating from less polluted sites." These authors analyzed concentrations of PBDE and other contaminants in a sample of fish oil products sold in Switzerland, finding low concentrations (PBDE ranges from 0.069 to 0.78 mg/g) "similar to concentrations reported for fish from the relatively uncontaminated Pacific."

Although it is likely that PBDE levels in even the crude oil obtained from these tuna would be relatively low, this has not been tested because, as will be clear from the remainder of this discussion, there is no health concern associated with ONC's intended use of refined tuna oil.

The literature regarding removal of PBDEs from food products, including fish oils, is sparse as compared with the relatively mature literature addressing many other environmental pollutants. As was apparently noted by FDA (based on the comment that "the success of other standard oil purification processes has been questioned"), Oterhals et al. (2007) found that effective active carbon adsorption of persistent organic pollutants is dependent on dispersive electronic interactions affected by sorbate planarity and steric effects, and so active carbon is more effective at reducing coplanar dioxin-like compounds and polycyclic aromatic hydrocarbons than polychlorinated biphenyls and PBDEs.

However, ONC does not depend solely on activated carbon to reduce levels of persistent organic pollutants, but rather on other methods well described in the published literature, including steam deodorization involving reduced pressure and elevated temperature. Oterhals et al. (2010) studied the ability of a process matching ONC's steam deodorization step to reduce levels of persistent organic pollutants in fish oil, including polycholorinated dibenzo-*p*-dioxins and dibenzofurans, dioxin-like polycholorinated biphenyls, and PBDEs. They used 15 samples of crude fish oil and tracked the concentrations before and after processing of six PBDE congeners—BDE-28, -47, -99, -100, -153, and -154—as well as total PBDEs. A 2-factorial design was used in which the experimenters varied both the feed rate and the evaporator

temperature, holding the evaporator pressure constant. In several of the samples, levels of BDE-28, -100, -153, and -154 were reduced to below detection limits. The greatest achieved reduction of BDE-47 was 99.996%, but the greatest reduction of BDE-99 was only 84.6%. The greatest reduction of total PBDEs in any of the 15 samples was 98.6% and the mean reduction over the 15 samples was 62.8%. Following a discussion of the relative efficacy of the various experimental conditions, the authors' conclusion was that "The experimental conditions reduced the chemical concentration of all the studied congeners."

While it is apparent that variation in evaporator pressure and temperature, as well as changes in feed rate, can affect the efficacy of the PBDE reduction process, these published results fully support the ability of the ONC processing methods to lower PBDE concentrations in the crude oil.

2b. Please briefly summarize the significance of this information in relation to the intended use of tuna oil in formulas intended for term and preterm infants.

As noted above, concentrations of PBDEs in ONC's refined tuna oil are very low, and the potential exposure of infants to PBDEs from the intended use of refined tuna oil is orders of magnitude below levels regarded as safe.

The results of analyses of PBDE levels in 4 lots of refined tuna oil are presented in Appendix B of the GRAS notice. While numerous congeners were tested, we here focus on 4 of them—BDE-47, -99, -153, and -209—because these congeners were selected for a toxicity assessment by the European Food Safety Authority (EFSA) in the most extensive research published to date on health effects of PBDEs (EFSA 2011). Further, as discussed in more detail in the following paragraph, only BDE-47 and BDE-99 were detected in the analyses of refined tuna oil—all other congeners were present at only non-detectable levels in all 4 lots tested.

In the analyses of ONC's refined tuna oil, BDE-47 was detected in all 4 lots tested at a mean concentration of 0.089 ng/g (range 0.078 to 0.115 ng/g), while BDE-99 was detected in only one of the 4 lots, at a concentration of 0.055 ng/g. The mean level of BDE-99 in the 4 lots tested, making the conservative assumption that it was present at the limit of detection (LOD) in those lots where it was not actually detected, was 0.050 ng/g. BDE-153 and -209 were not detected in any tested lot; again making the conservative assumption that these congeners were present in all lots at their LODs, the mean concentrations would have been 0.081 and 2.482, respectively. (This latter apparently high level, of course, is an artifact resulting from the relatively high LOD of the test for BDE-209 and not an indication that this congener is actually present at such a level.)

