

Appendix I.

Expert Panel Report

Date 12.02.01
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Your letter of
Your ref:

MERCK

Merck KGaA Darmstadt
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Dear Mr. Andrew Fairfull:

In response to your e-mail from 4.2.01 we would like to inform you the followings

Firstly, enclosed for your files is a copy of the Expert Panel Report, including the signatures of the Expert Panel. The „GRAS“ status of HiDHA Tuna Oil product intended for use in place of Menhaden Oil in traditional foods per 21 C.F.R. 184.1472(a)(3), and in dietary supplements providing up to 1 g/day HiDHA Tuna oil.

Secondly, as you know, many substances added to foods are not food additives and do not require approval by the Food and Drug Administration (FDA) prior to use. These substances, popularly known as “GRAS” substances, are considered to be “generally recognized as safe.” As stated in the Federal Food, Drug, and Cosmetic Act (FDCA), a GRAS substance is

generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use.

21 U.S.C. § 321(s). If a substance is found to be GRAS under the intended conditions of use, FDA approval or notification is not required prior to such use. FDA acknowledges that “under the 1958 amendment [to the FDCA], a substance that is GRAS for a particular use may be marketed for that use without agency review and approval.” 62 Fed. Reg. 18938, 18939 (April 17, 1997). FDA also recognizes that companies “have the right to make independent GRAS determinations on food substances.” 53 Fed. Reg. 16544, 16545 (May 10, 1988).

A specially-convened independent panel of qualified scientific experts recently evaluated the HiDHA Tuna Oil product for use in foods and dietary supplements. After a critical review of the scientific evidence, including, e.g., physical and chemical identity information, manufacturing process, publicly available safety data, corroborating unpublished safety data, the intended uses and consumption estimates, the Expert Panel concluded that, based on scientific procedures, the HiDHA Tuna Oil product is GRAS under the conditions of its intended use in foods.

Accordingly, based on its GRAS status, the Hi-DHA Tuna Oil product may be legally marketed for the conditions of use in foods that were evaluated by the Expert Panel.

If you have any further questions, please do not hesitate to contact us.

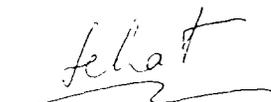
Sincerely,

ppa



Dr. Martin

1 V



Dr. Sehat

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**EXPERT PANEL REPORT:
THE "GRAS" STATUS OF HDHA (DOCOSAHEXAENOIC ACID) TUNA OIL
PRODUCTS INTENDED FOR USE IN PLACE OF MENHADEN OIL
IN TRADITIONAL FOODS PER 21 C.F.R. 184.1472(a)(3), AND IN DIETARY
SUPPLEMENTS PROVIDING UP TO 1 GM/DAY HDHA TUNA OIL**

October 18, 2000

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**EXPERT PANEL REPORT:
THE "GRAS" STATUS OF HiDHA (DOCOSAHEXAENOIC ACID) TUNA OIL
PRODUCTS INTENDED FOR USE IN PLACE OF MENHADEN OIL
IN TRADITIONAL FOODS PER 21 C.F.R. 184.1472(a)(3), AND IN DIETARY
SUPPLEMENTS PROVIDING UP TO 1 GM HiDHA TUNA OIL/DAY**

October 18, 2000

1.0 Introduction

The undersigned, an independent panel of recognized experts (the "Expert Panel"), qualified by their scientific training and relevant national and international experience in evaluating the safety of food and food ingredients, were requested by Merck KGaA ("Merck") and Clover Corporation Limited ("Clover") to conduct a comprehensive and critical evaluation of the available pertinent data and information to determine whether the intended use of the HiDHA* Tuna Oil products manufactured by Clover and marketed by Merck would be "generally recognized as safe" ("GRAS"). The HiDHA Tuna Oil products would be added to traditional foods in place of menhaden oil consistent with good manufacturing practices and the applications described in 21 C.F.R. 184.1472(a)(3). HiDHA Tuna Oil products would also be used in dietary supplements that provide daily intakes of up to 1000 mg. HiDHA Tuna Oil per day. The qualifications of the Expert Panel members are evidenced in their *curricula vitae*, provided in Appendix 1.

2.0 Basis for GRAS Status

The Expert Panel members reviewed proprietary data and information concerning the manufacture and intended use of five HiDHA Tuna Oil products: HiDHA 23N Food, HiDHA 25S Food, HiDHA 25S Softgel, HiDHA 25F Softgel and Driphorm HiDHA Bake. Specifically, the Panel reviewed data and information relating to the physical and chemical properties of the HiDHA Tuna Oil products, the method of manufacture and processing, and the stability of the HiDHA products, the conditions of intended use and the estimated daily intakes resulting from use of the HiDHA Tuna Oils in dietary supplements and as a substitute for menhaden oil in traditional foods. The Expert Panel independently and critically evaluated data and information relevant to the safety and toxicity of tuna oils, docosahexaenoic acid ("DHA") and eicosapentaenoic acid ("EPA") that was found in a search of the published scientific literature from January 1, 1997 through July 14, 2000 conducted by George W. Burdock, Ph.D. and made available to the Panel. In addition, the Panel considered the comments published by the U.S. Food and Drug Administration ("FDA") during the menhaden oil GRAS affirmation rulemaking through June 5, 1997, and other data and information deemed appropriate or necessary by the Panel.

* Registered trademark of Clover Corporation

Following independent, critical evaluation of such data and information, the members of the Expert Panel conferred several times, both with and without representatives of Merck. The Panel then discussed the data and information, individually and collectively, in a series of conference calls. The Expert Panel unanimously concluded that under the conditions of intended use in place of menhaden oil in conventional foods per 21 C.F.R. 184.1472(a)(3) and in dietary supplements of up to 1 gm/day, the HiDHA Tuna Oil products, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, are "generally recognized as safe" ("GRAS") based on scientific procedures. This report summarizes the data and information considered by the Expert Panel and the basis for the Panel's conclusion.

3.0 Chemistry of HiDHA Tuna Oil

To date there have been well over 8,000 publications dealing with fish oils and n-3 (also called "omega-3") fatty acids. Fish oils by their very nature are complex materials. These products have no single chemical name since, like other edible vegetable oils, they consist of complex mixtures of glycerides, fatty acids, unsaponifiables and phospholipids. The CAS registry number for fish oils is 8016-13-5, for menhaden oil it is 8002-50-4, herring oil 68153-06-0 and for cod liver oil 8001-69-2. The CAS number for fish oil, 8016-13-5, is generally used for fish oils, such as tuna oil, that do not have a unique CAS number.

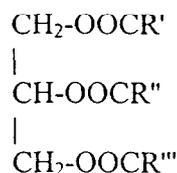
As in the case of other food lipids, fish oils consist mainly of a mixture of triglycerides of various long chain fatty acids with small amounts of mono and diglycerides. The fatty acids that characterize fish oils are similar to those in the various edible vegetable oils and animal fats differing principally in their relatively higher proportion of polyunsaturated fatty acids with five and six double bonds, and tuna oil differs from the other fish oils in the ratio of the C20:5 n-3 (EPA) to the C22:6 n-3 (DHA) fatty acids as indicated below in Table 1:

Table 1. Selected fatty acid content of some common oils and fats (g/100g).

FATTY ACID	TUNA	MENHADEN	SOYBEAN	PEANUT	COTTON SEED	BUTTER	LARD	CANOLA
4:0						3.2		
6:0						1.9		
8:0						1.1		
10:0						2.5	0.1	
12:0						2.8	0.5	
14:0	3.0	9.0	0.2	0.1	0.8	10.1	1.4	
16:0	20.0	19.0	10.7	9.5	22.0	26.3	23.7	4.8
18:0	6.0	3.0	3.9	2.3	2.3	12.1	13.0	1.5
16:1	4.5	12.0	0.3	-	0.8	2.3	2.6	0.5
18:1	15.0	13.0	22.8	45.6	18.1	25.1	40.9	53.2
22:1	1.0	-	-	-	-	-	-	0.2
18:2	1.5	1.0	50.8	31.0	50.3	2.3	10.0	22.2
18:3	1.0	1.0	6.8	-	0.4	1.4	1.4	11.0
20:5	6.0	14.0	-	-	-	-	-	-
22:6	26.5	8.0	-	-	-	-	-	-

Tuna oil has a complex chemical composition similar to other edible lipids. The fatty acids of tuna oil are essentially similar to those of other edible lipids and like them they have a characteristic range of fatty acids. Fatty acids are present possessing 20 and 22 carbon-atoms that, in tuna oil, are polyunsaturated in the *cis* configuration. There is also a minor occurrence of small amounts of the C22:1 isomers C22:1n-9 erucic acid and C22:1n-11 cetoleic acid with erucic acid amounting to less than 0.50% which are in line with the concentrations for menhaden oil. (The C22:1 n-9 (erucic acid) ranges from 0.10 – 0.30% and the C22:1 n-11 (cetoleic acid) + C22:1 n-13 ranges from less than 0.10% - 0.80% in menhaden oil.)

Our examination of the composition of tuna oil shows that it falls within the broad pattern of characteristics exhibited by edible oils as a whole. Tuna oil consists mainly of triglycerides with small amounts of mono- and diglycerides. The triglycerides are esters of fatty acids with chains generally of 14 to 22 carbon atoms and glycerol:



where R represents the fatty acid chains attached to the 1, 2 and 3 positions of the glycerol molecule identified by the superscripts. In mono- and diglycerides, one or two fatty acids, respectively, are esterified with glycerol in the various combinations provided by its three carbon atom positions.

We have used the following terminology to identify fatty acids. The term a:b is used for a straight chain, aliphatic fatty acid having a total of "a" carbon atoms in the molecule with "b" methylene-interrupted ethylenic bonds. Thus stearic acid, a saturated fatty acid (no double bonds), containing 18 carbon atoms is designated 18:0. Oleic acid, a fatty acid with a single double bond and containing 18 carbon atoms is specified as 18:1. The designation 18:1 does not identify the position of the bond or its configuration and can therefore represent monoenoic fatty acids other than oleic with 18 carbon atoms and a single double bond in other positions.

The position of double bonds is indicated using the expanded nomenclature, a:b n-x, in which "n" signifies that the position of the first ethylenic bond is determined relative to the methyl group of the molecule, and "x" is equal to the number of carbon atoms from the terminal methyl group to the first double bond. Thus 18:3n-3 is the shorthand notation for alpha-linolenic acid, an octadecatrienoic acid, with the structure shown below:



Unsaturated fatty acids may be regarded as belonging to families of which the terminal structures are the same, so that the fatty acids designated with n-3, n-6, and n-9 are said to belong to the linolenic, linoleic and oleic acid families, respectively.

In addition to the above variations in configuration, fatty acids of the same a:b n-x structure may exist as alternative geometric isomers. In the *cis* form, the sections of the molecule on both sides of the double bond lie on the same side, the molecule being bent back on itself. In the *trans* form, the sections bend in opposite directions. The two forms have different physical properties and potential metabolic implications. Most naturally occurring isomers have the *cis* configuration.

The comprehensive fatty acid analyses for a range of food lipids published by Sheppard et al. (Sheppard, A. J., J. I. Iverson, and J. I. Weihrauch, 1978). Composition of selected dietary fats, oils, margarines, and butter, in fatty acids and glycerides are provided in Volume 1 of the Handbook of Lipid Research, ed. A. Kuksis, Plenum Publ. Co., New York., and reported in the menhaden oil GRAS affirmation petition. These data provide a means for comparing the composition of tuna oil with a variety of other vegetable oils, animal fats and menhaden oil used in the human diet. All analyses were conducted by the same scientists. Representative figures from the Handbook of Lipid Research are shown in Table 2 below.

The principal fatty acids of tuna oil are mostly straight-chained, aliphatic, monocarboxylic fatty acids, with carbon atom chain lengths of 14 to 22, occurring in even numbers of carbon atoms. In common with other food lipids, fatty acids of chain length up to 18 carbon atoms are present in fully saturated form. At chain lengths of 18 or more carbon atoms, fully saturated fatty acids have limited solubility at body temperature.

Table 2. Fatty acid analysis for a range of food lipids.

