GR

ORIGINAL SUBMISSION

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January 15, 2002

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FOOD ADDITIVE SAFETY

UPS NEXT DAY

Alan Rulis, Ph.D.
Director
The Office of Premarket Approval (HFS200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
Room 1253
College Park, MD 20740-3835

Re: GRAS Notification Tuna Oil

Dear Dr. Rulis:

Enclosed herewith are three copies of Clover Corporation Limited's GRAS notification for Tuna Oil. Please copy me on any communications with the company. Email to hamishd@clovercorp.com.au and anthony.young@piperrudnick.com or a phone call to me (202/861-3882) will elicit a prompt response.

Sincerely yours,

Anthony L. Young
Counsel for
Clover Corporation Limited

ALY/jek
Enclosures

000205

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8 January 2002

Alan Rulis, Ph.D.
Director
The Office of Premarket Approval (HFS-200),
Center for Food Safety and Applied Nutrition,
Food and Drug Administration,
200 C St. SW.,
Washington, DC 20204,
United States of America.

Re. GRAS Notification – Tuna Oil

Dear Dr. Rulis:

Clover Corporation Limited hereby submits this premarket notification in support of its claim that use of tuna oil in conventional foods is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act (the act) because Clover Corporation has determined that such use is GRAS. It is the position of Clover Corporation that tuna oil is generally recognized as safe for use in food under the same conditions as are presently established for menhaden oil (21 C.F.R. §184.1472(a)(3)).

Signature: .....

Date: 08-01-2002

Authorized official: **Hamish Drummond**
Managing Director

Name of notifier: **Clover Corporation Limited**

Address of notifier: **Level 5, 231 Miller Street,**
P.O. Box 458,
North Sydney NSW 2060,
Australia.

The "notified substance": **tuna oil**

The applicable conditions of use of tuna oil:

Maximum Level of use of HiDHA® tuna oils

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

The basis for GRAS determination: **Through scientific procedures**

Statement re data and information:	**The data and information that are the basis for Clover Corporation's GRAS determination are attached for the Food and Drug Administration's review.**
Detailed information:	**Information is provided in the attached document and appendices (which include an Expert Report).**

Please direct any communications to Hamish Drummond at the following e-mail address:

hamishd@clovercorp.com.au
or by facsimile at +61 2 9956 7696

Copies of any communications should also be directed to counsel for Clover Corporation, Anthony L. Young, at anthony.young@piperrudnick.com. Mr. Young may be reached by telephone at 202-861-3882 and by facsimile at 202-223-2085.

Clover or its counsel will respond as promptly as possible to any communications from the Administration.

Sincerely yours,

Hamish Drummond
Managing Director

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TO

THE OFFICE OF PREMARKET APPROVAL (HFS-200)
CENTRE FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
200 C St SW
WASHINGTON, DC 20204
UNITED STATES OF AMERICA

NOTIFICATION OF GRAS STATUS FOR THE USE OF TUNA OILS IN CONVENTIONAL FOODS

FROM CLOVER CORPORATION LIMITED

December 2001

Contact:
Mr Hamish Drummond,
Managing Director,
Clover Corporation Limited
Level 5, 231 Miller Street,
P.O. Box 458,
North Sydney NSW 2060,
Australia

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1. Purpose of Submission

Clover Corporation submits this notification to the Food and Drug Administration (FDA) with respect to the use of tuna oil as an ingredient in conventional foods. It is the position of Clover Corporation that tuna oil is generally recognized as safe for use in food under the same conditions as are presently established for menhaden oil (21 C.F.R. §184.1472(a)(3)).

We submit that use of tuna oil as specified in this document is consistent with the Federal Food, Drug and Cosmetic Act (FDC Act), which states that 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."

An Expert Panel reported on October 18, 2000 on

> **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.**

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 product, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, are "generally recognized as safe" ("GRAS") based on scientific procedures."**

The full Export Panel Report is attached as Appendix I.

2. Description of the products

i) Chemistry

Fish oils are, by their very nature, complex materials consisting of a complex mix of glycerides, fatty acids, unsaponifiables and phospholipids. The CAS registry number for fish oils is 8016-13-5. This number is used for fish oils such as tuna oil, which do not have a unique CAS number. As stated in the Expert Panel Report (Appendix I, page 2):

> "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 characterise 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 other fish oils in the ratio of the C20:5 n-3 (EPA) to the C22:6 n-3 (DHA) fatty acids...".

Note that the ratio of EPA:DHA in tuna oil (around 1:4) is similar to that of human breast milk.

Table 1: Fatty Acid Composition of Tuna and Menhaden Oils

FATTY ACID	TUNA	MENHADEN
14:0	3.0	9.0
16:0	20.0	19.0
18:0	6.0	3.0
16:1	4.5	12.0
18:1	15.0	13.0
22:1	1.0	-
18:2	1.5	1.0
18:3	1.0	1.0
20:5	6.0	14.0
22:6	26.5	8.0

Reference: Expert Panel Report Appendix I, page 2

ii) Specifications

The HiDHA® tuna oil can be produced in a number of forms all of which are derived from the same raw tuna oil material and are processed in the same plant using essentially the same process route. The only differences are the degree of bleaching (number of bleach cycles) and winterization (the temperature of crystallization) employed during processing.

Table 2: Specifications for HiDHA® tuna oil products

Specification	HiDHA® Tuna Oil	HiDHA® Tuna Oil Powders	HiDHA® Tuna Oil Emulsions
Appearance	Pale yellow oil	White to light beige powder	Light beige emulsion
Odour	Faint fresh fish	bland	bland
%Total Fat	100%	24-30%	25% min
Fatty Acid Profile*:			
Docosahexaenoic Acid (DHA), %*	25-28%	18-25%	25-28%
Eicosapentaenoic Acid (EPA), %*	5-8%	4-8%	5-8%
Total omega-3 fatty acid content, %*	32-40%	26-34%	32-40%
Acid Value, mgKOH/g	1.0	**	**
Peroxide Value, meq O_2/kg	5	**	**
p-Anisidine Value	20	**	**
Colour, Gardner	4	**	**
Unsaponifiable Matter, %	2	**	**
Lead, ppm	0.1	**	**
Mercury, ppm	0.1	**	**
Cadmium, ppm	0.1	**	**
Arsenic, ppm	0.1	**	**
Total Heavy Metals, ppm	2	**	**
DDT, ppm	0.05	**	**
DDE, ppm	0.05	**	**
HCB, ppm	0.05	**	**
PCB, ppm	0.1	**	**
Lindane, ppm	0.05	**	**
Yeast and Mould (cfu/g)	<100		
Standard Aerobic Plate Count (cfu/g)	<100	<1000	<1000
Enterobacteriaceae (cfu/g)	<100		
E. coli	ND in 1g		
Salmonella spp.	ND in 10g	ND in 25g	ND in 25g
Coliforms (37°C)		ND in 0.1g	ND in 0.1g
Coagulase positive staph.		ND in 0.01g	ND in 0.01g
Baccillus cereus		ND in 0.01g	ND in 0.01g

* Fatty acid composition is expressed as a percentage of fatty acid methyl esters prepared for GC analysis [% area method]

** The fat content of these products (HiDHA® Tuna Oil Powders and HiDHA® Tuna Oil Emulsions) have the same values as those listed in column 2 of the Table.

The HiDHA® tuna oil can be combined with additional approved ingredients to increase its range of applications in baked, liquid and semi-liquid conventional foods.

Table 3: Ingredient Listing for HiDHA® tuna oil products

HiDHA® Tuna oil products	Ingredients	FDA Regulatory Status
HiDHA® Tuna oil	HiDHA® Tuna oil Mixed natural tocopherols	 21 C.F.R. § 182.3890
HiDHA® Tuna oil Powders	HiDHA® Tuna oil Mixed natural tocopherols Hydrogenated palm oil Mono- and diglycerides of edible fatty acids Ascorbyl palmitate Modified food starch Citric acid Maltodextrin Sodium caseinate Sodium ascorbate Lecithin dl-alpha tocopherol Propyl gallate Butylhydroxyanisole (BHA)	 21 C.F.R. § 182.3890 GRAS 21 C.F.R. § 184.1505 21 C.F.R. § 182.3149 21 C.F.R. § 172.892 21 C.F.R. § 184.1033 21 C.F.R. § 184.1444 21 C.F.R. § 182.1748 21 C.F.R. § 182.3731 21 C.F.R. § 184.1400 21 C.F.R. § 182.3890 21 C.F.R. § 184.1660 21 C.F.R. § 182.3169
HiDHA® Tuna oil Emulsions	HiDHA® Tuna oil Mixed natural tocopherols Sodium caseinate Whey protein isolate Glucose	 21 C.F.R. § 182.3890 21 C.F.R. § 182.1748 GRAS 21 C.F.R. § 168.121

Data sheets for products presently available are included in Appendix II.

Details of analytical methods are provided in the Expert Panel Report, page 9.

iii) Manufacturing

HiDHA® tuna oil is manufactured from raw tuna oil, a by-product of the edible tuna canning industry in American Samoa. Selected tuna fish are responsibly

fished in the Southern Pacific Ocean using dolphin friendly methods. The Southern Pacific Ocean provides a sustainable catch of fish from a pollution-free environment. Tuna found in these waters have higher levels of DHA compared to tuna of other oceans. Harvested species include skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*), albacore (*Thunnus alulunga*), and bigeye (*Thunnus obesus*). All of these species have been widely consumed throughout history by humans. The tuna stocks are under no threat and the existing level of harvesting is sustainable.

Tuna are caught by high speed fishing vessels and are immediately blast frozen to below -35° C and kept at that temperature until the vessel arrives at the cannery.

The stabilized raw tuna oil is imported to Australia where it is refined to ensure the final product is stable and consistent to strict specifications.

Table 4: Specifications for raw tuna oil

Specification	Limits
Product name	Stabilized Crude Tuna Fish Oil
Description	Dark brown, free flowing liquid
Docosahexaenoic acid (DHA), %	23.5 min
Eicosapentaenoic acid (EPA), %	7.5 min
Total omega-3 fatty acids	33.0 min
Iodine value	175 min
Moisture and impurities, %	1.0 max

Unsaponifiable matter, %	2.0 max
Free fatty acids, %	4.0 max
Anisidine number	240 max
Cholesterol, mg/g	22 max
Vitamin A (retinol), I.U./ml	500 max
Vitamin D3, I.U./ml	700 max
Arsenic, ppm	0.10 max
Cadmium, ppm	0.10 max
Copper, ppm	0.50 max
Lead, ppm	0.50 max
Mercury, ppm	0.10 max
PCBs, ppm	0.05 max
DDT/DDE, ppm	0.05 max
HCB, ppm	0.05 max
Lindane, ppm	0.02 max

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 in 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.