As was calculated in the GRAS notice, the estimated 90th percentile intake of refined tuna oil under its intended conditions of use in infant formula is 160 mg/kg bw/day. Consequently, the estimated 90th percentile exposures to the 4 PBDE congeners are:

BDE-47: 0.014 ng/kg bw/day
BDE-99: 0.008 ng/kg bw/day
BDE-153: 0.013 ng/kg bw/day
BDE-209: 0.397 ng/kg bw/day

In its assessment, EFSA (2011) determined that, for the European population, the highest dietary exposure to PBDEs is due to BDE-47 and -209; BDE-47 was the highest congener in "fish and other seafood" and "food for infants and small children" while BDE-209 was the highest in all other food categories. The estimated mean chronic dietary exposure for BDE-47 is from 0.29 (lower bound) to 1.91 (upper bound) ng/kg bw/day; for BDE-209 the estimated mean dietary exposure is from 0.35 to 2.82 ng/kg bw/day.

As a result of these exposures, PBDEs are found in human milk. EFSA (2011) determined that the BDE-47 concentration in human milk in Europe is 0.14 to 3.0 ng/g fat; BDE-99 is 0.03 to 1.1 ng/g fat; BDE-153 is 0.10 to 2.4 ng/g fat, and BDE-209 is 0.21 to 2.9 ng/g fat. EFSA (2011) estimated the exposure of breastfed infants to PBDEs from their mothers' milk, providing estimates for infants with "average" and "high" milk intake. EFSA exposure estimates for these 4 PBDE congeners are shown in Table 1, along with the estimated 90th percentile exposure from the intended use of ONC's refined tuna oil. The table also includes, in the final column, a "PBDE Exposure Ratio" calculated by dividing both the lower-bound estimated exposure to breastfed infants with average milk intake and the upper-bound estimated exposure to breastfed infants with high milk intake by the 90th percentile estimated exposure from ONC's refined tuna oil.

Table 1.

PBDE Congener	Breastfed Infants		90th Percentile Intake From Formula with ONC Refined Tuna Oil (ng/kg bw/day	PBDE Exposure Ratio
	Infants With Average Milk Intake (ng/kg bw/day)	Infants With High Milk Intake (ng/kg bw/day)		
BDE-47	0.64 to 13.8	0.96-20.6	0.014	46 to 1471
BDE-99	0.14 to 5.05	0.14 to 7.57	0.008	18 to 946
BDE-153	0.46 to 11.0	0.69 to 16.5	0.013	35 to 1269
BDE-209	0.96 to 13.3	1.44 to 20.0	0.397	2 to 50
Source of intake estimates for breastfed infants: EFSA 2011				

As is apparent, the potential exposure to PBDEs from the intended use of ONC's refined tuna oil is far below that of breastfed infants. (And, it must again be noted, congeners BDE-153 and BDE-209 were not actually detected in ONC's oil; the exposure estimates are based on the assumption that all tested samples contained these congeners at the LOD.)

In addition to estimating the exposure of breastfed infants to PBDEs, EFSA (2011) conducted risk assessments for BDE-47, -99, -153, and -209. The critical endpoint for all PBDE congeners was determined to be neurodevelopmental effects in mice. Using the results of experimental studies, EFSA (2011) derived $BMDL_{10}$ values (i.e., lower 95% confidence limits for benchmark responses of 10%) for the 4 congeners. EFSA's $BMDL_{10}$ values are:

BDE-47: 309 µg/kg bw

BDE-99: 12 µg/kg bw

BDE-153: 83 µg/kg bw

BDE-209: 1700 µg/kg bw

Table 2 was constructed based on these $BMDL_{10}$ values, comparing them with the estimated 90th percentile exposure to each congener from the intended use of ONC's refined tuna oil in infant formula and developing a margin of exposure (MOE) for each congener.

Table 2.

PBDE Congener	$BMDL_{10}$ (µg/kg bw)	90th Percentile Intake From Formula with ONC Refined Tuna Oil (µg/kg bw/day	Margin of Exposure
BDE-47	309	0.000014	22,071,429
BDE-99	12	0.000008	1,500,000
BDE-153	83	0.000013	6,384,615
BDE-209	1700	0.000397	4,282,115
Source of $BMDL_{10}$: EFSA 2011			

As can be seen, the MOE for each of the 4 PBDE congeners is well over one million, and that for BDE-47 exceeds 20 million. EFSA (2011) explained the use of the MOE as follows:

> "Usually a MOE of 100, covering uncertainties and variability with respect to kinetic and dynamic differences between animal species and humans (factor 4 × 2.5 = 10) and within the human population (factor 3.2 × 3.2 = 10), is considered sufficient to conclude that there is no health concern. Since the MOE approach is based on a body burden comparison between animals and humans, the potential kinetic differences have been accounted for. Equally, by focussing on the body burden associated with a $BMDL_{10}$ for neurobehavioural effects in mice induced during a relevant period for brain development, and applying this body burden to the entire life span in humans, individual difference in susceptibility has been covered. Therefore, the calculated MOE should be sufficient to cover intraspecies differences in sensitivity for the effects observed. This implies that an MOE larger than 2.5 might indicate that there is no health concern" (p 5).