FATTY ACID	TUNA	MEN-HADEN	SOY-BEAN	SUN-FLOWER	PEANUT	COTTON SEED	CANOLA	OLIVE	SESAME	CORN	SAF-FLOWER	BUTTER	LARD	BEEF TALLOW	CHICKEN FAT
4:0												3.2			
6:0												1.9			
8:0												1.1			
10:0						0.5						2.5	0.1	0.1	0.2
12:0			0.1			0.4			0.3			2.8	0.5	0.9	1.0
14:0	3.0	9.0	0.2	0.1	0.1	0.8			0.1		0.10	10.1	1.4	3.7	1.2
16:0	22.0	19.0	10.7	5.8	9.5	22.0	4.8	11.5	9.4	10.7	6.4	26.3	23.7	24.8	23.8
18:0	6.0	3.8	3.9	4.1	2.3	2.2	1.5	2.3	4.8	1.7	2.5	12.1	13.0	18.7	6.4
20:0	1.0		0.2	0.3	1.4	0.2	0.6	0.4	0.6	0.3	0.5		0.8		
Sat.	32	33.3	15.1	10.3	17.3	26.1	6.9	14.2	15.2	12.7	9.5	62.3	39.5	48.2	32.6
14:1												1.5		1.6	
16:1	3.0	13.3	0.3	0.1		0.8	0.5	1.0	0.3	0.1	0.6	2.3	2.6	4.7	5.8
18:1	21.0	15.5	22.8	21.7	45.6	18.1	53.2	71.5	39.1	24.6	11.9	25.1	40.9	36.0	39.7
20:1	1.0	1.7			1.2		1.0		0.2						
22:1	3.0	0.7					0.2		0.4						
Mono	28	31.2	23.1	21.8	46.8	18.9	54.9	72.5	40.0	24.7	12.5	28.9	43.5	42.3	45.5
18:2	1.0	2.0	50.8	66.4	31.0	50.3	22.2	8.2	40.0	57.3	73.3	2.3	10.0	3.7	16.5
18:3	1.0	1.0	6.8	0.3		0.4	11.0	0.7	0.5	0.8	0.5	1.4	1.4	0.6	1.1
18:4	1.9	2.4													
20:4	2.0	1.0													
20:5	6.0	12.5													
22:5	2.0	1.7													
22:6	22.0	7.9													
PUFA	36	28.5	57.6	66.7	31.0	50.7	33.2	8.9	40.5	58.1	73.8	3.7	11.4	4.3	17.6

4.0 Specifications

The HiDHA Tuna Oils consist of four products in oil form, HiDHA 23N Food, HiDHA 25S Food, HiDHA 25S Softgel, and HiDHA 25F Softgel, and one product in powder form, Driphorm HiDHA Bake. All products are derived from the same raw tuna oil material and processed in the same plant using essentially the same process route (see manufacturing description in Section 5.0 below). The only differences are the degree of bleaching (number of bleach cycles) and winterization (the temperature of fractional crystallization) employed during processing. The HiDHA 23N Food is a refined, non-winterized oil containing a minimum of 23% DHA. The HiDHA 25S Food is a refined, semi-winterized oil containing no less than 25% DHA. These two products are intended for use in traditional foods.

The HiDHA 25S Softgel and the HiDHA 25F Softgel are intended for use in the soft gelatin capsules of dietary supplements. These products are subjected to a low-temperature crystallization process (winterization) that enables them to remain clear and resistant to haze formation at low temperatures. The HiDHA 25S Softgel is a refined, semi-winterized oil containing at least 25% DHA. The HiDHA 25F Softgel is a refined, fully-winterized (at 0°C) oil that contains a minimum of 25% DHA.

The four HiDHA Food (23 N and 23 S) and Softgel (25 S and 25F) products contain 2000 ppm of an antioxidant. The antioxidant consists of 70% mixed natural tocopherols and 30% vegetable oil. The tocopherols are listed as GRAS in 21 C.F.R. § 182.3890 for use in foods in accordance with good manufacturing practice. The specific ingredients found in the Driphorm HiDHA Bake product are discussed further in Section 5.0 below.

The specifications for the five HiDHA Tuna Oil products are as follows:

Table 3. Specifications for the HiDHA Tuna Oil products.

SPECIFICATION	HiDHA 23N Food	HiDHA 25S Food	HiDHA 25S Softgel	HiDHA 25F Softgel	Driphorm HiDHA Bake
Appearance	Cloudy, pale yellow oil	Cloudy, pale yellow oil	Clear, pale yellow oil	Clear, pale yellow oil	Fine, light-tan powder
Odor	Faint fresh fish	Faint fresh fish	Faint fresh fish	Faint fresh fish	Bland
Docosahexaenoic Acid (DHA), %	23% minimum	25% minimum	25% minimum 28% maximum	25% minimum 28% maximum	18% minimum 25% maximum
Eicosapentaenoic Acid (EPA) %	5% minimum	5% minimum	5% minimum 8% maximum	5% minimum 8% maximum	4 % minimum 6% maximum
Total Omega 3 Fatty Acid Content	32% minimum	34% minimum 40% maximum	34% minimum 40% max.	34% minimum 40% maximum	26% minimum 34% maximum
Acid Value, mg KOH/g	0.5 maximum	0.5 maximum	1.0 maximum	1.0 maximum	NA
Peroxide Value, O ₂ /kg	1.0 maximum	1.0 maximum	5.0 maximum	5.0 maximum	NA
p-Anisidine Value	10 maximum	10 maximum	20 maximum	20 maximum	NA
Color, Lovibond 1” Gardner	0.5R, 10.0Y 4 maximum	0.5R, 10.0Y 4 maximum	1.0R, 20.0Y 7 maximum	1.0R, 20.0Y 7 maximum	NA
Unsaponifiable Matter, %	2.0% maximum	2.0% maximum	2.0% maximum	2.0% maximum	NA
Cold Test	NA	Passes 8°C for 5.5 hours	Passes 8°C for 5.5 hours	Passes 0°C for 3 hours	NA
Lead, ppm	0.1 max.	0.1 maximum	0.1 maximum	0.1 maximum	NA
Mercury, ppm	0.1 maximum	0.1 maximum	0.1 maximum	0.1 maximum	NA
Cadmium, ppm	0.1 maximum	0.1 maximum	0.1 maximum	0.1 maximum	NA
Arsenic, ppm	0.1 maximum	0.1 maximum	0.1 maximum	0.1 maximum	NA
Total Heavy Metals, ppm	2.0 maximum	2.0 maximum	2.0 maximum	2.0 maximum	NA
DDT ppm	0.05 maximum	0.05 maximum	0.05 maximum	0.05 maximum	NA

SPECIFICATION	HiDHA 23N Food	HiDHA 25S Food	HiDHA 25S Softgel	HiDHA 25F Softgel	Driphorm HiDHA Bake
DDE, ppm	0.05 maximum	0.05 maximum	0.05 maximum	0.05 maximum	NA
HCB, ppm	0.05 maximum	0.05 maximum	0.05 maximum	0.05 maximum	NA
PCB, ppm	0.10 maximum	0.10 maximum	0.10 maximum	0.05 maximum	NA
Lindane, ppm	0.05 maximum	0.05 maximum	0.05 maximum	0.05 maximum	NA
Yeast and Mould (cfu/g)	<100	<100	<100	<100	NA
Standard aerobic plate count (cfu/g)	<100	<100	<100	<100	<1000
Enterobacteriaceae (cfu/g)	<100	<100	<100	<100	NA
E. coli	ND in 1 g.	ND in 1 g.	ND in 1 g.	ND in 1 g.	NA
Salmonella spp.	ND in 10 g.	ND in 10 g.	ND in 10 g.	ND in 10 g.	ND in 25 g.
Coliforms (37°C)	NA	NA	NA	NA	ND in 0.1 g.
Coagulase positive staph.	NA	NA	NA	NA	ND in 0.01 g.
Baccillus cereus	NA	NA	NA	NA	ND in 0.01 g.

Several analytical methods are employed to determine compliance with specifications. The analytical methods from the Official Methods and Recommended Practices of the American Oil Chemists' Society (AOCS) 5th Edition First Printing, (AOCS 1998) that are applied to the HiDHA products are:

<u>Determination</u>	<u>Official Method</u>
Iodine value	Cd 1-25 (1997)
Unsaponifiable matter	Ca 6b-53 (1997)
Color (Gardener)	Td 1a-64 (1997)
Color Lovibond (British Std.)	Cc 13e-92 (1997)
Acid Value/Free fatty acid	Ca 5a-40 (1997)
Peroxide value	Cd 8b-90 (1997)
p-Anisidine Value	Cd 18-90 (1997)
Cold Test	Cc 11-53 (1997)
Docosahexaenoic Acid	Ce 1b-89 (1999) % area method
Eicosapentaenoic Acid	Ce 1b-89 (1999)% area method
Total Omega 3 Fatty Acids	Ce 1b-89 (1999) % area method

Additional analytical methods used for the HiDHA products are:

<u>Determination</u>	<u>Official Method</u>
Microbiological tests	Consulchem M35*
Lead	MAL 130 [⊗]
Mercury	MAL 056 [♦]
Cadmium	MAL 130 [⊗]
Arsenic	MAL 005 [†]
Total Heavy Metals	Food Chemicals Codex IV p. 760
DDT	JAOAC Vol. 67 No. 2 (1984)
DDE	JAOAC Vol. 67 No. 2 (1984)
HCB	JAOAC Vol. 67 No. 2 (1984)
PCB	JAOAC Vol. 67 No. 2 (1984)
Lindane	JAOAC Vol. 67 No. 2 (1984)

* An internal method used by Consulchem P/L Laboratory for determining microbial levels in oils, which includes Standard Aerobic Plate Count (SAPC), Enterbacteriaceae, E. Coli, Salmonella, Yeasts and Molds, based on BP1993, AS 1766 and AS 4275.

⊗ An internal method used by Dunn Son and Stone Laboratory to determine copper, cadmium, lead and iron in vegetable and marine oils, based on APHA 3110 (20th ed).

♦ An internal method used by Dunn Son and Stone Laboratory to determine mercury in fish products, based on AOAC 977.15 to suit fish oils.

† An internal method used by Dunn Son and Stone laboratory to determine arsenic in foods, based on APHA 3114B to suit fish oils.