The Expert Panel Report (Appendix I, pages 10-17) provides full details on the manufacturing process in American Samoa and Australia for HiDHA® tuna oil and a range of products based on HiDHA®.

iv) Analytical data

Detailed analytical data is provided in the Expert Panel Report (Appendix I, pages 17-29). Data on tuna oil has been available for over 30 years. Data on raw tuna oil used in the production of HiDHA® from 1997 to 1999 indicates that only about 14 fatty acids make up the major portion of the composition of the oil and the relative fractions are:

- Saturates 29-30%
- Monounsaturates 22-23%
- Polyunsaturates 40-42%
- Ratio of EPA:DHA ranges from 0.22-0.27

The data indicate that the fatty acid content 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 for other fish oils is of the order of 0.65-2.5 and usually 1.0 or greater.

There is a very slight trace of *trans* fatty acids in the raw tuna oil amounting to less than 1.0% C16:1 *trans* accounting for more than half of the total *trans* fatty acids. Processing has very little effect on the total *trans* fatty acids in the oil.

Table 11 in the Expert Panel Report (Appendix I, page 28) demonstrates the minor variability between five batches of refined tuna oil in 1998 – 1999. This is summarised in Table 5.

Table 5: Comparison 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
Date	10.3.99	14.7.99	18.3.99	19.5.99	9.11.98
Saturates, %	29.36	32.52	28.41	27.62	28.00
Monounsaturates, %	22.01	23.38	22.79	23.31	23.33
Polyunsaturates, %	43.34	39.92	44.16	44.77	44.71
DHA, %	26.04	25.1	26.62	26.74	26.92
EPA, %	7.33	6.00	7.09	6.67	6.61
EPA/DHA	0.28	0.24	0.27	0.25	0.25

v) Environmental contaminants

The HiDHA® tuna oils contain extremely low levels of environmental contaminants because:

- The oil is deliberately sourced from specific tuna species harvested only in clean waters;
- The oil is extracted only from the body musculature; and
- The oil is "fail-safe" purified using advanced processing techniques.

The waters in the south and central regions of the South Pacific Ocean are relatively unpolluted hence oil extracted from the fish are much less contaminated with industrial and agrochemical residues than fish oil products from the more polluted waters of the Northern hemisphere.

No oil is extracted from the liver or any part of the viscera of the tuna fish further reducing the risk of contamination as these organs primarily accumulate fat-soluble environmental contaminants.

Following is a summary of processing steps used in the purification of HiDHA® tuna oil.

Table 6: Processing Steps for Purification of HiDHA® tuna oil

The Clover tuna oil refining plant was designed specifically for refining tuna oil and uses unit processes that are standard in the edible oil industry. The factory is listed with 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.

See Expert Panel Report (Appendix I, pages 29-37) for full details of analytical results.

3. Safety aspects

Clover Corporation achieves GRAS status based on scientific procedures by comparing the HiDHA® tuna oil to menhaden oil.

i) Product integrity

Controlled manufacturing procedures, strict product specifications and continuous product monitoring ensures that Clover's HiDHA® tuna oil products are consistently of a high standard and have a low threshold of contaminants.

ii) Product stability

HiDHA® tuna oil is sensitive to air, light and extended periods at elevated temperatures. The product may be stored for 2 years from date of manufacture in the unopened original drum, in cool (10-25° C), dry conditions (relative humidity less than 75%). After opening the original container, the oil will remain stable if thoroughly flushed with an inert gas (nitrogen, argon, etc) and resealed under inert gas. Only the following are suitable materials for equipment coming into contact with tuna oil: stainless steel, aluminum, glass, enamel or food-approved plastic.

Recent technological advances have led to the development of an expanded range of HiDHA® tuna oil products that offer greater flexibility of use in a stable form. Microencapsulation of tuna oil with permitted ingredients and additives has allowed the production of oxygen-sensitive tuna oils in a form, which has good physical stability and enhanced resistance to oxidation.

Example 1.
The manufacture of the Driphorm HiDHA® Bake microencapsulated tuna oil involves standard unit processes of dispersion of ingredients, heating, homogenisation and drying to produce a fine powder.

Example 2.
The Gelphorm HiDHA® microencapsulated tuna oils are in the form of an emulsion and involves standard unit processes of dispersion of ingredients, heating and homogenisation. The encapsulant materials are different as outlined in the Data Sheets (see Appendix II).

iii) Intake exposure levels

HiDHA® tuna oil is intended for use as a replacement for menhaden oil in conventional foods under the conditions prescribed in 21 C.F.R. § 184.1472(a)(3). 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 and 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 and DHA are listed in the Table below, and it is within these same limitations that HiDHA® tuna oils will be used in conventional foods.

Table 7: Maximum Level of use of HiDHA® tuna oils

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

a) Historical intakes

Fish oils have been part of the human diet for millennia with communities living close to the coast continuing to consume regular and significant quantities of fish and therefore fish oils.

b) Conventional food use

It has been estimated that the average intake of EPA and DHA in the USA is about 0.1 g/day. In two Japanese studies intakes of EPA and DHA in Japan were shown to be 1.5 and 4.2 grams/day respectively (Bruinsma et al. 2000).

Product formulation examples:

The following examples are realistic and recommended quantities of HiDHA® tuna oil in a range of foods. Examples 1 – 4 represent foods containing 30 mg or 60 mg EPA and DHA in a serving. Examples 5 – 6 represent foods containing 165 mg EPA and DHA in a serving.

Example 1:
60 mg EPA and DHA in a serving of bread (in Australia this is 2 slices) requires an addition rate of only 0.5% HiDHA® tuna oil. This is the likely maximum level that would be recommended for HiDHA® tuna oil to bread. (Note that 21 C.F.R. § 184.1472(a)(3) permits up to 1% menhaden oil in bread as served.)

Example 2:
60 mg EPA and DHA in a serving of yogurt (200g) requires an addition rate of only 0.1% HiDHA® tuna oil. (Note that 21 C.F.R. § 184.1472(a)(3) permits up to 4% menhaden oil in yogurt as served.)

Example 3:
30mg EPA and DHA in a slice of processed cheese (20g) requires an addition rate of only 0.5% HiDHA® tuna oil. (Note that 21 C.F.R. § 184.1472(a)(3) permits up to 5% menhaden oil in cheese as served.)

Example 4:

60mg EPA and DHA in a serving of edible oil spread (10g) requires an addition rate of 2% HiDHA® tuna oil. (Note that 21 C.F.R. § 184.1472(a)(3) permits up to 20% menhaden oil in fats and oils as served.)

Example 5:
165 mg EPA and DHA in a cereal-based snack food (35g) requires an addition rate of 1.6% HiDHA® tuna oil. (Note that 21 C.F.R. § 184.1472(a)(3) permits up to 5% menhaden oil in snack foods as served.)

Example 6:
165 mg EPA and DHA in a serving of edible oil spread (10g) requires an addition rate of 5.5% HiDHA® tuna oil. (Note that 21 C.F.R. § 184.1472(a)(3) permits up to 20% menhaden oil in fats and oils as served.)

Unofficial recommended daily intakes of EPA and DHA are listed in Table 8. At this stage there are no official RDAs for omega-3 fatty acids in the USA or Australia.

Table 8: Unofficial recommended daily intakes of EPA and DHA

Organisation	**Recommendations for daily intakes**
International Society for the Study of Fatty Acids and other Lipids (Simopoulos, 2000)	• 650 mg EPA and DHA for adults • minimum 220 mg EPA for adults • minimum 220 mg DHA for adults • For pregnant and lactating women ensure 300 mg DHA
British Nutrition Foundation (1992)	• 1145 mg EPA and DHA for women • 1430 mg EPA and DHA for men (Calculated from recommendation for a desirable population intake of 0.5% energy from very long chain omega-3s, which equates to about 8g EPA/DHA per week for women and 10g per week for men.)
American Heart Association (2000)	• 900 mg EPA and DHA for secondary cardiovascular disease prevention

c) Infant formula use

Whilst this submission is requesting affirmation of GRAS status for HiDHA® tuna oil, it may of interest that Clover Corporation provides tuna oil to Nutricia Australia for Karicare First Infant Formula. The

product, launched in February 1997 was presented as a unit of standard infant formula with 7 capsules of tuna oil and gamma-linolenic acid attached under the lid. From December 1998 onwards 0.2% DHA and 0.4% AA were encapsulated into the final product.

Since the end of 1997, 584,963 units of Karicare formula containing tuna oil have been distributed to retail pharmacy and grocery. Each unit provides one week's supply hence the equivalent of over 4 million daily serves of product have been supplied. In that time, 204 complaints in relation to the Karicare formula were registered with the manufacturer, Nutricia. (These complaints were not related to the tuna oil component but to other issues such as missing scoops, foreign body, poor miscibility, etc.) Products were retrieved where possible and tested by the manufacturer for a range of parameters. In every case the results have met with specifications for the tuna oil. (Appendix III).

iv) Safety studies

a) Biodisposition data (Absorption, Distribution, Metabolism and Excretion)

From Expert Panel Report (Appendix I, page 40):

> EPA and DHA are incorporated into circulating triglycerides in the same proportion that they appear in the dietary oil (Bronsgeest-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, 1967). EPA and DHA show specificity for different phospholipids (Mori et al., 1987). Cholesterol esters become enriched with EPA but not with DHA (Holub et al., 1987).
>
> Essentially, DHA is metabolized as are other fats although, being a bigger molecule, the reaction rates may be lower.