The exposures to PBDE congeners from ONC's refined tuna oil—which exceed this 2.5 safety threshold by factors of at least 600,000—do not in any way compromise the safety of the intended use of the oil in infant formulas. Since the EFSA (2011) report is generally available to the scientific community, and since its preparation by a recognized authoritative body shows that the conclusions are generally accepted by qualified scientists, it strongly supports the GRAS status of ONC's refined tuna oil.

ONC once again affirms that the intended use of refined tuna oil is both safe and GRAS.

References

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Edwards, Alison

From: Ramos-Valle, Moraima
Sent: Thursday, July 28, 2011 3:23 PM
To: Carlson, Susan; Ditto, Mary D; Mattia, Antonia
Cc: Twaroski, Timothy P; Edwards, Alison; Dinovi, Michael J; Chanderbhan, Ronald F
Subject: FW: Questions on GRN 379
Attachments: Response to Q1a.docx

FYI, I just received this. Did we sent him a note?

From: Jim Heimbach [mailto:jheimbach@va.metrocast.net]
Sent: Thursday, July 28, 2011 3:18 PM
To: Ramos-Valle, Moraima
Subject: Questions on GRN 379

Dear Moraima--

Our response to part a of FDA's first question (Q1a) is attached. This completes our responses to the questions.

Thanks for your patience--
Jim

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Q1a. Please describe deodorization and steam deodorization conditions (e.g., temperature, vacuum), providing citation to published data supporting this methodology, where applicable. If certain details of the method of manufacture are considered confidential by the notifier, these details may be omitted with citation of publicly-available reference(s) (that address removal of contaminants that may be of health concern) and a statement that the conditions employed by ONC meet or exceed those conditions described in those references.

It is first important to understand that the low level of contaminants in ONC's refined tuna oil is due to a multitude of factors and so it is not appropriate to single out one of them—e.g., the steam deodorization or the treatment with activated carbon—and search for evidence supporting the efficacy of that treatment alone in reducing contaminant levels. Obtaining a fish oil with acceptably low levels of contaminants begins with sourcing the oil from fish caught in the relatively uncontaminated open Pacific Ocean rather than more contaminated waters such as coastal zones or the North Sea. It continues with obtaining the crude tuna oil from canners operating under cGMP. Further removal of those contaminants that are present occurs through a cascade of processing steps that include alkali refining, short-path distillation (also known as molecular distillation), bleaching-clay adsorption, treatment with activated carbon, and finally steam deodorization.

Each of these processing steps is maximally effective against certain contaminants and less effective against others. In combination, they have a proven track record (as shown by the analyses of ONC's refined tuna oil that were included with the GRAS notice) in producing a refined tuna oil of sufficient purity that the tiny levels of contaminants remaining in the oil cannot be regarded as potentially constituting a health threat to the infants consuming formula with refined tuna oil added at the intended level.

The overall efficacy of these methods has achieved general recognition in the oil processing industry both through published studies and through years of practice. Most published research addresses only a single process, although Sprague et al (2010) described "double" decontamination of fish oil to remove persistent organic pollutants through a 2-step process—an initial adsorption using activated carbon that removed about 90% of the PCDD/F, followed by a thin-film deodorization step that removed up to 95% of PCBs as well as pesticides and other contaminants, free fatty acids, and peroxides. The authors reported:

> "Previously, removal of 90% of PCDD/F from fish oil could be achieved using activated carbon treatment alone. However, removal of DL-PCB was less effective and required the use of high-temperature deodorisation that could cause oxidation of HUFA. More effective removal of both PCDD/F and DL-PCB can be achieved using activated carbon coupled with short-path distillation as was used in the present study" (Sprague et al. 2010).

Both of these processes are part of ONC's standard processing for its tuna oil.

The recognized conditions for these steps are described below, along with recent published studies of efficacy in the removal of contaminants, especially persistent organic pollutants. In most cases, the exact conditions employed by ONC are not reported other than to state that they meet or exceed the conditions described. However, FDA should be aware that the members of the GRAS Expert Panel, which included experts in chemistry and fish-oil processing (George Pauli and Tony Bimbo), had complete access to all details of ONC's processing conditions in reaching their decision that these methods and conditions are generally recognized as able to produce tuna oil of appropriate purity and that the intended use of the refined oil is both safe and GRAS.

Neutralization (Alkali Refining)

The refining steps for marine oils are essentially the same as those for other edible oils and have been thoroughly described in the literature (e.g., Mounts 1980). Alkali refining temperature parameters are 70° - 80°C for the refining and 90°C for the water washing step. The vacuum drying step is at 58 mmHg to reduce the moisture content to 0.1%. ONC's neutralization step is fully consistent with these conditions.