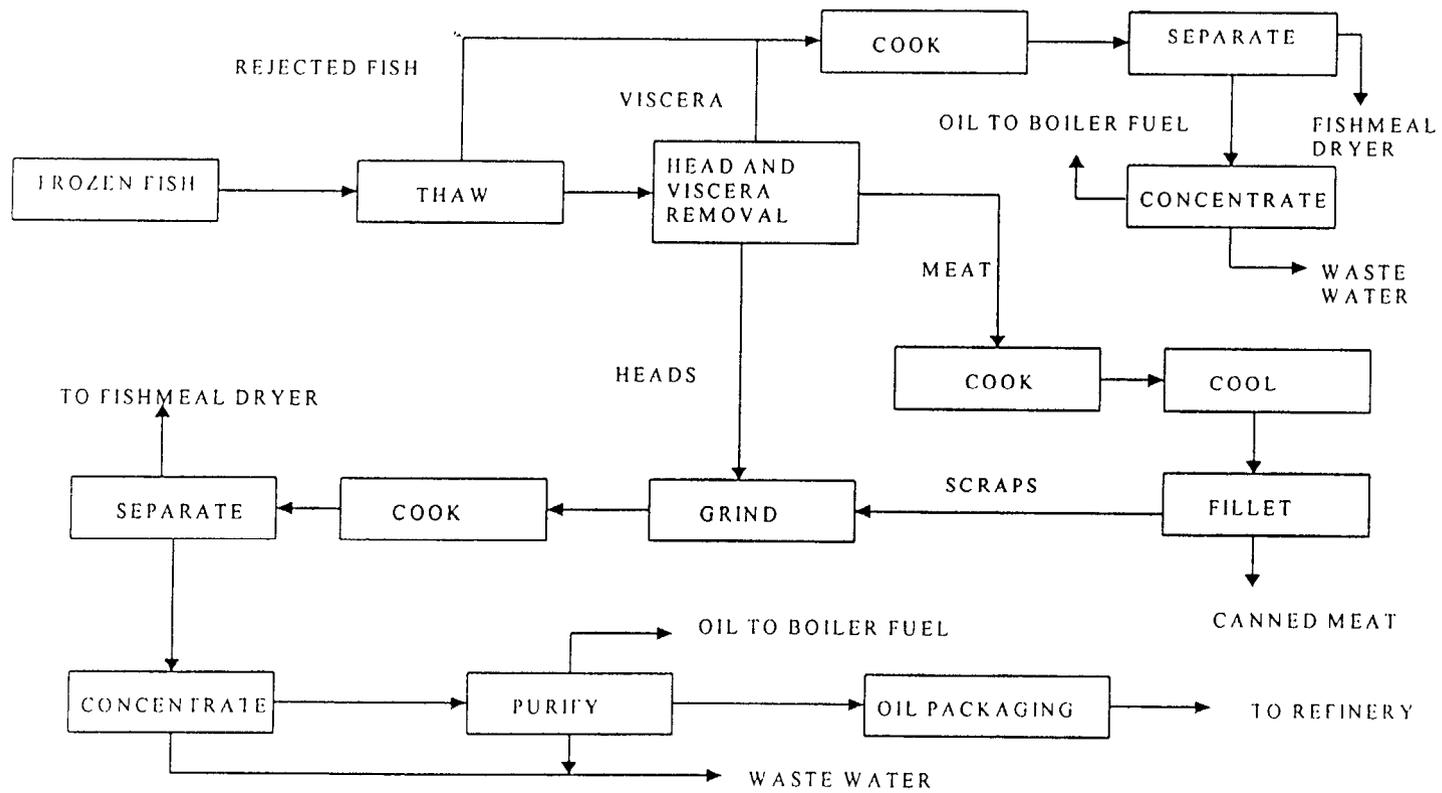
5.0 Manufacturing

HiDHA Tuna Oil is manufactured from Raw Tuna Oil, a by-product of the edible tuna canning industry. The tuna species harvested for production of Raw Tuna Oil come from the relatively unpolluted, pristine waters of the South and Central Pacific Ocean. These species include skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*), albacore (*Thunnus alulunga*), and bigeye (*Thunnus obesus*). All of these species have been widely consumed by humans since time immemorial.

The canneries in American Samoa from which the raw material comes are all dolphin friendly (i.e. there is no by-catch of dolphins) and have been that way since the beginning of the 1990's. The tuna fishery in the South and Central Pacific Ocean is the largest in the world with total catches exceeding 1 million short tons annually since 1989 with minor variations from year to year. Annual fluctuations in catch are, in general related to many factors, such as the process of reproduction, survival of fish especially in the early stage of life and oceanographic conditions such as EL Nino's effect, economic factors such as consumer demand and the market price for tuna. The tuna stocks are under no threat and the existing level of harvesting is sustainable.

Tuna are caught by high speed fishing vessels using longlines or a device called a medina cloth which enables dolphins diving deep to escape through the open bottom of the net. The catch is immediately blast frozen to below -35° C and kept at that temperature until the vessel arrives at the cannery. The tuna are removed from the cold storage and allowed to partially thaw to -5° C before further processing. The head is then removed and used for the production of Raw Tuna Oil. The intestines (viscera) including the liver are removed, segregated and processed separately in a completely separate processing plant. Oil from the processing of the heads is not mixed with oil from the processing of the viscera. The eviscerated tuna bodies are cooked for a short period of time and filleted, with the choice meat going forward to the canning operation. The off-cuts from filleting, the skin, the frame, red muscle meat and the belly flaps are combined with the heads and further processed as shown in the flow diagram in Figure 1.

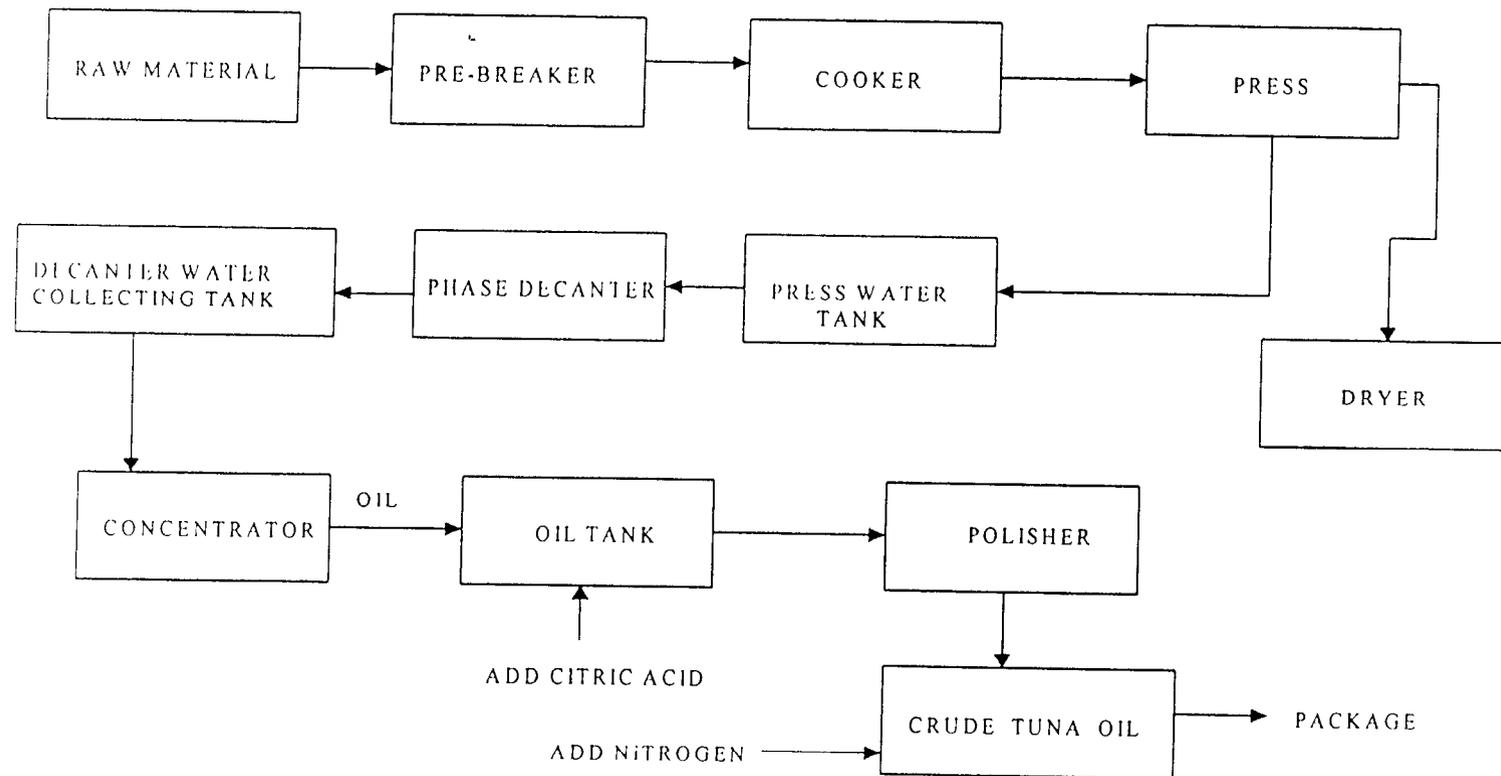
FIGURE 1. TUNA PROCESSING OPERATION



The liquid stream (presswater) generated in the short residence time cooker is separated from fine solids in a decanter centrifuge and the Raw Tuna Oil is separated from the aqueous phase with a separator centrifuge. The oil is then treated with a citric acid solution to chelate pro-oxidant metal ions. The moisture in the Raw Tuna Oil at this stage is less than 0.20% and most of the metal ions capable of initiating oxidation of the oil are removed along with the aqueous phase in this step of the process.

The Raw Tuna Oil is then pumped into a stainless steel storage tank and cooled to ambient temperature while being continuously purged with food grade nitrogen. At the end of the production day, the raw tuna oil is pumped into containers that have been purged with nitrogen and are sealed after filling with oil under a nitrogen blanket. The stabilized Raw Tuna Oil is then shipped to the refinery in Australia for further processing. A flow diagram showing more detail in the production of the Raw Tuna Oil is shown in Figure 2.

FIGURE 2. CRUDE TUNA OIL PROCESSING DETAILS



The purchasing specifications for the Raw Tuna Oil are provided in Table 4 below.

Table 4. Specifications for Raw Tuna Oil.

SPECIFICATION	LIMITS
Product Name	Stabilised Crude Tuna Fish Oil
Description	Dark Brown Free Flowing Liquid
Docosahexaenoic Acid (DHA), %	23.5% minimum
Eicosapentaenoic Acid (EPA), %	7.5%, minimum
Total Omega-3 Fatty Acids, %	33.0% minimum
Iodine Value	175 minimum
Moisture and Impurities, %	1.0% maximum
Unsaponifiable Matter, %	2.0% maximum
Free Fatty Acids, %	4.0% maximum
Anisidine Number	240 maximum
Cholesterol, mg/g	22 maximum
Vitamin A (Retinol), I.U./ml	500 maximum
Vitamin D3, I.U./ml	700 maximum
Arsenic, ppm	0.10 maximum
Cadmium, ppm	0.10 maximum
Copper, ppm	0.50 maximum
Lead, ppm	0.50 maximum
Mercury, ppm	0.10 maximum
PCB's, ppm	0.05 maximum
DDT/DDE, ppm	0.05 maximum
HCB, ppm	0.02 maximum
Lindane, ppm	0.02 maximum

The refining process includes the application of all or some of the accepted food processing techniques of winterization, degumming, neutralization, bleaching, and deodorization, as appropriate, to provide oils that may be used for foods. The tuna oil refining plant was designed specifically for refining tuna oil and uses unit processes that are standard throughout the edible oil industry. The factory is licensed by the Australian Therapeutic Goods Administration and operates in accordance with pharmaceutical standard Good Manufacturing Practices ("GMPs"). The premises and the manufacturing processes have qualified for ISO 9002 and AQIS certification, and are operated to comply with a strict environmental protection code. An overview of the tuna oil refining process that converts the Raw Tuna Oil into HiDHA Tuna Oil is shown in Figure 3.

As noted above, the five HiDHA Tuna Oil products, HiDHA 23N Food, HiDHA 25S Food, HiDHA 25S Softgel, HiDHA 25F Softgel and Driphorm HiDHA Bake, are derived from the same Raw Tuna Oil using the same fundamental process. In the Driphorm HiDHA Bake product, the tuna oil is microencapsulated to produce a dry powder, whereas the other four HiDHA products remain in the oil form. The main differences among the four oils are the extent to which the oils are bleached and winterized. The oils intended for use in traditional foods, HiDHA 23N Food and HiDHA 25S Food must have better oxidative stability, color and taste than the oils used in dietary supplements. This means that they must undergo repeated cycles of bleaching to ensure very low acid values, peroxide values and anisidine numbers. The color of the oil is also improved by this intensive adsorption treatment. The HiDHA 25S Food differs from the HiDHA 23N Food in terms of the DHA content (25% vs. 23%) and the winterization treatment. The N designation means the product was subjected to no winterization while the S means a semi-winterization step (at 5° C) was employed.

The two grades of HiDHA Tuna Oil intended for use in the soft gelatin capsules of dietary supplements, HiDHA 25S Softgel and HiDHA 25F Softgel, must be of high quality especially in terms of appearance. These oils must be clear and transparent even at low temperatures. To ensure this, the oils are subjected to the low temperature crystallization process termed winterization. Product HiDHA 25F Softgel has been given a more complete or full winterization treatment at 0° C (denoted by F).

The Driphorm HiDHA Bake produced from the HiDHA 23N Food using a patent-pending microencapsulation process. Essentially, the process is based on the use of high shear mixers for manufacturing a stable emulsion with an average droplet size of less than 2 microns. The oil is mixed with a carbohydrate matrix material and or whey protein concentrate along with emulsifiers, antioxidants, edible mineral salts, humectants, artificial flavoring, and chelating agents, all of which have GRAS or Food Additive approval. The resultant mixture forms a stable emulsion which is then spray dried by conventional methods to form a free flowing powder containing about 23% oil.

The manufacturing processes described previously are all state of the art processes which have been previously described in the menhaden oil GRAS petition. The microencapsulation process employs food grade ingredients to form the stable emulsion and a spray drying process that is used to produce a variety of edible food products including dry skim milk powder and protein concentrates among others. Microencapsulation is used to produce edible flavorings including spices, vitamin and mineral mixes. Microencapsulated fish oil products are currently available in the United States and being marketed for use in a variety of edible foods such as breads and soup mixes. All the ingredients used in the manufacture of the Driphorm HiDHA Bake powder are permitted for use in foods as shown in Table 4 below:

Table 4. Ingredients used in manufacture of Driphorm HiDHA Bake.