Recent studies by Yep Y.L. and colleagues at RMIT University, Melbourne determined the acute and chronic effects of human consumption of bread containing microencapsulated tuna oil. In the acute study the proportion of DHA and total omega-3 polyunsaturated fat were significantly increased in plasma triacylgycerol. In the chronic study, total omega-3 polyunsaturated fatty acids in the plasma phospholipid fraction were significantly increased. The authors concluded that low dose long chain omega-3 fatty acids consumed as microencapsulated fish oil-enriched bread, increased the long chain omega-3 fatty acids in plasma of human subjects (Appendix IV)

The Childhood Asthma Prevention Study is presently being conducted by the Children's Hospital at Westmead, Sydney, following evidence that increases in the prevalence of asthma during the 1980s and 1990s has been significant. The study will research the effects of a healthy diet with a favourable balance of omega-3 and omega-6 fatty acids and adequate antioxidants together with house dust mite avoidance on protection against the development of asthma or symptoms of wheeze in children who are prone to this illness. (Appendix V)

A principal component of the dietary intervention involves the use of HiDHA® tuna oil soft gel capsules. The capsules have been used by approximately half of the 600 children enrolled in the study. Over 5 years 1,056,000 individual capsules will be used in the study.

The plasma phospholipid levels of the babies in the study show that the HiDHA® tuna oil capsules have altered red cell plasma fatty acid membrane composition and are an effective measurable intervention for the purpose of investigating risk factors associated with the development of asthma.

The study is now in its third year with parents being asked to give their child one capsule per day. Some parents choose to give 2 capsules/day. To date, no children have had a negative effect from taking HiDHA® tuna oil capsules. This continuing study has demonstrated the bioavailability of fatty acids from tuna oil in the study subjects.

b) Preclinical data

From Expert Panel Report (Appendix 1, pages 40-41):

> 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. HiDHA® tuna oil is 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 Aterburn (1998) (Kyle et al., 1998). *In vitro* and *in-vivo* toxicological 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 (US FDA 1993). These potential safety issues are discussed below. 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 as safe (US 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.

c. Clinical data

From Expert Panel Report (Appendix I, pages 41- 45):

- General considerations

In humans it has been 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 (Conquer and Holub, 1996 and 1997).

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 were extensively discussed in FDA's Final Rule on Health Claims for Omega-3 Fatty Acids and Coronary Heart Disease (US 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 triacylgycerol 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 84, thromboxane A2, and prostaglandin E2). EPA correspondingly produces n-3 eicosanoids (e.g., leukotriene 85, 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 et al., 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).

- 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).

- Glucose levels in non-insulin 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.

- 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. HDL_3-cholesterol was decreased by EPA and HDL_2-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 HDL_2-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.

- Comparison of DHA-rich tuna oils to EPA-rich fish oil (Appendix VI)

In 1997 the CSIRO Division of Human Nutrition, Australia, compared DHA-rich tuna oil with commercial MaxEPA-type fish oil on acceptability and cardiovascular risk factors. The study participants were adult males, who consumed eight 1 g capsules per day for two 6-week periods. The results indicated that both supplements were well tolerated with fewer unfavourable comments about the DHA-rich tuna oil than the comparator. Both oils resulted in high circulating levels of omega-3 fatty acids. The results also demonstrated that DHA-rich tuna oil is as efficacious as EPA-rich fish oil in counteracting hypertriglyceridaemia and thrombotic tendency. Both supplements reduced serum

thromboxane production (40%) and plasma triglycerides (26%), with a modest elevation of LDL cholesterol (6%). Neither oil supplement affected total or HDL cholesterol and there were no clinically relevant changes in blood pressure.

The study also examined effects on production of pro-inflammatory cytokines, viz. interleukin 1b (IL1b) and tumor necrosis factor (TNFa). There was no significant difference between the two supplements with an overall effect of 21% reduction in IL1b. There was no consistent effect on TNFa. The authors concluded that comparison to other studies indicates that both the dose and duration of treatment were adequate to demonstrate cytokine inhibition.

- Other considerations (Appendix I, pages 44-45)

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).

4. Regulatory status in Australia

In Australia, foods are regulated by the Australia New Zealand Food Authority through the Food Standards Code (Australia New Zealand Food Authority, 2000). Dietary supplements are regulated through the Therapeutic Goods Administration as are pharmaceuticals. The overlap between foods and drugs has been recognized by the authorities in Australia and is presently being addressed.

i) Australia New Zealand Food Authority

Fish oils, including tuna oil, are considered foods and as such, when included as an ingredient in a food (such as bread or yogurt), must be declared in the statement of ingredients on the label of that food. Standard 1.2.4 (Labelling of Ingredients) Clause 4 states:

> *"Ingredients must be declared in the statement of ingredients using –*
>
> *(a) the common name of the ingredient; or*
> *(b) a name that describes the true nature of the ingredient; or*
> *(c) where applicable, a generic name set out in the Table to this clause."*

The Table to Clause 4 states that fats or oils must be qualified as to whether the source is animal or vegetable.

Standard 1.2.3 (Mandatory Warning and Advisory Statements and Declarations) Clause 4 states that fish and fish products must be declared on the label of a package of food.

As stated previously, claims relating to the omega-3 content of foods are also regulated. Standard 1.2.8 (Nutrition Information Requirements) (Div 3) Clause 13 (3) and (4) states:

> *(3) A nutrition claim must not be made in relation to the omega-3 fatty acid content of a food, unless the food satisfies the requirements of subclause (2) and contains no less than -*
>
> *(a) 200 mg alpha-linolenic acid per serving; or*
> *(b) 30 mg total eicosapentaenoic acid and docosahexaenoic acid per serving.*
>
> *(4) A nutrition claim must not be made that a food is a 'good source' of omega-3 fatty acid or words of similar import, unless the food satisfies the requirements of subclause (2) and contains no less than 60 mg total eicosapentaenoic acid and docosahexaenoic acid per serving* (Australia New Zealand Food Authority, 2000)

ii) Australian Therapeutic Goods Administration

Whist this Submission is in respect of GRAS affirmation of HiDHA® tuna oil for use in conventional foods, for completeness the following approvals have been provided by the Therapeutic Goods Administration (TGA) in Australia.

Milkarra capsules produced by Clover Corporation and containing HiDHA® tuna oil are registered with the TGA as "MILKARRA capsule blister pack", ARTG Registration Number AUST R 55013 with commencement date of registration being 7 June 1996.

Gazette Notice 13, 3 April 1996 stated that the TGA gives notice that:

> *"the prohibited representation described in paragraph (a) below, being a representation that is necessary for the appropriate use of the therapeutic goods described in paragraph (b) below, may be included either on the label of the package of those goods or in information included in the package in which those goods are contained:*
>
> *(a) a representation to the effect that the goods described in paragraph (b) below may assist normal neurological development in infants whose diets are deficient in long chain polyunsaturated fatty acids;*
> *(b) MILKARRA capsule blister pack*
>
> *supplied as an over-the-counter drug by Clover Corporation Pty Ltd."*

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Appendix I.

Expert Panel Report

Date 12.02 01
Division/Dept CHN
Responsible Dr. N. Sehat
Phone ++49 61 51/726060
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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 to1 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 (FDC Act), 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 FDC Act], 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. i.V

Dr. Martin Dr Sehat

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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/DAY HiDHA TUNA OIL

October 18, 2000

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3. Material Safety Data Sheets

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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:

$$\begin{array}{l} CH_2\text{-}OOCR' \\ | \\ CH\text{-}OOCR'' \\ | \\ CH_2\text{-}OOCR''' \end{array}$$

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:

$$CH_3\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$$

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

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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

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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.

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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:

Determination	Official Method
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:

Determination	Official Method
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

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FIGURE 2. CRUDE TUNA OIL PROCESSING DETAILS

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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.

FIGURE 3. TUNA OIL REFINING PROCESS

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.

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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
14:0	3.14	2.88	3.01	2.81	2.51	2.55
15:0	0.92	1.00	0.86	0.80	0.87	0.79
16:0	18.77	20.17	17.40	16.77	16.83	16.89
17:0	1.06	1.30	0.99	0.99	1.02	0.98
18:0	4.80	5.88	4.63	4.82	4.97	4.83
20:0	0.28	0.66	1.02	0.67	0.79	0.72
22:0	0.21	0.28	0.20	0.49	0.45	0.19
24:0	0.21	0.26	0.28	0.27	0.26	0.27
aturates	29.36	32.52	28.41	27.62	28.00	27.02
14:1	0.21	0.19	0.21	0.18	0.21	0.19
15:1	0.12	0.12	0.11	0.10	0.10	0.10
16:1 n-9	0.33	0.28	0.31	0.29	0.53	0.33
16:1 n-7	3.85	3.77	3.84	3.80	3.83	3.46
16:1 n-6	0.68	0.56	0.61	0.62	0.48	0.54
17:1	0.65	0.78	0.71	0.75	0.78	0.80
18:1 n-9	11.51	12.91	12.21	12.79	13.26	13.73
18:1 n-7	2.36	2.24	2.27	2.14	2.26	2.09
18:1 n-6	0.10	0.15	0.11	0.10	0.17	0.17
20:1 n-11	0.44	1.03	0.64	0.92	0.39	0.25
20:1 n-9	0.17	0.14	0.14	0.14	0.14	0.12
22:1 n-11	0.43	0.37	0.45	1.03	0.48	0.32
22:1 n-9	0.62	0.29	0.75	0.21	0.15	0.17
24:1	0.55	0.56	0.53	0.54	0.56	0.57
onounsaturates	22.01	23.38	22.79	23.31	23.33	22.87
16:2 n-4	0.10	0.10	0.10	0.10	0.10	0.11
8:2 n-6	1.63	1.09	1.50	1.50	1.21	1.29
0:2 n-6	0.19	0.20	0.21	0.27	0.22	0.28
6:3 n-3	0.89	1.13	0.91	0.66	0.38	0.88
8:3 n-6	0.23	0.22	0.25	0.26	0.33	0.21
8:3 n-3	0.79	0.50	0.76	0.71	0.53	0.53
0:3 n-6	0.26	0.22	0.27	0.26	0.20	0.24
8:4 n-3	1.08	0.38	1.20	1.61	1.35	1.63
0:4 n-6	1.67	1.86	1.82	2.00	2.03	1.99
0:4 n-3	0.20	0.20	0.20	0.32	0.20	0.29
2:4 n-6	0.26	0.17	0.25	0.29	2.03	0.22
0:4 n-3	0.20	0.20	0.20	0.32	0.20	0.29
0:5 n-3	7.33	6.00	7.09	6.67	6.61	7.04
2:5 n-6	1.58	2.57	1.83	1.91	2.20	1.83
2:5 n-3	1.11	1.18	1.16	1.28	1.29	1.23
2:6 n-3	26.04	25.1	26.62	26.74	26.92	27.36
lyunsaturates	43.34	39.92	44.16	44.77	44.71	45.13
ers	5.29	4.18	4.64	4.30	3.96	4.98
A/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)	Batch142F (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

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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

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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
Trans 14:1		
Trans 16:1	0.5	0.64
Trans 18:1 n-9	0.3	0.13
Trans 18:2 n-8	0.2	0.19
Total *Trans*	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.