Deodorization (Short Path Distillation, Molecular Distillation)

Molecular distillation was first used in the National Oceanic and Atmospheric Administration Biomedical Test Materials Program (NOAA 1989) as a substitute for steam deodorization. The process used by NOAA was a 2-stage system. In the first stage the oil was heated to 100°C under a vacuum of 1 mmHg. In this stage, peroxides were destroyed and some low-boiling volatile material was removed. The oil was also degassed in the first stage. The first stage discharge then went to the second stage which operated at 260°C and a vacuum of 0.5 mmHg. In this stage cholesterol, pesticides, and PCBs were removed.

Standard conditions call for a pressure range of 0.001 to 1 mbar (0.00075 to 0.75 mmHg) and a temperature range of 200° - 220°C (Albers and Graverholt 2006). USDA researchers demonstrated that deodorization of soybean oil, contaminated with TCDD-^{14}C to a level of 3.3 ppm, at standard conditions for 1.5 hours reduced the concentration of the contaminant by less than half (Mounts et al. 1976), but that extending deodorization to 3 hours greatly reduced the concentration.

In order to gain greater efficacy, ONC processes tuna oil under greater vacuum and higher temperature than specified by Albers and Graverholt (2006).

Albers and Graverholt (2006) reported that short-path distillation offers the advantage of distillation at considerably reduced pressure and thus reduced evaporation temperature. These economical distillation rates can be achieved in the fine vacuum range, i.e. within the pressure range of 0.001 to 1 mbar. In short-path distillation, the mixture of substances to be evaporated is distributed as a very thin film onto the evaporator surface. According to Albers and Graverholt (2006), approximately 95% of the PCBs, dioxins, and furans can be removed under these conditions. While no data were presented, the authors claimed that similar reductions were achieved for PBDEs.

Decolorization (Adsorbing, Bleaching)

Addison et al. (1974) described the removal of organochlorine pesticides and biphenyls from pre-treated (alkali-refined and neutralized) marine oils by means of clay. The recommended conditions for vacuum bleaching are a temperature of 105°C and pressure of 80 mmHg for 15-20 minutes (Brekke 1980). ONC's decolorization of tuna oil is performed at slightly lower temperature and substantially lower pressure, increasing its efficacy.

Activated Carbon Treatment

Published recommendations for conditions during treatment with activated carbon are more varied. The temperature suggested by DeKock et al. (2002) is 80°C while Maes et al. (2005) recommend 70°C, both for 30 minutes, at pressures of 23 and 37 mmHg, respectively. A more recent regimen, published by Oterhals et al. (2010), employs a brief exposure (1 – 10

seconds) at greatly elevated temperature (200° – 228°C) and lower pressure (0.016 – 0.018 mmHg). The conditions established by ONC for processing of tuna oil are close to those of DeKock et al. (2002).

In research at USDA, bleaching with activated carbon almost totally removed the TCDD-^{14}C from soybean oil initially contaminated to a concentration of 3.68 ppm (Mounts et al. 1976). Neither the length of bleaching time nor the amount of bleaching agent significantly influenced the efficacy of the treatment.

Maes et al. (2005) reported that treatment of contaminated cod liver oil (5.4 ppt TEQ polychlorinated dibenzo-*p*-dioxins and dibenzofurans [PCDD/F] and 18.1 ppt TEQ dioxin-like PCBs) with 0.5% activated carbon, removed almost all PCDD/F and up to 80% of the dioxin-like PCBs. The researchers performed the tests at 70°C and 37 mmHg vacuum for 30 minutes with carbon dosages of 0.1 and 0.5%.

Steam Deodorization

Multiple published sources generally agree on the optimum conditions for steam deodorization, with Brekke (1980), DeKock et al. (2002) and Hernandez (2011) recommending temperatures of 190° - 210°, 150° - 200°, and 180° - 190°C, respectively, and pressures of 1 – 6, 1 – 2, and 1 – 6 mmHg, respectively. DeKock et al. (2002) recommend a processing time of 45 – 90 minutes, while Hernandez (2011) recommends 15 – 120 minutes. ONC's steam deodorization is performed at near the average of the recommended temperature ranges and near the lower boundary of the recommended pressures.

In summary, ONC relies on a series of processing steps to assure a final product, refined tuna oil, of exceptional purity. Each of these processes alone is performed under conditions that meet or exceed those recommended in generally accepted published sources. The combination of processes meets or exceeds all standards for the oil-processing industry, and consistently produces refined tuna oil with contaminant residues far below levels of concern.

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