Ingredient	FDA Regulatory Status
HiDHA Tuna Oil	
Hydrogenated peanut oil	GRAS
Mono- and Diglycerides of edible fatty acids	21 C.F.R. § 184.1505
Ascorbyl palmitate	21 C.F.R. § 182.3149
Modified food starch	21 C.F.R. § 172.892
Citric acid	21 C.F.R. § 184.1033
Maltodextrin	21 C.F.R. § 184.1444
Sodium caseinate	21 C.F.R. § 182.1748
Sodium ascorbate	21 C.F.R. § 182.3731

6.0 Analytical Data

The fatty acid composition of tuna oil has been known for some time. Early data on the fatty acid profile was generated by packed column gas chromatography and this was later replaced by the currently acceptable method of capillary column gas chromatography (both AOCS and AOAC approved methods). The early data is presented here only to show that data has been available on tuna oil for over 30 years. In 1967, Gruger reviewed the fatty acid composition of a number of fish, shellfish, and liver oils. Tuna oils were included and are reproduced in Table 5 below.

Table 5. Fatty acid composition of tuna oils, determined by packed column gas chromatography.

Fatty Acid	Tunny	Albacore	Blue Fin	Blue Fin	Blue Fin darkmeat	Skipjack	Yellowfin
C14:0	5.9	3.7	1.9	4.5	4.9	7.0	2.6
C15:0		1.0		0.6	0.7	1.0	0.6
C16:0	19.2	29.3	15.4	22.1	25.7	24.0	27.1
C16:1	11.5	6.3	4.8	2.8	3.5	6.3	4.4
C17:0		1.2	1.2	0.8	0.7	1.1	2.1
C18:0	4.6	6.1	8.2	6.1	12.5	3.0	7.5
C18:1	12.8	16.6	21.2	21.7	14.5	16.2	17.8
C18:2	2.2	0.7	1.7	0.8	0.6	2.1	0.9
C18:3	1.2	0.6				1.2	0.4
C18:4	4.4	2.2		0.9	1.0	0.5	
C20:1	1.2	2.7	7.3	6.3	3.2	2.0	1.1
C20:4	2.7	1.2		1.0	1.4	3.0	3.6
C20:5	13.9	6.5	6.8	6.4	5.5	13.2	4.6
C22:1	1.3	2.0	2.0	5.4	2.1		
C22:5	2.2	0.8	4.5	1.4	1.8	1.5	1.3
C22:6	17	17.6	24.4	17.1	21.0	17.3	22.2

Generally, these data show that about 15 fatty acids account for most of the composition of tuna oil from various species and that in most cases the DHA content exceeds the EPA content. The 15 fatty acids are generally present to some degree or other in most of the edible fats and oils that are currently in the U.S. diet.

In 1998, Bimbo published a paper in the American Oil Chemists publication, *Inform*. The paper entitled "Guidelines for Characterizing Food Grade Fish Oil" included a table reporting the typical fatty acids in some commercially available marine oils. The data was collected from producers of these oils worldwide and included tuna oil in the table. This table is of interest because it contains relatively recent information collected from manufacturers and was generated with new analytical methodology, capillary column gas chromatography as opposed to the packed column methodology presented in the previous table. The data are reproduced in Table 6 below:

Table 6. Fatty acid composition of marine oils, determined by capillary column gas chromatography.

Fatty Acid	Anchovy	Jack Mackerel	Menhaden	Sardine	Capelin	Herring	Mackerel	Norway Pout	Sand Eel	Sprat	Tuna
C14:0	9	8	9	8	7	7	8	5	7		3
C15:0	1	1	1	1					1		1
C16:0	17	18	19	18	10	17	14	12	13	17	22
C16:1	13	8	12	10	10	6	7	4	5	7	3
C17:0	1	1	1	1							1
C18:0	3	3	3	3	1	2	2	3	2	2	6
C18:1	10	16	11	13	14	14	13	10	7	16	21
C18:2	1	1	1	1	1	1	1	1	2	2	1
C18:3	1	1	1	1	1	2	1	1	1	2	1
C18:4	2	2	3	3	3	3	4	3	5		1
C20:1	1	2	1	4	17	15	12	13	12	10	1
C22:1	1	1		3	15	19	15	17	18	14	3
C20:5	22	13	14	16	8	6	7	9	11	6	6
C22:5	2	2	2	2		1	1	1	1	1	2
C22:6	9	15	8	9	6	6	8	14	11	9	22
Others*	7	8	14	7	7	1	7	7	4	14	6
EPA/DHA	2.44	0.87	1.75	1.78	1.33	1.00	1.00	0.64	1.00	0.67	0.27

*Other fatty acids include C16:2, C16:3, C16:4, and C20:4.

Source: Inform Vol. 9, No. 5 (May 1998) pages 473-481.

These data also show that the composition of the major marine oils of commerce can be described with 15 fatty acids, and that tuna oil can be differentiated from the other marine oils by the ratio of EPA to DHA. While most of the other oils have an EPA to DHA ratio of 0.6 –2.44, tuna oil has a ratio of 0.27. This ratio should make it quite easy to distinguish tuna oil from other marine oils and from other edible fats and oils when incorporated into foods. Typical gas chromatograms for the HiDHA 23N Food, the HiDHA 25S Food, the HiDHA 25F Softgel and the HiDHA 25S Softgel products are provided in Appendix 2. The actual fatty acid data computed from the chromatograms appears in the following Table 7.

Table 7. Typical fatty acid composition of the 4 HiDHA Tuna Oil products.*

Fatty Acid	HiDHA 23N Code 7005 Batch EC101	HiDHA 23N Code 7005 Batch EG141	HiDHA 25S Code 8505 Batch EC181	HiDHA 25S Code 8501 Batch EE191	HiDHA 25S Code 8501 Batch DK091	HiDHA 25F Code 9004 Batch EA 201
C14:0	3.14	2.88	3.01	2.81	2.51	2.55
C15:0	0.92	1.00	0.86	0.80	0.87	0.79
C16:0	18.77	20.17	17.40	16.77	16.83	16.89
C17:0	1.06	1.30	0.99	0.99	1.02	0.98
C18:0	4.80	5.88	4.63	4.82	4.97	4.83
C20:0	0.28	0.66	1.02	0.67	0.79	0.72
C22:0	0.21	0.28	0.20	0.49	0.45	0.19
C24:0	0.21	0.26	0.28	0.27	0.26	0.27
Saturates	29.36	32.52	28.41	27.62	28.00	27.02
C14:1	0.21	0.19	0.21	0.18	0.21	0.19
C15:1	0.12	0.12	0.11	0.10	0.10	0.10
C16:1 n-9	0.33	0.28	0.31	0.29	0.53	0.33
C16:1 n-7	3.85	3.77	3.84	3.80	3.83	3.46
C16:1 n-6	0.68	0.56	0.61	0.62	0.48	0.54
C17:1	0.65	0.78	0.71	0.75	0.78	0.80
C18:1 n-9	11.51	12.91	12.21	12.79	13.26	13.73
C18:1 n-7	2.36	2.24	2.27	2.14	2.26	2.09
C18:1 n-6	0.10	0.15	0.11	0.10	0.17	0.17
C20:1 n-11	0.44	1.03	0.64	0.92	0.39	0.25
C20:1 n-9	0.17	0.14	0.14	0.14	0.14	0.12
C22:1 n-11	0.43	0.37	0.45	1.03	0.48	0.32
C22:1 n-9	0.62	0.29	0.75	0.21	0.15	0.17
C24:1	0.55	0.56	0.53	0.54	0.56	0.57
Monounsaturates	22.01	23.38	22.79	23.31	23.33	22.87
C16:2 n-4	0.10	0.10	0.10	0.10	0.10	0.11
C18:2 n-6	1.63	1.09	1.50	1.50	1.21	1.29
C20:2 n-6	0.19	0.20	0.21	0.27	0.22	0.28
C16:3 n-3	0.89	1.13	0.91	0.66	0.38	0.88
C18:3 n-6	0.23	0.22	0.25	0.26	0.33	0.21
C18:3 n-3	0.79	0.50	0.76	0.71	0.53	0.53
C20:3 n-6	0.26	0.22	0.27	0.26	0.20	0.24
C18:4 n-3	1.08	0.38	1.20	1.61	1.35	1.63
C20:4 n-6	1.67	1.86	1.82	2.00	2.03	1.99
C20:4 n-3	0.20	0.20	0.20	0.32	0.20	0.29
C22:4 n-6	0.26	0.17	0.25	0.29	2.03	0.22
C20:4 n-3	0.20	0.20	0.20	0.32	0.20	0.29
C20:5 n-3	7.33	6.00	7.09	6.67	6.61	7.04
C22:5 n-6	1.58	2.57	1.83	1.91	2.20	1.83
C22:5 n-3	1.11	1.18	1.16	1.28	1.29	1.23
C22:6 n-3	26.04	25.1	26.62	26.74	26.92	27.36
Polyunsaturates	43.34	39.92	44.16	44.77	44.71	45.13
Others	5.29	4.18	4.64	4.30	3.96	4.98
EPA/DHA	0.28	0.24	0.27	0.25	0.25	0.26

* HiDHA 25S Food and HiDHA 25S Softgel originate from the same oil.

A number of batches of Raw Tuna Oil have been analyzed to determine the variation in the fatty acid composition of the oil. These data appear in Tables 8 and 9 below, and represent batches of Raw Tuna Oil produced in Samoa in April and May 1997, and analyzed during June 1997. From these data, we find that the total amount of saturated fatty acids in tuna oil is between 27 and 30%. The major fatty acids in this fraction are C14:0 less than 3.3%, C16:0 less than 18%, and C18:0 less than 5%. The mono-unsaturated fraction is between 22 and 25% of which the major fractions are C16:1n-7 0.5%, and C18:1n-9 less than 14%.

There is a very slight trace of *trans* fatty acids in raw tuna oil amounting to less than 1.0% with C16:1 *trans* accounting for more than half of the total *trans* fatty acids. Processing of the raw tuna oil into the Hi DHA Tuna Oil products has very little effect on the total *trans* fatty acids in the oil as can be seen in Table 9.

The total polyunsaturated fraction which includes both the n-3 and n-6 fatty acids is between 40 and 50%. The n-6 fraction ranges from 6-8% and is comprised of C18:2n-6 (linoleic acid) less than 2% and C20:4n-6 (arachidonic acid) approximately 2%. The erucic acid fraction C22:1 n-9 is less than 0.5% in tuna oil. The total n-3 fraction is between 32 and 40% of which C18:4n-3 is around 1%, C20:5 n-3 is between 5 and 7%, C22:5n-3 is about 2% and C22:6n-3 is between 24 and 30%. The ratio of EPA to DHA ranges from 0.22 to 0.27.

Table 8. Fatty acid composition of Raw Tuna Oil produced in April and May 1997.