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.

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
Ortho PCBs	0.39	0.80	0.44
NON-*Ortho* 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-*ortho* PCBs	*Ortho*-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 (µg/kg).

Polyaromatic hydrocarbon ("PAH")	PAH µg/kg		Conversion Factor PAH/BaP	BaP equiv. µg/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
ã-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 used 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.

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 (Bronsgeest-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 ecosanoids (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 arrythmic 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. HDL_3-cholesterol was decreased by EPA and HDL_2-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 HDL_2-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.

BY:

Anthony Bimbo	10/21/2000 Date
Joseph F. Borzelleca, Ph.D.	24 October 2000 Date
Walter Glinsmann, M.D.	Oct. 19, 2000 Date
David Kritchevsky, Ph.D.	26 October 00 Date

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Distinguished Service Award, 1986

National Italian - American Foundation Award
Excellence in Medicine and Community Service, 1987

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Distinguished Alumnus Award, 1987

Virginia Commonwealth University - School of Basic Health Sciences
Outstanding Faculty Award, 1987

Virginia Commonwealth University - School of Basic Health Sciences, Dept. of Pharmacology and Toxicology
Professor of the Year- 1992

American College of Toxicology
Distinguished Service Award- 1997

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AWARDS

HHS Secretary's Award for Excellence in Public Service, Implementing the Food Labeling Initiative, 1993.

PHS Citation for exemplary and creative work as a management clinician in nutrition research and investigative matters, 1989

FDA Award of Merit for Leadership, Health Hazard Evaluations, 1988

PHS Unit Commendation, Food Irradiation Safety Evaluation, 1988

PHS Citations for Outstanding Management Performance, 1986-91

PHS Meritorious Service Medal for sustained high quality leadership and outstanding contributions to nutrition and food safety, 1986

FDA Award of Merit for Leadership, Aspartame Clinical Investigation Team, 1986

FDA Commissioner's Special Citation to the Research Involving Human Subjects Committee for unique and outstanding performance to the Food and Drug Administration by promoting research while ensuring the protection of the human subjects involved, 1984

PHS Commendation Medal for sustained high quality leadership and professional accomplishments in planning, implementing, and evaluating nutrition research at the Food and Drug Administration, 1982

FDA Commendable Service Award as a member of the Monosodium Glutamate Review Group, 1997

RECENT INVITED LECTURES AND SEMINARS

June 14, 1999 — *Scientific Concepts, Standards, and Subtantiation*
American Association of Cereal Chemists on Functional Foods: Strategies for the Food Industry, Newport Beach, California

March 30,1999 — *Distinguishing a Nutrient from a Drug*
Ceres Forum® on What is a Nutrient? Georgetown University Conference Center, Washington, DC

June 19, 1998	*The Role of Clinical Investigations in Establishing Safety and Efficacy for Functional Foods* Course: A Global Perspective on Regulatory Approval for Food Ingredients, Nutraceuticals, and Dietary Supplements. Institute of Food Technologists '98 Atlanta, GA
April 18, 1998	*Functional Foods: Regulatory Considerations* Symposium: Functional Foods for Health Promotion – A Public Health Opportunity? Experimental Biology '98, San Francisco, CA
Oct. 27, 1997	*Functional Foods: Regulatory Considerations* Panel: Are Consumers Ready for Functional Foods? American Dietetic Association Annual Meeting, Boston, MA
March 13, 1997	*Overview of Research on Health Benefits from Specific Phytochemicals* Panel on Demystifying Functional Foods, Public Voice 20th Annual National Food Policy Conference, Washington, DC
Nov. 28, 1996	*Dietary Guidance and the U.S. Food Supply: Focus on Infants and Young Children* Health Visitors Association Centenary Conference, Harrogate, England
Oct.17	*U.S. Health Claims: Rational and Lessons Learned* Symposium on Health Claims for Foods in Canada, U. Toronto, Ontario, Canada
Feb. 26	*Statements of Nutritional Support: Claim Substantiation* Symposium on the New Structure/Function Claims Frontier Under DSHEA, National Nutritional Foods Association, Washington, DC
Feb. 9	*Regulatory Framework: United States* Workshop on Antioxidants, ILSI-Europe, Brussels, Belgium
Jan. 24, 1996	*Perspective on the Future of Designer and Functional Foods: Definitions, Regulatory Perspective, and Health Claims* Annual Meeting, ILSI, Cancun, Mexico
Oct. 2, 1995 Oct. 3	*Food Labeling and Food Safety Requirements in the United States* and *International Developments in Food Fortification, Foods for Special Dietary Use, and Functional Foods*, Doosan Technical Center, Seoul, Korea
Sept. 26 Sept. 28	*Functional Foods in North America* *Regulatory Aspects of Functional Foods* First International Conference on East-West Perspectives on Functional Foods, ILSI-Southeast Asia, Singapore
Sept.14	*Adverse Events Associated with Chemical Food Components* Safety Forum: Population Subgroups Requiring Special Food Safety Attention National Center for Food Safety and Technology, Argo-Summit, IL
July 12-13	*Health Benefits vs. Safety Concerns When Developing "Functional" Foods* and *Regulation of Food Components for Health Promotion and Disease Prevention* Nutracon '95: Nutraceuticals and Functional Foods, Las Vegas, NV
June 20, 1995	*Dietary Guidelines for Infants and Young Children* Public Voice Children and Nutrition Conference, Washington, DC

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CURRICULUM VITAE

DAVID KRITCHEVSKY, PhD

Pages 000339 to 000342 of curriculum vitae removed to conform to privacy protection procedures.

CLOVER RESEARCH CENTRE

TECHNICAL INFORMATION

CHROMATOGRAMS
TUNA FISH OIL

PRODUCT CODE: 7005

Please note:
GC calibrated against standards on a daily basis.

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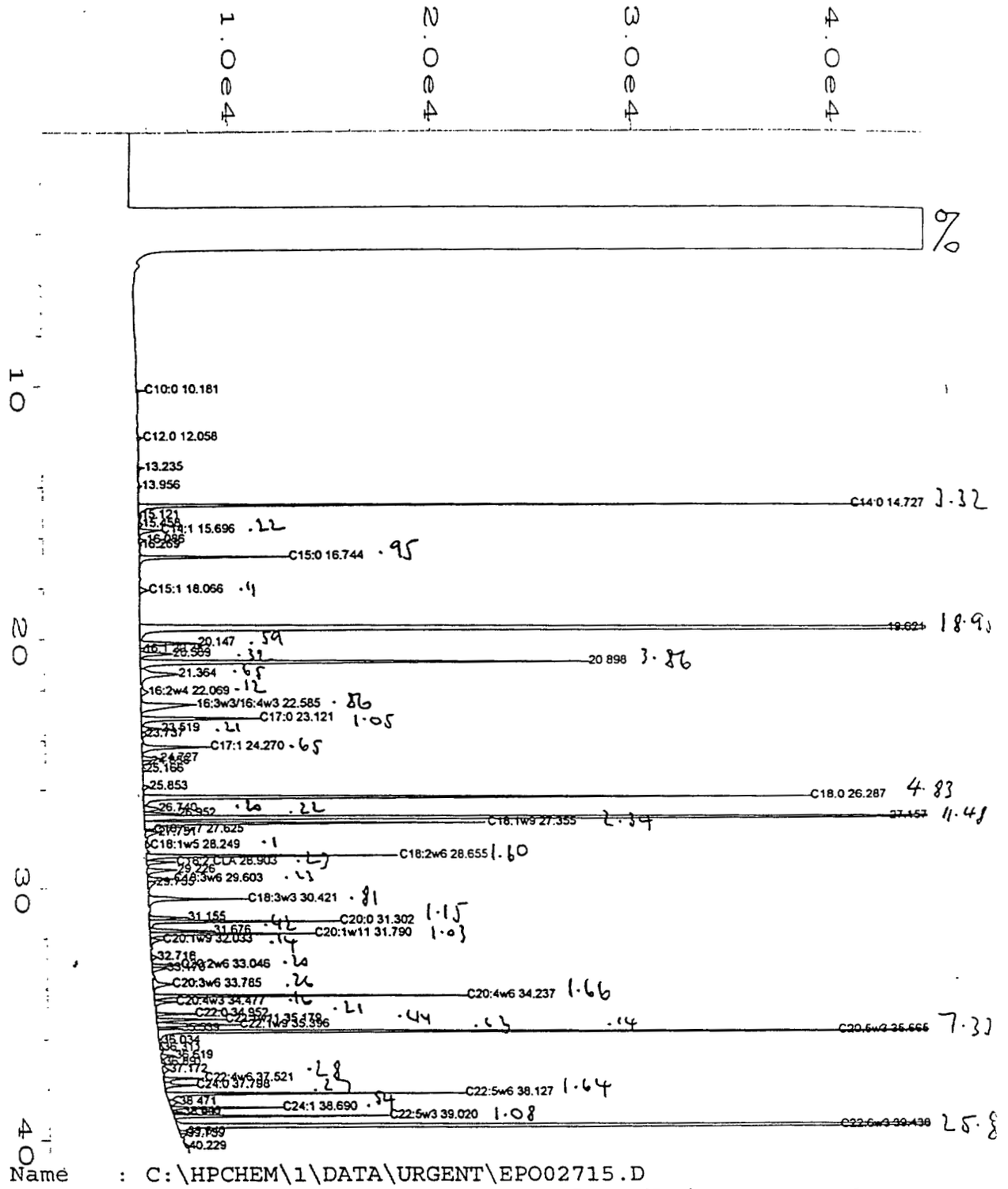

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Acquired on	: 17 Mar 99 11:25 AM	Instrument Method	: GEORGE1.MT
Report Created on	: 17 Mar 99 12:10 PM	Analysis Method	: GEORGE1.MT
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Multiplier	: 1	ISTD Amount	:
Sample Info	: Signal Noise-19. Tuna Fish Oil.Hi-DHA 25N3.7003.B.No.EC101 250ul.		