Fatty Acid	Batch 135F (5/16/97)	Batch 139F (5/20/97)	Batch 140F (5/21/97)	Batch 142F (5/23/97)	Batch 128F (5/9/97)	Batch 129F (5/12/97)	Batch 132F (5/13/97)	Batch 134F (5/15/97)
C14:0	2.7	2.8	2.9	2.9	2.8	2.8	2.7	2.7
C15:0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
C16:0	18.4	18.6	18.1	18.2	18.2	18.3	18.2	18.4
C17:0	1.3	1.3	1.2	1.2	1.3	1.3	1.3	1.3
C18:0	6.0	6.3	5.7	5.7	5.9	5.9	5.9	6.0
C20:0	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.4
C22:0	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3
Saturates	30	30.6	29.2	29.4	29.7	29.8	29.6	29.8
C16:1n-9	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
C16:1n-7	3.4	3.6	3.4	3.4	3.1	3.1	3.3	3.4
C17:1	0.9	0.9	0.8	0.8	0.8	0.9	0.9	0.9
C18:1n-9	13.6	12.7	13.0	13.1	12.3	12.4	12.9	13.5
C18:1n-7	2.1	2.2	2.2	2.2	1.9	1.9	2.1	2.1
C20:1n-11	1.3	1.5	1.5	1.5	1.5	1.5	1.8	1.3
C20:1n-9	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
C22:1n-11	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.5
C22:1n-9	0.3	0.2	0.4	0.4	0.5	0.5	0.3	0.3
C24:1n-9	0.6	0.6	0.6	0.6	0.5	0.5	0.6	0.6
Monoenes	23.0	22.5	22.9	22.9	21.5	21.6	22.6	23.0
C18:2n-6	1.3	1.2	1.4	1.4	1.4	1.4	1.2	1.3
C20:2n-6	0.4	0.5	0.4	0.4	0.5	0.5	0.5	0.4
C18:3n-6	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2
C18:3n-3	0.4	0.4	0.5	0.5	0.5	0.5	0.4	0.4
C20:3n-6	0.2	0.3	0.2	0.2	0.3	0.3	0.4	0.2
C18:4n-3	0.6	0.5	0.8	0.8	0.6	0.6	0.4	0.6
C20:4n-6	2.2	2.6	2.1	2.1	2.2	2.2	2.6	2.2

C20:5n-3	5.7	5.7	6.8	6.8	6.0	6.0	5.9	5.7
C22:4n-6	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
C22:5n-6	2.2	2.3	1.8	1.8	2.0	2.0	2.2	2.2
C22:5n-3	1.2	1.3	1.2	1.2	1.1	1.1	1.2	1.2
C22:6n-3	25.4	24.5	25.2	25.0	26.8	26.4	25.6	25.5
PUFA	39.8	39.6	40.8	40.5	41.6	41.2	40.8	39.9
Others	6.2	6.3	6.1	6.2	6.2	6.4		6.3
EPA/DHA	0.22	0.23	0.27	0.27	0.22	0.23	0.23	0.22

Table 9. Fatty acid composition of Hi DHA Tuna Oil showing levels of *trans* fatty acids.

Fatty Acid	Batch DF 161 January 7, 1998	Batch DC 231 July 24, 1998
C14:0	2.9	3.37
C15:0	0.8	1.06
C16:0	16.7	17.78
C17:0	1.0	1.17
C18:0	4.7	4.79
C20:0	1.1	1.31
C22:0	0.3	0.42
Saturates	27.5	29.90
<i>Trans 14:1</i>		
<i>Trans 16:1</i>	0.5	0.64
<i>Trans 18:1 n-9</i>	0.3	0.13
<i>Trans 18:2 n-8</i>	0.2	0.19
Total <i>Trans</i>	1.0	0.96
C16:1 n-9	0.3	0.30
C16:1 n-7	3.9	4.73
C17:1	0.8	0.80
C18:1 n-9	12.6	11.31
C18:1 n-7	2.2	2.42
C20:1 n-11	1.6	0.17
C20:1 n-9	0.3	0.34
C22:1 n-11	1.5	0.13
C22:1 n-9	0.2	0.47
C24:1 n-9	0.8	0.56
Monounsaturates	24.0	21.23
C18:2 n-6	1.3	1.40
C20:2 n-6	0.3	0.24
C18:3 n-6	0.3	0.33
C18:3 n-3	0.6	0.47
C20:3 n-6	0.2	0.22
C18:4 n-3	1.3	1.31
C20:4 n-6	1.9	1.86
C22:4 n-6	0.2	0.22
C20:5 n-3	6.8	6.37
C22:5 n-6	1.7	1.99
C22:5 n-3	1.3	1.24
C22:6 n-3	26	28.36
Polyunsaturates	41.9	41.00
Others	5.6	8.91
EPA/DHA	0.26	0.24

The fatty acid composition of two batches of Raw Tuna Oil produced in November 1999 are shown in Table 10. In this case, a more detailed analysis was done on the oil and many of the minor fatty acids were also reported.

Table 10. Fatty acid composition of Raw Tuna Oil produced in November 1999.

Fatty Acid	Lot 313H (11/9/99)	Lot 315H (11/11/99)
C8:0	<0.1	<0.1
C10:0	<0.1	<0.1
C11:0	<0.1	<0.1
C12:0	<0.1	<0.1
C14:0	2.66	2.76
C15:0	0.81	0.88
C16:0	19.2	19.6
C17:0	1.03	1.15
C18:0	5.00	5.36
C19:0	0.21	0.24
C20:0	0.24	0.28
C21:0	<0.1	<0.1
C22:0	0.14	0.14
C24:0	<0.1	<0.1
Saturates	29.29	30.41
C14:1	<0.1	<0.1
C15:1	<0.1	<0.1
C16:1	4.02	3.98
C17:1	<0.1	<0.1
C18:1	16.5	15.4
C20:1	2.59	2.24
C22:1	0.73	0.22
C24:1	0.80	0.72
Monounsaturates	24.64	22.56
C16:2	<0.1	<0.1
C18:2 n-6	1.04	1.11
C20:2 n-6	0.40	0.39
C22:2 n-6	<0.1	<0.1
C16:3	<0.1	<0.1
C18:3 n-6	0.47	0.56
C18:3 n-3	0.40	0.43
C20:3 n-6	0.14	0.15
C20:3 n-3	0.36	0.32
C22:3 n-3	<0.1	<0.1
C16:4	<0.1	<0.1
C18:4 n-3	0.56	0.58
C20:4 n-6	1.80	1.90
C20:4 n-3	0.55	0.47

C22:4 n-6	0.25	0.26
C20:5 n-3	6.82	6.54
C21:5 n-3	0.18	0.18
C22:5 n-6	1.09	1.39
C22:5 n-3	1.08	1.04
C22:6 n-3	26.1	26.7
Polyunsaturates	41.24	42.21
Others	4.71	4.94
EPA/DHA	0.26	0.25

The data presented for 1999 is a more in-depth analysis of the fatty acids in raw tuna oil. However, we find that there are still only about 14 fatty acids that make up the major portion of the composition of the oil and like the 1997 data, the relative fractions are still in the same proportion, that is the saturates 29-30%, the monounsaturates 23-25% and the polyunsaturates 41-42% and the ratio of EPA to DHA ranged from 0.25-0.26.

The data from these two periods plus the historical data presented indicate that the fatty acid composition of Raw Tuna Oil generally falls within the ranges of other marine oils with the distinction that the ratio of EPA to DHA is of the order of 0.25 while that ratio for other fish oils is of the order of 0.65-2.5 and generally 1.0 or greater.

The HiDHA Tuna Oils are manufactured from Raw Tuna Oil which has been extracted from the heads and off cuttings of the edible tuna. We would not expect to see many major differences in the composition of the oil since most of the oil is concentrated in the head and dark meat of the tuna. The fatty acid composition of 5 batches of refined tuna oil are presented in Table 11 for comparison to the historical and current raw tuna oil composition.

Table 11. Fatty acid composition of 5 batches of refined tuna oil.

Fatty Acid	Batch EC101 (3/11/99)	Batch EG141 (7/23/99)	Batch EC181 (4/9/99)	Batch EE191 (5/25/99)	Batch DK091 (12/7/98)
C14:0	3.14	2.88	3.01	2.81	2.51
C15:0	0.92	1.00	0.86	0.80	0.87
C16:0	18.77	20.17	17.40	16.77	16.83
C17:0	1.06	1.30	0.99	0.99	1.02
C18:0	4.80	5.88	4.63	4.82	4.97
C20:0	0.28	0.66	1.02	0.67	0.79
C22:0	0.21	0.28	0.20	0.49	0.45
C24:0	0.21	0.26	0.28	0.27	0.26
Saturates	29.36	32.52	28.41	27.62	28.00
C14:1	0.21	0.19	0.21	0.18	0.21
C15:1	0.12	0.12	0.11	0.10	0.10
C16:1 n-9	0.33	0.28	0.31	0.29	0.53
C16:1 n-7	3.85	3.77	3.84	3.80	3.83
C16:1 n-6	0.68	0.56	0.61	0.62	0.48
C17:1	0.65	0.78	0.71	0.75	0.78
C18:1 n-9	11.51	12.91	12.21	12.79	13.26
C18:1 n-7	2.36	2.24	2.27	2.14	2.26
C18:1 n-6	0.10	0.15	0.11	0.10	0.17
C20:1 n-11	0.44	1.03	0.64	0.92	0.39
C20:1 n-9	0.17	0.14	0.14	0.14	0.14
C22:1 n-11	0.43	0.37	0.45	1.03	0.48
C22:1 n-9	0.62	0.29	0.75	0.21	0.15
C24:1	0.55	0.56	0.53	0.54	0.56
Monounsaturates	22.01	23.38	22.79	23.31	23.33
C16:2 n-4	0.10	0.10	0.10	0.10	0.10
C18:2 n-6	1.63	1.09	1.50	1.50	1.21
C20:2 n-6	0.19	0.20	0.21	0.27	0.22
C16:3 n-3	0.89	1.13	0.91	0.66	0.38
C18:3 n-6	0.23	0.22	0.25	0.26	0.33
C18:3 n-3	0.79	0.50	0.76	0.71	0.53
C20:3 n-6	0.26	0.22	0.27	0.26	0.20
C18:4 n-3	1.08	0.38	1.20	1.61	1.35
C20:4 n-6	1.67	1.86	1.82	2.00	2.03
C20:4 n-3	0.20	0.20	0.20	0.32	0.20
C22:4 n-6	0.26	0.17	0.25	0.29	2.03
C20:4 n-3	0.20	0.20	0.20	0.32	0.20
C20:5 n-3	7.33	6.00	7.09	6.67	6.61
C22:5 n-6	1.58	2.57	1.83	1.91	2.20
C22:5 n-3	1.11	1.18	1.16	1.28	1.29
C22:6 n-3	26.04	25.1	26.62	26.74	26.92
Polyunsaturates	43.34	39.92	44.16	44.77	44.71
Others	5.29	4.18	4.64	4.30	3.96
EPA/DHA	0.28	0.24	0.27	0.25	0.25

The fatty acid profiles for the HiDHA Tuna Oil products show the same relative ratios of C20:5 to C22:6, and the same relative levels of saturates, monounsaturates and polyunsaturates. Thus, the refined tuna oil products are essentially similar in composition to the historical composition data for raw tuna oils and contain the same relative number of major fatty acids as the other marine oils, and edible fats and oils.

7.0 Environmental Contaminants

Tunas are a worldwide species, part of the family *Scombridae*. This taxonomic family includes all tunas, as well as bonitos, mackerels, seerfishes and the butterfly kingfish. The tunas are highly migratory and found throughout the world in most of the oceans. This migratory aspect allows them to possibly pickup environmental contaminants in their food which is then deposited into the fat. When the heads and red (dark) meat are rendered into Raw Tuna Oil and fishmeal, the contaminants will show up in the Raw Tuna Oil and in the fat in the fishmeal.

In the early stages of their preparation for food use, oils and fats generally contain non-triglyceride substances in small amounts. Some of these may detract from acceptability for given purposes because of flavors and odors which they impart to the lipid or because they reduce the stability or shelf life. Lipids intended for edible purposes are therefore refined to remove these substances and achieve national or international specifications while retaining, as far as possible, their desirable features. The non-triglyceride substances in Raw Tuna Oil and in other marine oils can be grouped according to their effects, i.e. hydrolytic (moisture, insoluble matter, free fatty acids, mono- and diglycerides, enzymes), oxidative (trace metals, oxidation products, phosphatides), catalyst poisons (phosphatides, oxidation products, compounds containing nitrogen, sulfur and halogens) and compounds with less well known effects, that may include traces of metallic or organic contaminants from the environment. Pigments and sterols are also present. Some of these non-triglyceride substances are known to have undesirable tastes and odors (hydrocarbons, terpenes, resins, sterols, waxes, and sugars).

In recent times, the treatment of crops with pesticides, defoliants and herbicides has resulted in residues of these substances in marine life. Industrial chemicals such as polychlorinated biphenyls (PCB's) are also known to accumulate in the lipids of marine animals. However, it has been demonstrated that such residues are satisfactorily removed by the normal processing procedures used in the production of edible oils. In these respects, Raw Tuna Oil does not differ particularly from other unrefined edible lipids, and it may be given similar treatments to achieve similar effects. Bimbo (1998) collected the consensus of marine oil experts on the effects that the various processing steps had on the removal of these non-triglyceride substances. His findings are reproduced in Table 12:

Table 12. Processing steps used to purify fats and oils.

PROCESSING STEP	PURPOSE
Oil storage	Remove insoluble impurities
Degumming	Remove phospholipids, sugars, resins, proteinaceous compounds, trace metals, and other materials.
Alkali refining	Remove free fatty acids, pigments, phospholipids, oil insoluble material, water-soluble material, and trace metals.
Water washing	Remove soaps.
Drying	Remove moisture.
Bleaching	Remove pigments, oxidation products, trace metals, sulfur compounds, and trace soaps.
Deodorization	Remove free fatty acids, mono-and diglycerides, aldehydes, ketones, chlorinated hydrocarbons, and pigment decomposition products.
Winterization	Remove higher melting triglycerides, enhance unsaturated triglycerides.
Vacuum stripping, or thin film distillation	Remove chlorinated hydrocarbons, fatty acids, oxidation products and cholesterol.

Figure 3 in section 5.0 above outlines the processing steps used to produce the HiDHA-Tuna Oils. A brief description of these processing steps and their effect on the quality of the oil follows.

Processing/refining Raw Tuna Oil in fact starts at the stage of storage following production, depending on its ultimate intended use. The main objective of “winterization” is to prevent clouding of the oil at refrigerator temperatures. If the oil is cooled or cold ambient conditions exist, the triglycerides with the highest melting points, known as stearines, will settle out, leaving a clear oil. This preferentially increases the unsaturated fatty acid content of the supernatant oil, known as the olein fraction, so that cold physical conditions maintained during storage at the producing plant can govern the composition of the oil to some extent by reducing the saturated fatty acid content.

The process of winterization is conducted in specially constructed tanks lined with polyurethane or epoxy coating that provide physically inert conditions in the tanks. The oil is slowly chilled to a working temperature of 0°C to allow formation of crystals of higher melting point triglycerides. Under warmer ambient conditions, or if the oil is warmed and stirred, this precipitation is prevented, and uniformity of composition is maintained.

“Degumming” is the term for the general removal of mucilage, consisting mainly of phosphatides and proteinaceous material to which is bound a proportion of the trace metal content. Its use is optional. Oil-soluble and non-hydratable gums are made insoluble in

the oil by treatment with 0.05 to 1.0% phosphoric acid, followed by removal of the precipitate and excess phosphoric acid by centrifugation.

“Alkali refining” removes free fatty acids by treatment of the heated oil at 90°C with sodium hydroxide (4N) in a continuous centrifugal line. The alkali neutralizes the free fatty acid content and reacts with phospholipids, nitrogen- and sulfur-containing compounds and some pigments, rendering them water-soluble so that these impurities are largely removed in the aqueous discharge from the centrifuge. Washing with water and centrifugation follows to remove soaps.

In the “bleaching” step, the oil is treated with activated clay, primarily to adsorb pigments, but this is also effective in reducing the content of oxidation products, trace metals, phosphorus and, to a lesser extent, sulfur compounds. The term “bleaching” is usually applied to this treatment, but it is obviously something of a misnomer, since more than color improvement is accomplished. The treatment is carried out at 82°C with amounts of clay that vary between 0.2 and 4.0% of the weight of the oil.

“Deodorization,” sometimes referred to as “steam stripping”, is the removal by vacuum steam distillation of small quantities of the more volatile components which are responsible for any odor or taste in edible lipids to obtain an organoleptically bland product. Before deodorization, the edible lipids may contain the volatile odor and flavor components originally present in the crude oil, the “soapy” type of odor created by neutralization and the “earthy” odor imparted by bleaching. These odors and flavors may be imparted by compounds at concentrations as low as 1 to 10 ppm or less. The deodorization process also serves to decrease the free fatty acid content of the lipid to 0.02 to 0.04%, to decompose any hydroperoxides present and to further improve color by decomposing some pigments.

The HiDHA Tuna Oils contain extremely low levels of environmental contaminants because:

- (1) The oil is deliberately sourced from specific tuna species harvested only in clean waters;
- (2) The oil is extracted only from the body musculature; and
- (3) The oil is “fail-safe” purified using advanced processing techniques.

The HiDHA Tuna Oils are produced only from four commercially harvested species found in the relatively unpolluted pristine waters of the South and Central regions of the South Pacific Ocean. This means that the tuna flesh and the body oil extracted from the fish are both much less contaminated with industrial/agrochemical residues than fish and fish oil products from the more polluted waters of the Northern Hemisphere.

The HiDHA Tuna Oils are produced only from the body musculature – no oil is extracted from the liver or any part of the viscera. This is an important precaution to take because any fat-soluble environmental contaminant would accumulate primarily in these organs.

The refining plant has developed physical processing techniques based on absorption chromatography and molecular distillation to remove the minor levels of any contaminants present to ensure that the final product contains levels which are barely detectable and well within all accepted limits. To limit contamination from sources other than the environment where the fish are caught, the tuna oil refining plant is licensed by the Australian Therapeutic Goods Administration in accordance with pharmaceutical standard GMP. The premises and the manufacturing processes have qualified for ISO 9002 and AQIS certification and are operated to comply with a strict environmental protection code. The data presented in Tables 13-21 demonstrate the purity of the HiDHA Tuna Oils with respect to PCB's and dioxins, polycyclic hydrocarbons (PAH's), pesticide residues and heavy metals.

Table 13. PCDDs and PCDFs in HiDHA Tuna Oil Samples (ng/kg).

COMPOUND	BATCH DC231	BATCH FC291	BATCH FD111
2378TCDD	0.07	0.05	0.20
12378 PeCDD	0.06	<0.02	0.26
123478HxCDD	0.04	0.10	0.51
123678HxCDD	0.12	<0.02	0.54
123789HxCDD	0.10	0.09	0.43
1234678HpCDD	0.87	0.24	1.13
OCDD	3.43	1.89	6.15
2378TCDF	0.03	0.08	0.33
12378PeCDF	<0.03	0.12	0.29
23478PeCDF	0.05	0.05	0.31
123478HxCDF	0.05	<0.02	0.39
123678HxCDF	0.04	<0.02	0.41
123789HxCDF	<0.05	0.05	0.41
234678HxCDF	0.08	<0.02	0.41
1234678HpCDF*	0.12	0.18	0.49
1234789HpCDF*	<0.16	0.07	0.57
OCDF*	1.43	0.55	4.48
TEQ upper	0.19	0.15	1.00

Whole sample basis.

* After repeat GC-MS analysis

Table 14. *Ortho*-PCBs in HI- DHA Tuna Oil (ng/kg).

CONGENER IUPAC NO.	BATCH DC231	BATCH FC291	BATCH FD111
18	0.19	<0.10	0.21
28	0.11	0.13	0.33
31	0.11	0.11	0.26
47	0.31	0.19	0.21
49	0.49	0.40	0.48
51	<0.10	<0.10	<0.10
52	0.94	1.01	1.10
99	1.80	2.34	1.80
101	3.82	6.10	4.50
105	0.25	1.20	0.28
114	<0.10	0.31	0.46
118	0.93	2.40	0.54
123	<0.10	0.26	0.17
128	0.68	1.74	0.65
138	5.76	16.02	6.09
153	8.37	22.47	8.91
156	0.10	0.31	<0.10
157	<0.10	0.19	<0.10
167	<0.10	0.26	<0.10
180	2.75	14.00	3.74
189	0.20	<0.10	<0.10
TEQ upper	0.39	0.80	0.44

Table 15. Non-*Ortho* PCBs in HiDHA Tuna Oil (ng/kg).

COMPOUND	BATCH DC231	BATCH FC291	BATCH FD111
PCB 77	0.94	0.90	2.23
PCB 81	0.29	0.15	0.17
PCB 126	0.13	0.30	0.46
PCB 169	<0.23	0.19	0.06
TEQ upper	0.02	0.03	0.05

Table 16. Total TEQs in HiDHA Tuna Oil (ng/kg).

COMPOUND	BATCH DC231	BATCH FC291	BATCH FD111
PCDDs & PCDFs	0.19	0.15	1.00
<i>Ortho</i> PCBs	0.39	0.80	0.44
NON- <i>Ortho</i> PCBs	0.02	0.03	0.05
TOTAL TEQ*	0.60	0.98	1.49

* including upper bound values.

Table 17 below illustrates the relatively low levels of these environmental contaminants in the HiDHA Tuna Oils compared to currently marketed products:

Table 17. Dioxin and PCB concentrations in fish oils (ng/kg).

Oil Type	Dioxins	Non- <i>ortho</i> PCBs	<i>Ortho</i> -PCBs	Total TEQ**
Cod Liver Oil, Bottles 1994	0.48 - 11	4.8 - 21	0.49 - 3.0	7.4 - 33
Cod Liver Oil, Capsules 1994		44	2.3 - 2.5	2.3 - 44
Cod Liver Oil, Bottles 1996	6.2 - 9.2	17 - 22	6.8 - 8.5	31 - 38
Cod Liver Oil, Capsules 1996	1.5 - 6.2	11 - 26	3.7 - 12	18 - 41
Halibut Liver Oil Capsules 1994	4.4 - 40	1.6 - 16	0.3 - 3.3	9.2 - 56
Halibut Liver Oil Capsules 1996	1.7 - 6.1	3.5 - 7.3	1.5 - 2.2	7.4 - 15
HiDHA TUNA OIL	0.19 - 0.3	0.02 - 0.04	0.39 - 0.40	0.60 - 0.74

** Totals differ from the sums of the concentrations due to rounding.

Source: Ministry of Agriculture, Fisheries and Food (1997). Dioxins and polychlorinated biphenyls in fish oil dietary supplements and licensed medicines. Food Surveillance Information Sheet No. 106, Food Safety Directorate, London, U.K.

The HiDHA Tuna Oil has also been assayed for Polyaromatic (cyclic) hydrocarbons (PAH's). These data appear in Table 18.

Table 18. Polyaromatic hydrocarbons in HiDHA Tuna Oil ($\mu\text{g}/\text{kg}$).

Polyaromatic hydrocarbon ("PAH")	PAH $\mu\text{g}/\text{kg}$		Conversion Factor PAH/BaP	BaP equiv. $\mu\text{g}/\text{kg}$	
	Batch FC 291	Batch FD 111		Batch FC 291	Batch FD 111
Acenaphthene	1.06	-	-		
Anthracene	0.14	0.36	-		
Benzo(a) anthracene	-	-	0.014		
Chrysene	-	-	0.013		
Fluorene	4.87	4.17	-		
Fluoranthene	0.60	-	0.020	0.012	
Phenanthrene	19.90	15.9	-		
Pyrene	<LOQ	<LOQ	0.130		
Sum light PAH	26.4	20.4			
Benzo(a)pyrene	0.06	-	1.00	0.06	
Benzo(b)fluoranthene	0.21	-	0.110	0.023	
Benzo(k) fluoranthene	0.05	<LOQ	0.070	0.004	
Benzo(g,h,i)perylene	-	<LOQ	0.030		
Diabenz(ah)anthracene	-	-	4.05		
Indenol(1,2,3-cd)pyrene	-	-	0.25		
Sum Heavy PAH	0.32	<LOQ			
Sum Total PAH	26.7	20.4			
Sum BaP equiv.				0.099	0

- = not detectable

LOQ = quantitation limit

Note: A conversion factor of PAH into BaP equivalents only exists for 10 PAH compounds.

Two batches of HiDHA Tuna Oil were analyzed for pesticide residues. The residues were extracted into ethyl acetate/cyclohexane and cleaned up by gel permeation chromatography. Analysis and quantification was by gas chromatography using mass spectrometric (ion trap) detection and electron capture detection. Confirmation of any residues found was by GC-MS (ion trap). The results are listed in Table 19 below.

Table 19. Pesticide residue analysis of HiDHA Tuna Oil.

PESTICIDE RESIDUE	BATCH 60560	BATCH DC231
Organochlorine residues	None Detected	None Detected
Organophosphorous residues	None Detected	None Detected

As shown in Table 20, organochlorine and organophosphorous compounds and their detection limits were also determined:

Table 20. Analysis of organochlorine and organophosphorous compounds and their detection limits in HiDHA Tuna Oil.