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Report Created on:	17 Mar 99 12:10 PM	Analysis Method	: GEORGE1.M
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
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Not all time reference peaks were found

Area Percent Report

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Multiplier	: 1	ISTD Amount	:
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Sig. 1 in C:\HPCHEM\1\DATA\URGENT\EPO02715.D

Pk#	Ret Time	Area	Height	Type	Width	Area %
1	10.181	1244	423	VV	0.045	0.0368
2	12.002	0	42	Fsho	0.000	0.0000
3	12.058	1733	264	PV	0.088	0.0513
4	13.235	1662	333	PV	0.071	0.0492
5	13.956	1044	183	BV	0.079	0.0309
6	14.727	112517	36044	VV	0.048	3.3286
7	14.937	0	213	Rsho	0.000	0.0000
8	15.121	1616	139	VV	0.150	0.0478
9	15.458	1413	188	VV	0.105	0.0418
10	15.696	7290	1072	VV	0.105	0.2157
11	16.086	2165	365	VV	0.086	0.0641
12	16.269	1016	128	VV	0.109	0.0300
13	16.744	32004	7377	VV	0.066	0.9468
14	17.048	0	45	Rsho	0.000	0.0000
15	17.991	0	174	Fsho	0.000	0.0000
16	18.066	3850	470	PV	0.115	0.1139
17	19.579	0	71998	Fsho	0.000	0.0000
18	19.621	640648	95439	VV	0.098	18.9526
19	20.147	19898	2865	VV	0.103	0.5887
20	20.382	1344	220	VV	0.092	0.0398

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Analysis Method : GEORGE1.MT
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22	20.898	130261	22324	VV	0.090	3.8536
23	21.235	0	420	Fsho	0.000	0.0000
24	21.364	21903	1881	VV	0.172	0.6480
25	22.029	0	278	Fsho	0.000	0.0000
26	22.069	4035	375	VV	0.150	0.1194
27	22.196	0	95	Rsho	0.000	0.0000
28	22.585	29124	2732	VV	0.146	0.8616
29	22.783	0	610	Rsho	0.000	0.0000
30	23.121	35312	5886	VV	0.091	1.0447
31	23.519	6931	978	VV	0.104	0.2051
32	23.737	2334	221	VV	0.141	0.0690
33	24.162	0	176	Fsho	0.000	0.0000
34	24.270	21890	3364	VV	0.096	0.6476
35	24.727	6114	894	VV	0.100	0.1809
36	24.858	3236	442	VV	0.102	0.0957
37	25.166	1751	152	VV	0.157	0.0518
38	25.853	2453	333	PV	0.105	0.0726
39	26.287	163393	33053	PV	0.077	4.8337
40	26.565	0	170	Rsho	0.000	0.0000
41	26.740	6878	740	VV	0.129	0.2035
42	26.952	7580	1669	VV	0.069	0.2242
43	27.157	388129	79025	VV	0.077	11.4822
44	27.355	78943	17026	VV	0.070	2.3354
45	27.567	0	448	Rsho	0.000	0.0000
46	27.625	2148	445	VV	0.071	0.0636
47	27.751	5397	658	VV	0.113	0.1597
48	28.211	0	169	Fsho	0.000	0.0000
49	28.249	2244	227	VV	0.137	0.0664
50	28.655	54155	12472	VV	0.066	1.6021
51	28.903	7771	1440	VV	0.082	0.2299
52	29.226	10002	1483	VV	0.097	0.2959
53	29.603	7860	1284	VV	0.089	0.2325
54	29.755	4216	428	VV	0.133	0.1247
55	29.932	0	122	Rsho	0.000	0.0000
56	30.355	0	1192	Fsho	0.000	0.0000
57	30.421	27526	4943	VV	0.079	0.8143
58	31.047	0	45	Fsho	0.000	0.0000
59	31.155	9453	1874	PV	0.074	0.2797
60	31.302	38776	9468	VV	0.062	1.1471
61	31.656	0	2338	Fsho	0.000	0.0000

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64	32.033	4789	614	VV	0.111	0.1417
65	32.718	2457	313	PV	0.112	0.0727
66	33.046	6581	1411	VV	0.069	0.1947
67	33.170	4675	724	VV	0.091	0.1383
68	33.679	0	200	Fsho	0.000	0.0000
69	33.785	8931	935	VV	0.132	0.2642
70	33.876	0	311	Rsho	0.000	0.0000
71	34.065	0	36	Rsho	0.000	0.0000
72	34.237	56067	15549	PV	0.055	1.6587
73	34.477	5364	1029	VV	0.076	0.1587
74	34.952	6966	1902	PV	0.056	0.2061
75	35.179	14877	3379	VV	0.066	0.4401
76	35.396	21145	4077	VV	0.077	0.6255
77	35.539	4582	1175	VV	0.059	0.1356
78	35.665	247657	73202	VV	0.053	7.3266
79	36.034	1123	136	VV	0.118	0.0332
80	36.311	1388	142	VV	0.127	0.0411
81	36.619	4424	688	VV	0.094	0.1309
82	36.854	0	132	Fsho	0.000	0.0000
83	36.891	1153	139	VV	0.114	0.0341
84	37.172	3304	342	VV	0.127	0.0978
85	37.521	9445	1993	VV	0.069	0.2794
86	37.773	0	1010	Fsho	0.000	0.0000
87	37.798	9226	1542	VV	0.086	0.2729
88	38.127	55286	14935	PV	0.056	1.6356
89	38.356	0	250	Rsho	0.000	0.0000
90	38.471	4054	479	VV	0.122	0.1199
91	38.559	0	201	Rsho	0.000	0.0000
92	38.690	18200	5550	VV	0.051	0.5384
93	38.840	1461	517	VV	0.043	0.0432
94	38.901	1473	518	VV	0.044	0.0436
95	39.020	36445	10650	VV	0.054	1.0782
96	39.273	0	185	Fsho	0.000	0.0000
97	39.438	874224	206643	VV	0.060	25.8626
98	39.640	2511	405	VV	0.083	0.0743
99	39.739	2078	313	VV	0.093	0.0615
100	40.217	0	154	Fsho	0.000	0.0000
101	40.229	1343	158	PV	0.142	0.0397

Data File Name	: C:\HPCHEM\1\DATA\URGENT\EPO02715.D		
Operator	: OB	Page Number	: 8
Instrument	: 5890-FID	Vial Number	: 6
Sample Name	: TO.PRODUCTION	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: 17 Mar 99 11:25 AM	Instrument Method	: GEORGE1.MT
Report Created on	: 17 Mar 99 12:10 PM	Analysis Method	: GEORGE1.MT
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
Multiplier	: 1	ISTD Amount	:

Total amount = 3.38026E+006

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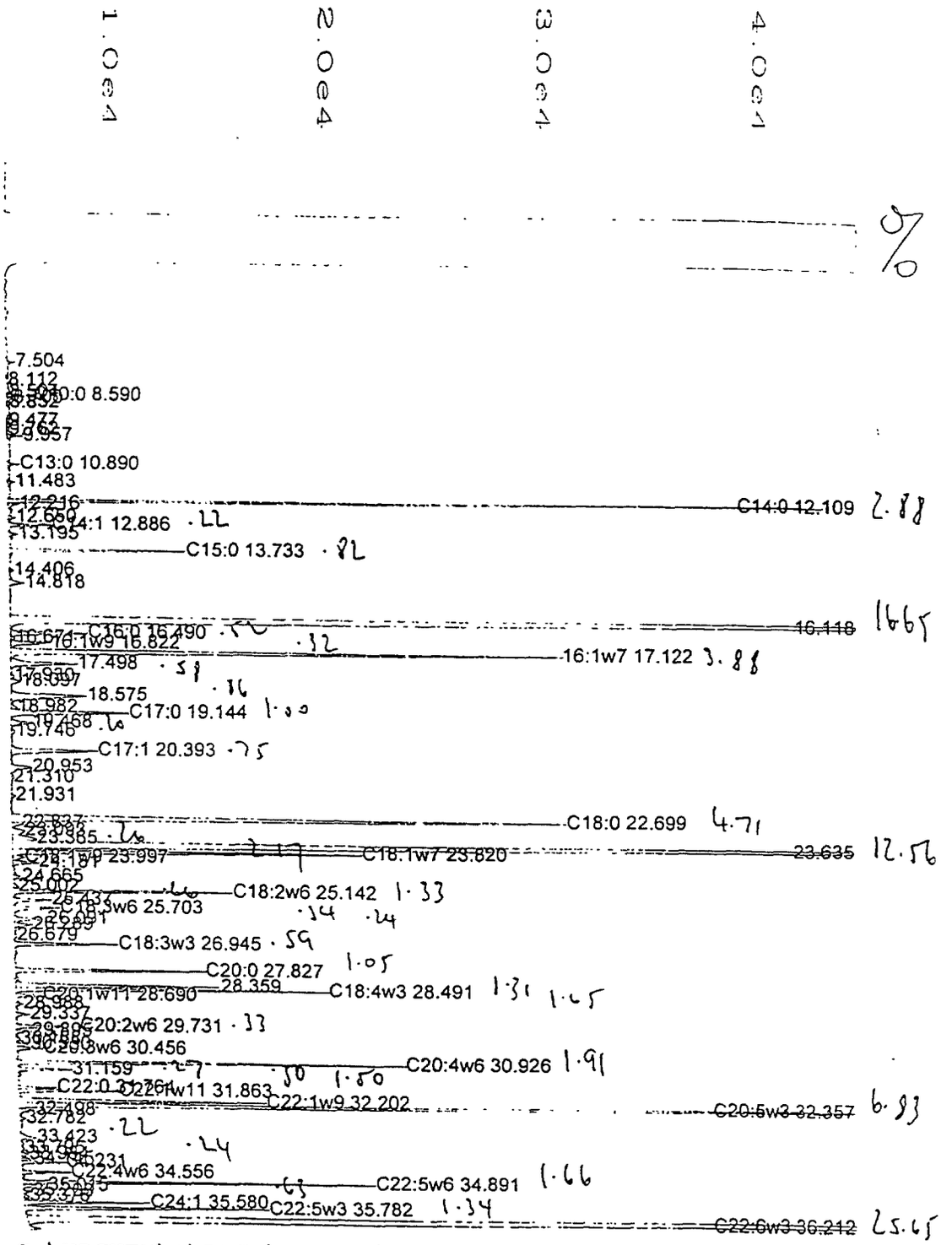
CLOVER RESEARCH CENTRE

TECHNICAL INFORMATION

CHROMATOGRAMS
TUNA FISH OIL

- PRODUCT CODE: 9004

Please note:
GC calibrated against standards on a daily basis.



Data File Name	: C:\HPCHEM\1\DATA\URGENT\EPO01874.D		
Operator	: OB	Page Number	: 1
Instrument	: 5890-FID	Vial Number	: 11
Sample Name	: PRODUCTION	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: 01 Jul 98 02:38 PM	Instrument Method	: GEORGE1.MTH
Report Created on	: 01 Jul 98 03:21 PM	Analysis Method	: GEORGE1.MTH
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
Multiplier	: 1	ISTD Amount	:
Sample Info	: Signal-16.Hi-DHA Tuna oil. B.N. DF161. 400ul/2ml.		

Data File Name	: C:\HPCHEM\1\DATA\URGENT\EPO01874.D		
Operator	: OB	Page Number	: 5
Instrument	: 5890-FID	Vial Number	: 11
Sample Name	: PRODUCTION	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: 01 Jul 98 02:38 PM	Instrument Method	: GEORGE1.M
Report Created on	: 01 Jul 98 03:21 PM	Analysis Method	: GEORGE1.M
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
Multiplier	: 1	ISTD Amount	:

Area Percent Report

Data File Name	: C:\HPCHEM\1\DATA\URGENT\EPO01874.D		
Operator	: OB	Page Number	: 1
Instrument	: 5890-FID	Vial Number	: 11
Sample Name	: PRODUCTION	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: 01 Jul 98 02:38 PM	Instrument Method	: GEORGE1.M
Report Created on	: 01 Jul 98 03:21 PM	Analysis Method	: GEORGE1.M
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
Multiplier	: 1	ISTD Amount	:
Sample Info	: Signal-16.Hi-DHA Tuna oil. B.N. DF161. 400ul/2ml.		

Sig. 1 in C:\HPCHEM\1\DATA\URGENT\EPO01874.D

Pk#	Ret Time	Area	Height	Type	Width	Area %
1	7.504	2069	430	VV	0.088	0.0602
2	8.112	1624	104	VV	0.190	0.0472
3	8.504	1272	133	VV	0.121	0.0370
4	8.590	2694	1207	VV	0.037	0.0784
5	8.700	1298	295	VV	0.059	0.0378
6	8.852	1074	141	VV	0.096	0.0313
7	9.477	1566	114	VV	0.169	0.0456
8	9.762	1522	102	VV	0.248	0.0443
9	9.944	0	448	Fsho	0.000	0.0000
10	9.957	2827	733	VV	0.065	0.0822
11	10.890	2372	580	VV	0.057	0.0690
12	11.483	2044	339	VV	0.081	0.0594
13	12.109	99057	37959	VV	0.041	2.8813
14	12.216	2363	521	VV	0.065	0.0687
15	12.650	1716	358	VV	0.068	0.0499
16	12.886	7648	2006	VV	0.057	0.2224
17	13.195	1776	426	VV	0.062	0.0517
18	13.733	28241	8219	VV	0.054	0.8214
19	14.406	1422	218	VV	0.093	0.0414
20	14.794	0	492	Fsho	0.000	0.0000
21	14.818	4241	685	VV	0.088	0.1234
22	16.118	572445	82990	VV	0.093	16.6507
23	16.490	18030	3583	VV	0.077	0.5244

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO01874.D
Operator : OB
Instrument : 5890-FID
Sample Name : PRODUCTION
Run Time Bar Code:
Acquired on : 01 Jul 98 02:38 PM
Report Created on: 01 Jul 98 03:21 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 6
Vial Number : 11
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.M
Analysis Method : GEORGE1.M
Sample Amount : 1
ISTD Amount :

24	16.671	1177	218	VV	0.079	0.0342
25	16.822	10389	1845	VV	0.086	0.3022
26	17.122	133264	25656	VV	0.082	3.8763
27	17.399	0	493	Fsho	0.000	0.0000
28	17.498	19958	3102	VV	0.095	0.5805
29	17.930	1203	166	VV	0.107	0.0350
30	18.097	2925	453	VV	0.100	0.0851
31	18.575	29675	3520	VV	0.121	0.8631
32	18.675	0	1290	Rsho	0.000	0.0000
33	18.982	1798	293	VV	0.091	0.0523
34	19.144	34293	5485	VV	0.097	0.9975
35	19.468	6715	963	VV	0.106	0.1953
36	19.746	1579	183	VV	0.125	0.0459
37	20.250	0	129	Fsho	0.000	0.0000
38	20.393	25893	4007	VV	0.101	0.7532
39	20.953	8053	994	VV	0.117	0.2342
40	21.060	0	290	Rsho	0.000	0.0000
41	21.283	0	108	Fsho	0.000	0.0000
42	21.310	1553	119	VV	0.171	0.0452
43	21.931	1323	201	VV	0.098	0.0385
44	22.662	0	19134	Fsho	0.000	0.0000
45	22.699	161957	25653	VV	0.094	4.7108
46	22.837	3178	507	VV	0.105	0.0924
47	23.093	5788	597	VV	0.133	0.1684
48	23.194	0	401	Rsho	0.000	0.0000
49	23.334	0	1010	Fsho	0.000	0.0000
50	23.365	9091	1134	VV	0.112	0.2644
51	23.606	0	53133	Fsho	0.000	0.0000
52	23.635	431683	63085	VV	0.096	12.5563
53	23.820	74644	16234	VV	0.071	2.1712
54	23.997	3858	555	VV	0.103	0.1122
55	24.181	6558	1225	VV	0.080	0.1907
56	24.665	2359	442	VV	0.080	0.0686
57	24.979	0	207	Fsho	0.000	0.0000
58	25.002	1498	233	VV	0.089	0.0436
59	25.142	45869	10244	VV	0.070	1.3342
60	25.437	8858	1770	VV	0.076	0.2576
61	25.703	11691	2138	VV	0.082	0.3400
62	26.091	8238	1547	VV	0.081	0.2396
63	26.289	4338	856	VV	0.076	0.1262
64	26.645	0	92	Fsho	0.000	0.0000

Data File Name : C:\HPCHEM\1\DATA\URGENT\EP001874.D
Operator : OB
Instrument : 5890-FID
Sample Name : PRODUCTION
Run Time Bar Code:
Acquired on : 01 Jul 98 02:38 PM
Report Created on: 01 Jul 98 03:21 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 7
Vial Number : 11
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MTH
Analysis Method : GEORGE1.MTH
Sample Amount : 1
ISTD Amount :

65	26.679	1036	126	VV	0.114	0.0301
66	26.945	20392	4794	VV	0.066	0.5931
67	27.827	36158	8858	VV	0.064	1.0517
68	28.359	44934	9544	VV	0.071	1.3070
69	28.491	56798	14637	VV	0.061	1.6521
70	28.690	5397	1233	VV	0.067	0.1570
71	28.988	1501	344	VV	0.067	0.0437
72	29.337	3005	636	VV	0.073	0.0874
73	29.731	11266	2925	VV	0.060	0.3277
74	29.895	2992	701	VV	0.066	0.0870
75	30.142	0	148	Fsho	0.000	0.0000
76	30.168	1212	181	VV	0.095	0.0352
77	30.330	2611	619	VV	0.065	0.0759
78	30.456	5168	1206	VV	0.066	0.1503
79	30.926	65509	17970	PV	0.058	1.9054
80	31.159	9433	2516	VV	0.058	0.2744
81	31.764	6275	1778	VV	0.055	0.1825
82	31.863	17293	4633	VV	0.057	0.5030
83	32.181	0	8584	Fsho	0.000	0.0000
84	32.202	51479	11529	VV	0.066	1.4974
85	32.357	234858	59974	VV	0.061	6.8313
86	32.498	3672	724	VV	0.073	0.1068
87	32.782	1305	277	VV	0.071	0.0379
88	33.336	0	203	Fsho	0.000	0.0000
89	33.423	4416	746	PV	0.083	0.1284
90	33.795	1018	150	VV	0.098	0.0296
91	33.984	1740	344	VV	0.072	0.0506
92	34.145	1930	558	VV	0.052	0.0561
93	34.231	7304	2081	VV	0.055	0.2125
94	34.556	8270	2240	VV	0.057	0.2406
95	34.891	57093	16367	VV	0.055	1.6607
96	35.015	4868	1147	VV	0.065	0.1416
97	35.208	1579	432	PV	0.057	0.0459
98	35.378	1384	263	VV	0.075	0.0403
99	35.580	21471	5849	VV	0.056	0.6245
100	35.782	46213	11346	VV	0.064	1.3442
101	36.181	0	127895	Fsho	0.000	0.0000
102	36.212	881681	165491	PV	0.077	25.6454
103	36.326	0	98	Rsho	0.000	0.0000