ORGANOCHLORINE COMPOUNDS		ORGANOPHOSPHOROUS COMPOUNDS	
Compound	mg/kg	Compound	mg/kg
Aldrin	0.005	Bromophos	0.05
2,4 DDE	0.005	Bromophos-ethyl	0.05
4,4' DDE	0.005	Chlorfenvinphos	0.05
2,4 DDT	0.01	Chlorpyrifos	0.05
4,4'' DDT	0.01	Chlorpyrifos-methyl	0.05
Dieldrin	0.005	Diazinon	0.05
Endosulfan A	0.01	Dichlorvos	0.05
Endosulfan B	0.01	Dioxathion	0.05
Endrin	0.005	Ethion	0.05
Cis-chlordane	0.05	Fenitrothion	0.05
Oxychlordane	0.05	Fonofos	0.05
Trans-chlordane	0.05	Fenchlorphos	0.05
Endosulfan sulfate	0.01	Malathion	0.05
Hexachlorobenzene	0.005	Parathion	0.05
α -HCH	0.005	Parathion-methyl	0.05
β -HCH	0.01	Phosalone	0.05
γ -HCH (lindane)	0.005	Pirimiphos-methyl	0.05
δ -HCH	0.01		
Heptachlor	0.005		
Heptachlor epoxide	0.005		
2,4 TDE	0.005		
4,4' TDE	0.005		
Mirex	0.05		
Cyfluthrin	0.05		
Cyhalothrin	0.05		
Cypermethrin	0.05		
Deltamethrin	0.05		
Fenvalerate	0.05		
Flucythrinate	0.05		
Permethrin	0.05		

Two batches of HiDHA Tuna Oil were analyzed for trace elements. The results appear in Table 21 below.

Table 21. Trace element analysis in HiDHA Tuna Oil (mg/kg).

ELEMENT	BATCH DC 231	BATCH FC 291	BATCH FD 111	BATCH E000331
Magnesium	0.10			
Calcium	13			
Iron	0.20			
Copper	<0.01			
Arsenic	<0.001**	<0.01	<0.01	<0.01
Selenium	0.07**			
Cadmium	<0.001	<0.01	<0.01	<0.10
Tin	0.008			
Mercury	<0.001**	<0.01	<0.01	<0.01
Lead	<0.01	<0.10	<0.10	<0.10
Heavy Metals as Lead		<1.00	<1.00	<1.00

** Indicates results obtained for low levels of analyte using hydride generation ICP-MS. This methodology is not UKAS accredited but the same quality control criteria have been applied.

The extensive data covering the HiDHA Tuna Oils clearly shows that the series of processing steps employed, remove or reduce all contaminants to levels that are either not detected under the conditions of the method or are well within all known regulatory levels for these compounds. A Material Safety Data Sheet (“MSDS”) for the HiDHA Tuna Oil series of products is provided in Appendix 3.

8.0 Intended Use

As described in the Introduction section above, the HiDHA Tuna Oil products are intended for use as replacements for menhaden oil in traditional foods under the conditions prescribed in 21 C.F.R. § 184.1472(a)(3), and in dietary supplements delivering up to 1.0 gram of oil per day.

8.1 Traditional Food Use

In affirming the GRAS status of menhaden oil, FDA established specific conditions for its use in traditional foods. The limiting factor in the menhaden oil GRAS affirmation was an increase in bleeding time, conservatively estimated to occur at intakes greater than 3 grams of EPA+DHA per person per day. The specific use limitations established by FDA in 21 C.F.R. § 184.1472(a)(3) to achieve commensurately limited exposure of EPA+DHA are listed below in Table 22, and it is within these same limitations that HiDHA tuna oils will be used in traditional foods:

Table 22. Maximum level of use in food.

Category of food	(% as served)
Cookies, crackers (21 C.F.R. § 170.3(n)(1))	5.0 percent
Breads, rolls (white & dark) (21 C.F.R. § 170.3(n)(1))	1.0 percent
Fruit pies, custard pies (21 C.F.R. § 170.3(n)(1))	7.0 percent
Cakes (21 C.F.R. § 170.3(n)(1))	10.0 percent
Cereals (21 C.F.R. § 170.3(n)(4))	4.0 percent
Fats, oils (21 C.F.R. § 170.3(n)(12)), but not in infant formula.	20.0 percent
Yogurt (21 C.F.R. § 170.3(n)(31))	4.0 percent
Cheese products (21 C.F.R. § 170.3(n)(5))	5.0 percent
Frozen dairy products (21 C.F.R. § 170.3(n)(20))	5.0 percent
Meat products (21 C.F.R. § 170.3(n)(29))	10.0 percent
Egg products (21 C.F.R. § 170.3(n)(11))	5.0 percent
Fish products (21 C.F.R. § 170.3(n)(13))	20.0 percent
Condiments (21 C.F.R. § 170.3(n)(8))	5.0 percent
Soup mixes (21 C.F.R. § 170.3(n)(40))	3.0 percent
Snack foods (21 C.F.R. § 170.3(n)(37))	5.0 percent
Nut products (21 C.F.R. § 170.3(n)(32))	5.0 percent
Gravies, sauces (21 C.F.R. § 170.3(n)(24))	5.0 percent

8.2 Dietary Supplement Use

Dietary supplements containing HiDHA Tuna Oils will be labeled for daily intakes of up to 1.0 gram oil per day. The HiDHA 25S and 25F Softgel preparations contain 5 to 8% EPA and 25 to 28% DHA, or 30 to 36% EPA+DHA. At the higher levels, this would add 0.36 gram EPA+DHA to total exposure.

1

9.0 Exposure

9.1 Presence in the Diet

Fish oils have constituted part of the human diet for centuries. It has been reported that there are formal 800-year-old Nordic regulations regarding fishing and, in fact, the cod fishery in Scandinavia has been well established for more than a millennium. Another report goes back even farther and mentions sources in the Bible and in early Greek and Roman writings. The first known clinical investigation to use cod liver oil was done by Dr. Samuel Kay at the Manchester Infirmary between 1752 and 1783. He found that cod liver oil gave relief to people suffering from rheumatism. Other work indicated it was effective in curing night blindness. These were published in a British scientific journal in 1783.

It was not until the early 1900s that cod liver oil was shown to be effective in the treatment of rickets. The active ingredient in the oil was found to be vitamin D, and the use of this oil then moved from a curative agent to a preventative agent. Up through World War II, cod liver oil was a primary source of Vitamins A and D. Then when scientists managed to produce these vitamins synthetically, the demand for cod liver oil dropped. People preferred vitamin tablets with no taste to that of liquid cod liver oil with its distinctive fishy flavor. Another report described studies in the USA, particularly the 19-year clinical trial conducted by Dr. Avery Nelson in Seattle. Dr. Nelson had heard about the effects of fish and cod liver oil consumption in Norway during World War II on the incidence of heart disease and decided to run similar tests in the US. His studies began in 1953 with patients referred to him by other physicians. His patients were advised to eat fatty fish at least 3 times per week as a main course meal. His results showed 4.5 times more deaths among patients who did not adopt the diet of fish compared to those who did.

In 1979, a paper describing the role of n-3 fatty acids in the prevention of cardiovascular diseases was published. From that point forward, the interest in fish oils as a source of these fatty acids has been increasing. Fish oil triglycerides have been offered in liquid form, capsules, tablets, and powders as natural products, reflecting the composition of the fish species processed.

9.2 Estimated Intakes

The levels of EPA and DHA in fish oils vary considerably but, on average, refined menhaden oil contains approximately 13-14% EPA and 7-9% DHA, whereas the HiDHA tuna oil contains 4-8% EPA and 18-25% DHA. Thus, exposure to traditional foods containing HiDHA tuna oil as a replacement menhaden oil would result in approximately a 25% increase in the combined amount of EPA and DHA, a greater than 50% reduction in EPA exposure and more than a doubling of DHA exposure. Total daily exposure to EPA and DHA from proposed uses of HiDHA Tuna Oil in both traditional foods (~3.75 grams) and dietary supplements (~0.36 gram) would approximate 4 grams per person per day.

The relative changes in EPA and DHA exposure from use of menhaden oil are described above.

10.0 Safety Studies

10.1 Biodisposition Data (Absorption, Distribution, Metabolism, and Excretion)

EPA and DHA are incorporated into circulating triglycerides in the same proportion that they appear in the dietary oil (Bronsgest-Schoulte et al. 1981). Their in vivo peroxidation is the same as that seen for oleate or linoleate (Higdon et al. 2000). Initial hydrolysis of fish oils is lower than that seen for other fats (Bottino et al. 1967). EPA and DHA show specificity for different phospholipids (Mori et al. 1987). Cholesterol esters become enriched with EPA but not DHA (Holub et al. 1987).

Essentially, DHA is metabolized as are other fats although, being a bigger molecule, the reaction rates may be lower.

10.2 Preclinical Data

The safety of fish oils, including tuna oil, is assumed from their long history of ingestion as a component of the human diet. Fish oils are complex materials and consist mainly of a mixture of triglycerides of various long-chain fatty acids (similar to those in various edible vegetable oils) with small amounts of mono- and diglycerides. The HiDHA Tuna Oil products are derived from raw tuna oil and manufactured using standard processes, resulting in products with relatively high concentrations of EPA and DHA. Of particular importance to an evaluation of a HiDHA oil as a substitute for menhaden or other fish oils is its relatively higher DHA content. Preclinical studies on DHA have largely been conducted because of its potential use in infant formulas or in dietary supplements to increase formula or breast milk concentrations to levels consistent with those occurring naturally in breast milk in populations consuming balanced mixed diets. Most of these studies have been conducted with a single cell algal source of DHA alone or in combination with an AA-rich oil derived from *Mortierella alpina* at ratios that approximate those found in human breast milk. A summary of these preclinical toxicological evaluations is provided by Kyle and Arterburn (1998). *In vitro* and *in-vivo* toxicology evaluations showed no evidence of mutagenicity, clastogenicity, or subchronic or chronic toxicity in rats fed 1.25 grams of DHA/kg b.w./day.

Long-chain polyunsaturated fatty acids ("PUFAs") are essential for normal structure and function of many tissues. For example, the eicosanoids are oxygenation products of C-20 polyunsaturated fatty acids including the n-3 fatty acid EPA and the n-6 fatty acid AA. Eicosanoids are found in plants and animals, and form a class of compounds that are biologically very active in both an autocrine and paracrine manner. Major biological targets include smooth muscle of the gastrointestinal, respiratory, reproductive and vascular systems; platelets and monocytes; the central nervous system; autonomic presynaptic nerve terminals; sensory nerve endings; endocrine organs; adipose tissue; and the eye. The half-lives of eicosanoids are very short, seconds to minutes. Eicosanoid

precursors, e.g., EPA and AA, can be synthesized respectively from the essential fatty acids alpha-linolenic (18:3, n-3) and linoleic (18:2, n-6), but not very efficiently, and often must be ingested as part of an adequate diet (Emken et al. 1994).

Synthesis of DHA from EPA involves an elongation step (22:5, n-3) prior to desaturation. The reverse conversion (retroconversion) from EPA to DHA involves the removal of a 2 carbon unit from the carboxyl end of the molecule, thus leaving the methyl (omega) end of the molecule intact. It also involves removal of the double bond at the Δ -4 position. It is not entirely clear whether the increased levels of EPA following ingestion of DHA is fully due to retroconversion or also involves a degree of feedback inhibition such that lesser amounts of EPA are converted to DHA. Detailed studies on plasma and tissue levels of DHA and EPA with varied DHA/EPA combinations are not available.

The safety of EPA and DHA can be assumed from their long history of ingestion as part of the human diet. The FDA has critically evaluated available information on the safety of the n-3 fatty acids, EPA and DHA, and identified only three potential safety issues: prolongation of bleeding time, modification of glycemic control, and elevation of LDL cholesterol (FDA 1993). These potential safety issues are discussed below in Section 10.3. In 1997, following a critical evaluation of all information available, the FDA concluded that consumption of up to 3 grams/day of EPA and DHA in menhaden oil is generally recognized safe (FDA 1997). The safety of EPA and DHA at the current level of use in traditional foods has been recently supported by an extensive critical review by a task group formed by the Consumer Healthcare products Association, the Council for Responsible Nutrition, and the National Fisheries Institute (Wright 2000). This review updates the relevant scientific literature from 1993 until March 2000. It was submitted to FDA in April 2000 in response to the agency's request for scientific data and information concerning health claims for omega-3 (n-3) fatty acids. Of particular importance to this GRAS evaluation was the fact that newer evidence supports the view that DHA as opposed to EPA is safer with respect to earlier defined safety issues, and that no new adverse safety issues have been raised by the presence of an increased DHA:EPA ratio in fish oil.

10.3 Clinical Data

10.3.1 General Considerations

In humans it has been repeatedly shown that ingesting DHA has different effects than EPA, although ingesting DHA alone can also result in increased levels of EPA (Conquer and Holub, 1996 and 1997; Nelson et al. 1997; Vidgren et al. 1997). This increase in EPA following the ingestion of DHA is dose dependent and appears to be due largely to retroconversion as opposed to feedback inhibition. Retroconversion is in the range of 1.4% when normal levels of dietary DHA are consumed (Brossard et al. 1996), but rise to approximately 12% when dietary levels of DHA exceed 1.2 g/day (Conger and Holub, 1996 and 1997). As noted in section 10.2, the degree of retroconversion under conditions where the dosage and ratios of DHA to EPA are systematically varied has not been defined. The fact that ingestion of DHA can result in significant increases in tissue

(platelet) EPA levels has been demonstrated by Nelson et al. (1997). This work is discussed under section 10.3.2.

Clinical evaluations of fish oils containing EPA and DHA and, to a lesser extent, esters of these fatty acids have been extensive. The basic effects of these highly unsaturated fatty acids as of 1992-3 were extensively discussed in FDA's Final Rule on Health Claims for Omega-3 Fatty Acids and Coronary Heart Disease (U.S. FDA 1993) and in the report of the British Nutrition Foundation's Task Force on Unsaturated Fatty Acids: Nutritional and Physiological Significance (1992). A potentially beneficial effect of both EPA and DHA is a lowering of plasma triacylglycerol and Very Low Density Lipoprotein ("VLDL") cholesterol levels that occur when one or both are ingested in amounts approximating 2 or more grams per day. The degree of effect depends on the type of patient and the composition of other fatty acids in the diet (Simopoulos 1991; Harris 1996).

As noted above, a particularly important aspect of any benefit or safety evaluation involving the addition of these fatty acids to food products is the fact that EPA (20:5, n-3) competes with AA (20:4, n-6) for the production of bioactive lipid derivatives, termed eicosanoids. AA acts as a precursor for lipoxygenase and cyclooxygenase producing n-6 eicosanoids (e.g., leukotriene B₄, thromboxane A₂, and prostaglandin E₂). EPA correspondingly produces n-3 eicosanoids (e.g., leukotriene B₅, thromboxane A₃, and prostaglandin E₃). The effect of DHA on eicosanoid formation is likely mediated by the degree to which EPA levels are raised, but other DHA/EPA interactions may well be involved, such as the ratio of DHA to EPA. Different intake ratios of n-6 and n-3 PUFAs can alter processes involved in clotting, immunity, glucose regulation, and lipoprotein metabolism and possibly other reactions involving specific eicosanoids, such as vascular wall reactivity and susceptibility to cardiac arrhythmic behavior. There also is some evidence that there is a significant decrease in the capacity to synthesize EPA and DHA from the essential fatty acid alpha linolenic acid (18:3, n-3) in infants and possibly in older individuals (Uauy, Treen and Hoffman 1982). Thus the relative effects of EPA and DHA in these groups may differ from those in the general population.

The primary safety issues with fish oils containing EPA and DHA were identified by FDA in the 1997 Final Rule affirming the GRAS status of menhaden oil. They were: (1) an increase in bleeding time with fish consumption and supplemental intakes of EPA and DHA from fish oils; (2) increased glucose levels in some studies with non-insulin-dependent diabetics which used fish oils in the range of 4.5 to 8.0 g/person/day; and (3) an increase in LDL-cholesterol or apo B (apolipoprotein B being a major functional component of LDL which contributes to atherogenesis).

10.3.2 Bleeding Time

Bleeding time was the principal determining factor in FDA's decision to limit EPA+DHA intake in the final rule on menhaden oil, in part, because it was easily quantified and referenced to adverse events related to bleeding. In contrast, the other variables considered i.e., elevated blood glucose and LDL cholesterol levels, have risks associated with them that are variable in a continuous manner over time and are also influenced by

many other factors that are not always clearly identifiable. Furthermore, the mechanism involved in bleeding time and clotting is linked to known changes in eicosanoid metabolism and platelet membrane composition. A shift in eicosanoid metabolism from AA precursor to EPA precursor enriches platelet membranes with EPA and changes eicosanoid production in a manner which lessens the ability of platelets to aggregate and form a thrombus.

The effects of low (<50mg/day) and high (6 g/day in triglyceride form) DHA diets were tested in healthy adult males under metabolic ward conditions (Nelson et al. 1997). After 30 days on a control (Western) diet of natural foods with low DHA, one group was maintained on the control diet and another received the high DHA diet for a 60 day period. No significant differences were noted between the groups in platelet aggregation in the presence of ADP, collagen, and AA. Also no differences were noted in prothrombin time, partial thromboplastin time, antithrombin III levels, or bleeding time. However, platelet contents of both DHA and EPA increased in the high DHA group even though essentially no dietary EPA was present. DHA levels were twice those of EPA and possibly this influenced bleeding time. Whatever the mechanism involved, it was clear that DHA administration, as compared with EPA administration, resulted in a lesser risk with respect to prolonged bleeding time and related clotting factors.

In another study by Agren et al. (1997), the effects of feeding a fish diet, a fish oil diet, or a DHA-containing diet was examined. Collagen-induced platelet aggregation decreased with the fish and fish oil diets, as would be anticipated, but remained unchanged with the DHA-containing diet. These results are consistent with those of Nelson et al. (1997).

10.3.3 Glucose Levels in Non-Insulin-Dependent Diabetics

The FDA has questioned whether increased consumption of menhaden oil containing EPA and DHA would adversely impact glycemic control in diabetics, but ultimately concluded that intakes of 3 grams/day would be generally recognized as safe (U.S. FDA 1997). This area has been thoroughly addressed by Wright (2000). Sixteen studies were reviewed by Wright and while there was a trend toward an increase in fasting glucose levels with fish oils, there were non-significant effects on glycated hemoglobin, insulin levels, and other endpoints relevant to the control of blood glucose concentration.

Earlier, Friedberg et al. (1998) reviewed fish oil and glycemic control in diabetes and performed a meta analysis on 26 studies involving fish oils (predominantly menhaden oil) with daily EPA/DHA intakes of up to 5.4 grams of EPA and 2.3 grams of DHA. Friedberg found only the typical large plasma triglyceride-lowering effect and minimal effects on fasting blood glucose levels which actually declined in insulin-dependent diabetics.

A pertinent very recent study was conducted by Mori et al. in mildly hyperlipidemic men (2000). In this double-blind and placebo-controlled trial, 4 grams of purified EPA, DHA, or olive oil was given for 6 weeks. Both EPA and DHA increased fasting insulin levels. EPA, but not DHA, tended to increase fasting glucose levels.

In summary, there are mixed effects of EPA and DHA on glucose metabolism in diabetics, but consumption levels of HiDHA oil from tuna would appear to be beneficial in terms of triglyceride lowering and have less of an effect of higher EPA-containing fish oils on glycemia.

10.3.4 Cholesterol Metabolism

In terms of potentially adverse effects of fish oils on cholesterol levels, and particularly LDL-cholesterol metabolism, HiDHA tuna oil would seem to be equivalently safe or safer than menhaden oil because of differential effects of EPA and DHA on cholesterol metabolism. The recent task group report to FDA (Wright 2000) extensively reviewed the effects of fish oils based largely on the work of Harris (1996) an analysis of unpublished primary data supporting existing publications.

More recently, Davidson et al. (1997) and Mori et al. (2000) provided additional information on the safety of DHA with respect to cholesterol metabolism by using respectively DHA supplements in triglyceride form and fish oil preparations purified by the Fish Oils Test Materials Program at the U.S. National Institutes of Health. In patients with combined hyperlipidemia, Davidson gave, in a well-controlled study, DHA at 1.25 or 2.5 grams/day. At the higher dose, LDL-cholesterol increased, serum triglycerides showed a marked decrease, and HDL-cholesterol increased.

Mori et al. (2000) focused on mildly hypercholesterolemic men who were otherwise healthy and gave 4 grams daily of the ethyl ester of EPA or DHA or olive oil for 6 weeks. Neither EPA nor DHA had any effect on total cholesterol, LDL-cholesterol or HDL-cholesterol. HDL₃-cholesterol was decreased by EPA and HDL₂-cholesterol was increased by DHA. LDL particle size increased with DHA but not with EPA. The differential effects of EPA and DHA on LDL-particle size is interesting because particle size was positively correlated with HDL-cholesterol and inversely with triglyceride change. These changes are somewhat difficult to interpret, but can generally be considered as indicating that DHA but not EPA improved serum lipid status in that it produced an increase in HDL₂-cholesterol and a shift to a larger LDL particle size which is considered to be less atherogenic than a smaller size particle.

In summary, the effects of DHA on serum lipids appear to be more favorable than those of EPA and therefore, the effects of HiDHA tuna oil with a higher ratio of DHA to EPA would be judged to be as safe, and probably safer, than an equivalent amount of menhaden oil with respect to changes in cholesterol metabolism.

10.3.5 Other Considerations

There are many other changes that occur with the increased ingestion of EPA and DHA, alone and in various combinations, but these changes have been considered beneficial and represent the basis for possible health claims in food labeling. The Wright (2000) submission to the FDA focuses on these changes. In particular, these changes focus on potentially beneficial effects related to the cardiovascular system, most of which are

summarized by Connor (2000). Of interest is a potentially beneficial effect of fish oil supplementation to reduce the risk to pre-term delivery in high-risk pregnancies demonstrated in a multicenter trial (Olsen et al. 2000).

11.0 Summary and Conclusions of the Expert Panel

The HiDHA Tuna Oil Products are produced from raw tuna oil derived from the head and off-cuttings of edible tuna using unit processes that are standard throughout the edible oil industry (e.g., winterizing, degumming, neutralization, bleaching and deodorization) in accordance with current pharmaceutical standard Good Manufacturing Practices. The refining plant is designed specifically for refining tuna oil, is licensed by the Australian Therapeutic Goods Association, has achieved ISO 9002 and AQIS certification, and complies with the applicable environmental protection laws. The chemical composition of tuna oil is a complex mixture of essentially the same fatty acids found in other edible oils but with a relatively increased content of DHA.

Fish oils have constituted a part of the human diet for centuries (as far back as Biblical times and early Greek and Roman periods). The HiDHA Tuna Oil products are intended for use as replacements for menhaden oil in traditional foods under the conditions of use prescribed in 21 C.F.R. § 184.147.2(a)(3), and in dietary supplements providing up to 1.0 gram of oil per day.

The qualitative and quantitative differences in action between DHA and EPA largely form the basis for evaluating the GRAS status of the HiDHA Tuna Oil products described in this report. The HiDHA Tuna Oil containing 18 to 28% DHA, approximately 2% of the 22:5 n-3 intermediate, and 4 to 8% EPA, is intended for use as a substitute for menhaden oil that contains 6.7% DHA, 2.5% of the intermediate, and 13.1 % EPA (mean values in the FDA GRAS affirmation proceeding). Because of (1) the difference in DHA and EPA concentrations in HiDHA Tuna Oil as compared with menhaden oil, and (2) the inclusion of dietary supplement use, the safety of exposure to approximately 4 grams of HiDHA Tuna Oil was evaluated.

Recent literature clearly documents that DHA and EPA have different effects on bleeding time, glucose metabolism, and cholesterol metabolism. The weight of the evidence firmly supports the view that HiDHA Tuna Oil has less potential for producing adverse health effects in these areas. Furthermore, additional studies on the health effects of DHA have yielded only potentially beneficial outcomes at the current intended use levels and at somewhat higher levels. Based on an evaluation of the current literature, the Expert Panel concluded that the intended uses of HiDHA Tuna Oil posed equivalent or less adverse risk to health than the current GRAS uses of menhaden oil.

We, the Expert Panel, having independently and collectively critically evaluated the data and information summarized in this document, conclude that the HiDHA Tuna Oil Products meeting appropriate specifications described herein and produced in accordance with current Good Manufacturing Practices are GRAS based on scientific procedures when used in traditional foods as replacements for menhaden oil under the conditions of use prescribed in 21 C.F.R. § 184.1472(a)(3), and in dietary supplements providing up to 1.0 gram of oil per day.

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