Total amount = 3.43797E+006

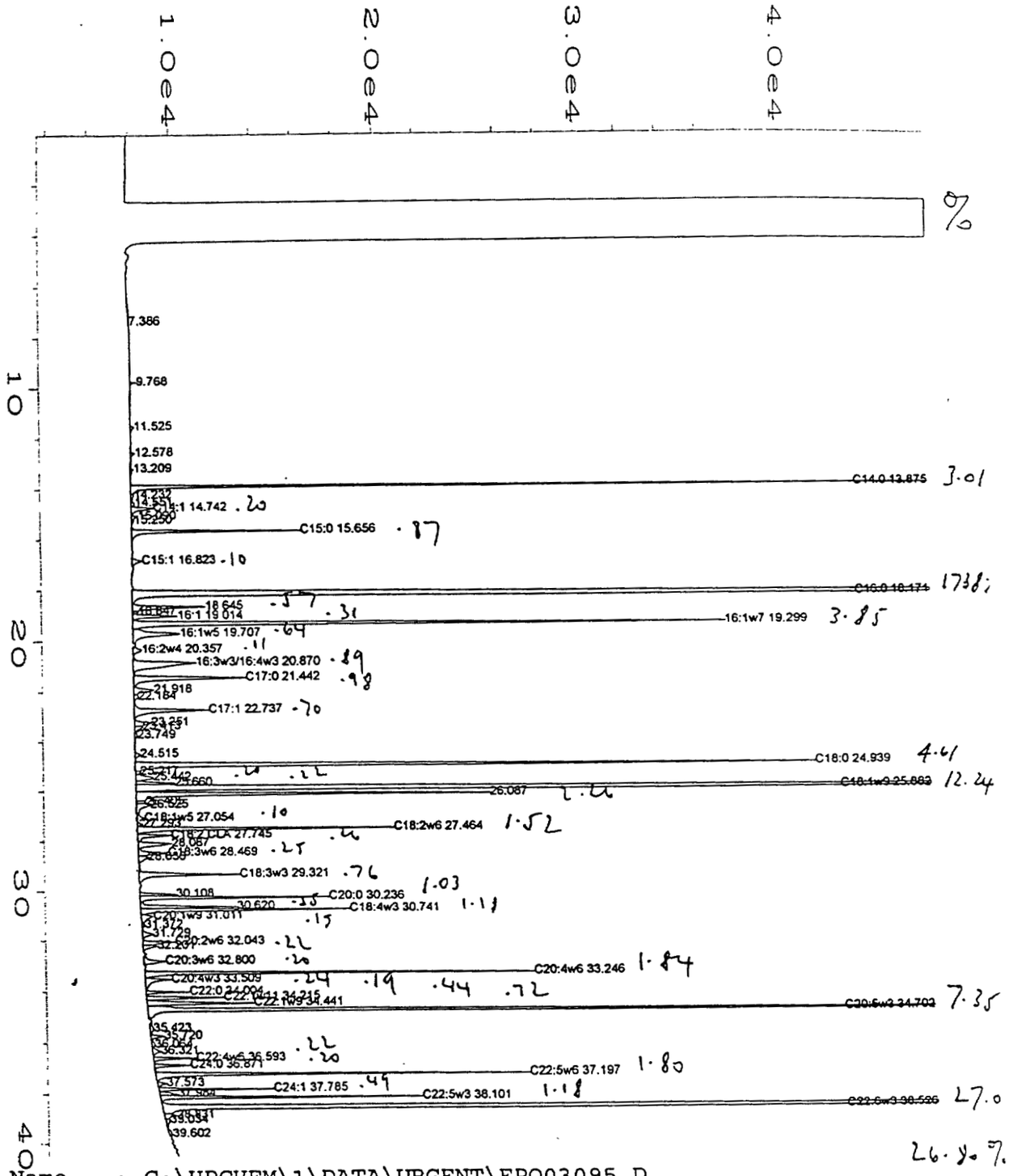
CLOVER RESEARCH CENTRE

TECHNICAL INFORMATION

CHROMATOGRAMS
TUNA FISH OIL

- PRODUCT CODE: 8505

Please note:
GC calibrated against standards on a daily basis.



26.9.7.

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO03095.D
Operator : OB
Instrument : 5890-FID
Sample Name : Prod.Tuna Oil
Run Time Bar Code:
Acquired on : 07 Jul 99 11:51 AM
Report Created on: 07 Jul 99 12:36 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1
Sample Info : Signal Noise 33.Tuna Fish Oil Hi-DHA 25S5 Prod.ID 8505 Batch No. EC181

Page Number : 1
Vial Number : 3
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MT
Analysis Method : GEORGE1.MT
Sample Amount : 1
ISTD Amount :

[1.0.7.7% acetone STD.

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO03095.D
Operator : OB
Instrument : 5890-FID
Sample Name : Prod.Tuna Oil
Run Time Bar Code:
Acquired on : 07 Jul 99 11:51 AM
Report Created on: 07 Jul 99 12:36 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 5
Vial Number : 3
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MT
Analysis Method : GEORGE1.MT
Sample Amount : 1
ISTD Amount :

Could not find time reference peak:
No peak of Number 30's description at 30.813 + 0.124 - 0.063 min.
Not all time reference peaks were found

Area Percent Report

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO03095.D
Operator : OB
Instrument : 5890-FID
Sample Name : Prod.Tuna Oil
Run Time Bar Code:
Acquired on : 07 Jul 99 11:51 AM
Report Created on: 07 Jul 99 12:36 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1
Sample Info : Signal Noise 33.Tuna Fish Oil Hi-DHA 25S5 Prod.ID 8505
Batch No. EC181

Page Number : 1
Vial Number : 3
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MT
Analysis Method : GEORGE1.MT
Sample Amount : 1
ISTD Amount :

Sig. 1 in C:\HPCHEM\1\DATA\URGENT\EPO03095.D

Pk#	Ret Time	Area	Height	Type	Width	Area %
1	6.864	0	35	Fsho	0.000	0.0000
2	7.386	2472	43	BV	0.695	0.0595
3	9.768	1301	330	BV	0.083	0.0313
4	11.493	0	104	Fsho	0.000	0.0000
5	11.525	2029	185	PV	0.148	0.0489
6	11.845	0	29	Rsho	0.000	0.0000
7	12.578	2153	232	PV	0.124	0.0519
8	12.796	0	46	Rsho	0.000	0.0000
9	13.209	1292	151	VV	0.115	0.0311
10	13.774	0	16	Fsho	0.000	0.0000
11	13.875	125011	42030	PV	0.093	3.0109
12	14.232	1541	143	VV	0.141	0.0371
13	14.551	1349	183	VV	0.117	0.0325
14	14.742	8135	1055	VV	0.118	0.1959
15	15.090	1925	327	VV	0.087	0.0464
16	15.250	1043	128	VV	0.112	0.0251
17	15.630	0	4498	Fsho	0.000	0.0000

Data File Name : C:\HPCHEM\1\DATA\URGENT\EP003095.D
Operator : OB
Instrument : 5890-FID
Sample Name : Prod.Tuna Oil
Run Time Bar Code:
Acquired on : 07 Jul 99 11:51 AM
Report Created on: 07 Jul 99 12:36 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 6
Vial Number : 3
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.M
Analysis Method : GEORGE1.M
Sample Amount : 1
ISTD Amount :

18	15.656	36198	8281	VV	0.067	0.8718
19	16.042	0	37	Rsho	0.000	0.0000
20	16.792	0	353	Fsho	0.000	0.0000
21	16.823	4167	471	PV	0.124	0.1004
22	18.171	721909	111488	VV	0.103	17.3873
23	18.645	23500	3600	VV	0.098	0.5660
24	18.847	1483	295	VV	0.084	0.0357
25	19.014	13039	2218	VV	0.091	0.3140
26	19.299	159988	29283	VV	0.085	3.8533
27	19.653	0	1491	Fsho	0.000	0.0000
28	19.707	26356	2316	VV	0.166	0.6348
29	20.357	4594	426	VV	0.163	0.1107
30	20.721	0	102	Fsho	0.000	0.0000
31	20.870	37037	3089	VV	0.165	0.8920
32	21.065	0	913	Rsho	0.000	0.0000
33	21.442	40730	5536	VV	0.111	0.9810
34	21.918	8612	980	VV	0.130	0.2074
35	22.184	2381	209	VV	0.172	0.0573
36	22.609	0	184	Fsho	0.000	0.0000
37	22.737	28848	3663	VV	0.115	0.6948
38	23.251	7270	829	VV	0.127	0.1751
39	23.413	3500	405	VV	0.144	0.0843
40	23.749	2023	138	VV	0.203	0.0487
41	24.515	2431	265	PV	0.129	0.0586
42	24.738	0	13	Rsho	0.000	0.0000
43	24.939	191405	33562	PV	0.089	4.6100
44	25.217	1545	253	VV	0.102	0.0372
45	25.442	8397	852	VV	0.156	0.2022
46	25.660	9290	1909	VV	0.074	0.2238
47	25.882	508252	87216	VV	0.091	12.2413
48	26.087	93942	17482	VV	0.082	2.2626
49	26.405	2376	455	VV	0.076	0.0572
50	26.525	7485	670	VV	0.154	0.1803
51	27.054	3891	360	VV	0.157	0.0937
52	27.173	0	115	Rsho	0.000	0.0000
53	27.293	1351	261	VV	0.078	0.0325
54	27.464	62959	12622	VV	0.076	1.5164
55	27.745	10764	1614	VV	0.100	0.2593
56	27.947	0	144	Rsho	0.000	0.0000
57	28.087	13590	1639	VV	0.122	0.3273
58	28.469	10288	1451	VV	0.102	0.2478

Data File Name : C:\HPCHEM\1\DATA\URGENT\EP003095.D
Operator : OB
Instrument : 5890-FID
Sample Name : Prod.Tuna Oil
Run Time Bar Code:
Acquired on : 07 Jul 99 11:51 AM
Report Created on: 07 Jul 99 12:36 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 7
Vial Number : 3
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.M
Analysis Method : GEORGE1.M
Sample Amount : 1
ISTD Amount :

59	28.636	5001	470	VV	0.149	0.1204
60	28.789	0	169	Rsho	0.000	0.0000
61	29.294	0	3442	Fsho	0.000	0.0000
62	29.321	31490	4978	VV	0.091	0.7584
63	30.074	0	1011	Fsho	0.000	0.0000
64	30.108	8769	1759	PV	0.076	0.2112
65	30.236	42680	9332	VV	0.071	1.0280
66	30.620	22630	4744	VV	0.071	0.5450
67	30.741	49007	10356	VV	0.072	1.1803
68	31.011	6254	575	VV	0.148	0.1506
69	31.372	1281	147	VV	0.124	0.0308
70	31.729	4873	500	VV	0.144	0.1174
71	31.935	0	46	Rsho	0.000	0.0000
72	32.043	9209	1566	VV	0.086	0.2218
73	32.201	4780	693	VV	0.098	0.1151
74	32.763	0	682	Fsho	0.000	0.0000
75	32.800	8419	1036	VV	0.116	0.2028
76	33.246	76551	19380	VV	0.062	1.8437
77	33.509	9943	1270	VV	0.108	0.2395
78	33.756	0	145	Rsho	0.000	0.0000
79	34.004	7941	2114	VV	0.059	0.1913
80	34.215	18123	3760	PV	0.074	0.4365
81	34.441	29675	5247	VV	0.085	0.7147
82	34.702	305262	83320	VV	0.058	7.3523
83	34.971	0	197	Rsho	0.000	0.0000
84	35.423	2838	178	VV	0.209	0.0684
85	35.720	4001	665	VV	0.093	0.0964
86	36.042	0	138	Fsho	0.000	0.0000
87	36.064	1870	142	VV	0.173	0.0450
88	36.321	3873	411	VV	0.129	0.0933
89	36.460	0	64	Rsho	0.000	0.0000
90	36.593	9120	2096	PV	0.067	0.2196
91	36.871	8183	1737	VV	0.073	0.1971
92	37.197	74675	18597	PV	0.062	1.7986
93	37.573	2709	430	VV	0.094	0.0653
94	37.685	0	74	Rsho	0.000	0.0000
95	37.785	20224	5673	VV	0.057	0.4871
96	37.984	4485	872	VV	0.072	0.1080
97	38.101	49043	12904	VV	0.060	1.1812
98	38.526	1121439	228315	VV	0.072	27.0100
99	38.712	0	464	Rsho	0.000	0.0000

Data File Name	: C:\HPCHEM\1\DATA\URGENT\EPO03095.D		
Operator	: OB	Page Number	: 8
Instrument	: 5890-FID	Vial Number	: 3
Sample Name	: Prod.Tuna Oil	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: 07 Jul 99 11:51 AM	Instrument Method	: GEORGE1.MT
Report Created on	: 07 Jul 99 12:36 PM	Analysis Method	: GEORGE1.MT
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
Multiplier	: 1	ISTD Amount	:

100	38.831	5150	610	VV	0.120	0.1240
101	39.034	2654	223	VV	0.162	0.0639
102	39.207	0	83	Rsho	0.000	0.0000
103	39.577	0	142	Fsho	0.000	0.0000
104	39.602	1928	162	PV	0.155	0.0464

Total amount = 4.15193E+006

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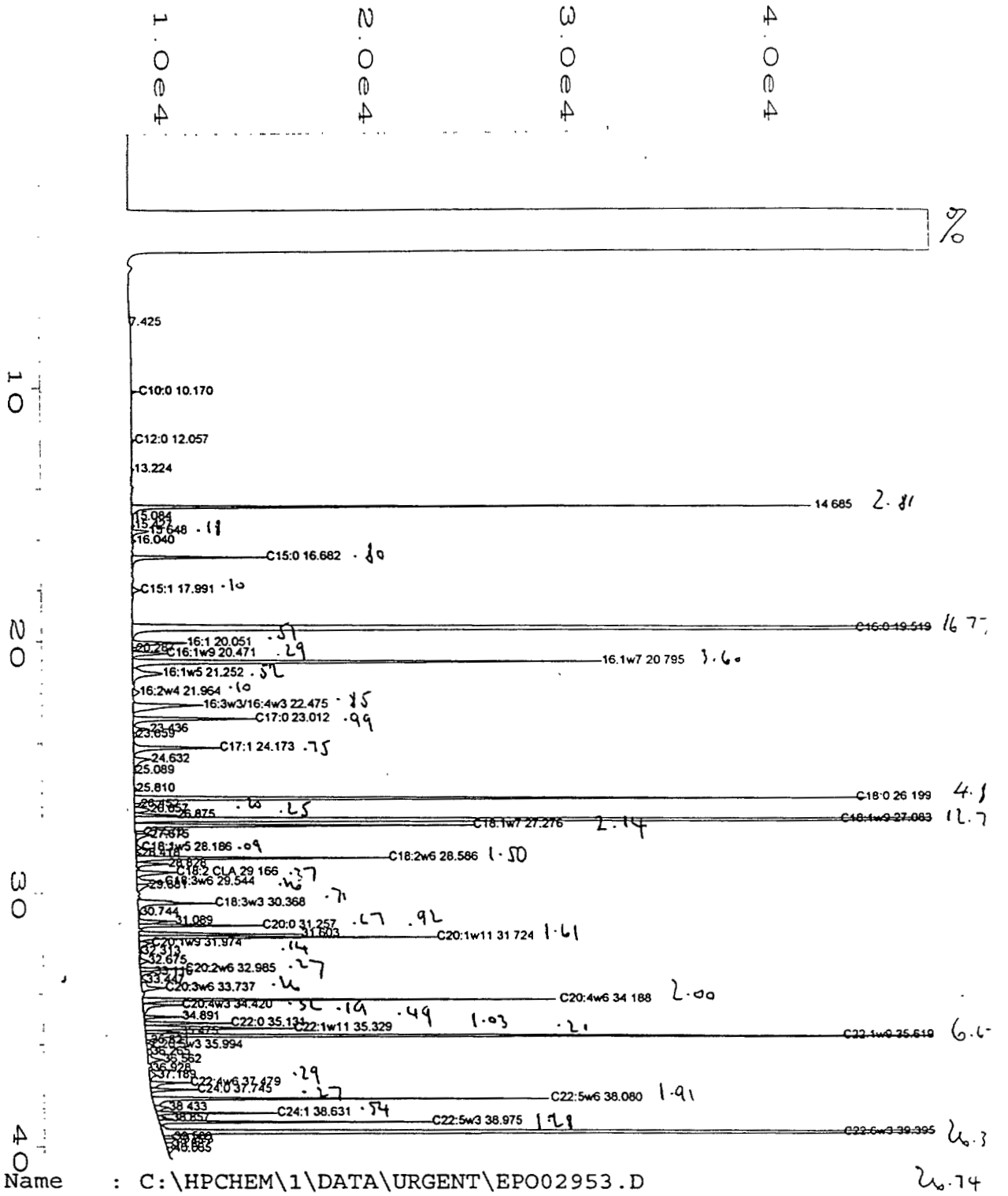
CLOVER RESEARCH CENTRE

TECHNICAL INFORMATION

CHROMATOGRAMS
TUNA FISH OIL

- PRODUCT CODE: 8501

Please note:
GC calibrated against standards on a daily basis.



Data File Name	: C:\HPCHEM\1\DATA\URGENT\EPO02953.D		
Operator	: OB	Page Number	: 1
Instrument	: 5890-FID	Vial Number	: 10
Sample Name	: ProductionTO	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: 25 May 99 11:28 AM	Instrument Method	: GEORGE1.M
Report Created on	: 25 May 99 12:15 PM	Analysis Method	: GEORGE1.M
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
Multiplier	: 1	ISTD Amount	:
Sample Info	: Signal Noise 28.TO.Product: 8501.Batch: EE191.250ul.		

000361

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO02953.D
Operator : OB
Instrument : 5890-FID
Sample Name : ProductionTO
Run Time Bar Code:
Acquired on : 25 May 99 11:28 AM
Report Created on: 25 May 99 12:15 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 5
Vial Number : 10
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MTH
Analysis Method : GEORGE1.MTH
Sample Amount : 1
ISTD Amount :

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO02953.D
Operator : OB
Instrument : 5890-FID
Sample Name : ProductionTO
Run Time Bar Code:
Acquired on : 25 May 99 11:28 AM
Report Created on: 25 May 99 12:15 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1
Comp . Info : Signal Noise 28.TO.Product: 8501.Batch: EE191.250ul.

Page Number : 1
Vial Number : 10
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MTH
Analysis Method : GEORGE1.MTH
Sample Amount : 1
ISTD Amount :

Fig. 1 in C:\HPCHEM\1\DATA\URGENT\EPO02953.D

Pk#	Ret Time	Area	Height	Type	Width	Area %
1	6.888	0	87	Fsho	0.000	0.0000
2	7.425	1530	38	PV	0.478	0.0413
3	10.170	1206	369	BV	0.048	0.0325
4	12.033	0	100	Fsho	0.000	0.0000
5	12.057	1509	172	BV	0.114	0.0407
6	12.212	0	40	Rsho	0.000	0.0000
7	13.224	1271	143	PV	0.118	0.0343
8	14.685	104274	33530	VV	0.048	2.8143
9	15.084	1242	104	VV	0.155	0.0335
10	15.250	0	46	Rsho	0.000	0.0000
11	15.427	1034	139	VV	0.105	0.0279
12	15.648	6807	850	VV	0.112	0.1837
13	16.040	1391	224	VV	0.091	0.0375
14	16.682	29720	6572	VV	0.068	0.8021
15	17.045	0	50	Rsho	0.000	0.0000
16	17.917	0	164	Fsho	0.000	0.0000
17	17.991	3602	398	VV	0.126	0.0972
18	19.519	621508	94276	VV	0.094	16.7740
19	20.051	19025	2683	VV	0.105	0.5135
20	20.287	1296	215	VV	0.090	0.0350
21	20.471	10860	1680	VV	0.100	0.2931
22	20.795	133298	23140	VV	0.089	3.5976
23	21.121	0	410	Fsho	0.000	0.0000
24	21.252	19103	1467	VV	0.189	0.5156
25	21.454	0	207	Rsho	0.000	0.0000
26	21.964	3515	341	VV	0.148	0.0949

Data File Name : C:\HPCHEM\1\DATA\URGENT\EP002953.D
Operator : OB
Page Number : 6
Instrument : 5890-FID
Vial Number : 10
Sample Name : ProductionTO
Injection Number :
Run Time Bar Code:
Sequence Line :
Acquired on : 25 May 99 11:28 AM
Instrument Method: GEORGE1.MT
Report Created on: 25 May 99 12:15 PM
Analysis Method : GEORGE1.MT
Last Recalib on : 17 NOV 93 08:32 AM
Sample Amount : 1
Multiplier : 1
ISTD Amount :

27	22.475	31563	3470	VV	0.127	0.8519
28	22.666	0	663	Rsho	0.000	0.0000
29	23.012	36733	6030	VV	0.092	0.9914
30	23.206	0	147	Rsho	0.000	0.0000
31	23.436	6247	836	VV	0.109	0.1686
32	23.659	1792	170	VV	0.136	0.0484
33	24.035	0	142	Fsho	0.000	0.0000
34	24.139	0	2586	Fsho	0.000	0.0000
35	24.173	27899	4277	VV	0.095	0.7530
36	24.449	0	51	Rsho	0.000	0.0000
37	24.632	8783	878	VV	0.137	0.2370
38	24.784	0	274	Rsho	0.000	0.0000
39	24.895	0	97	Rsho	0.000	0.0000
40	25.089	1378	108	VV	0.161	0.0372
41	25.810	1253	136	BV	0.124	0.0338
42	25.970	0	29	Rsho	0.000	0.0000
43	26.199	178533	35932	PV	0.077	4.8184
44	26.452	1895	307	VV	0.093	0.0511
45	26.657	7228	762	VV	0.153	0.1951
46	26.875	9392	2083	VV	0.069	0.2535
47	27.083	473730	90466	VV	0.077	12.7855
48	27.276	79284	16691	VV	0.073	2.1398
49	27.570	2513	492	VV	0.074	0.0678
50	27.675	6578	747	VV	0.123	0.1775
51	28.149	0	210	Fsho	0.000	0.0000
52	28.186	2917	275	VV	0.153	0.0787
53	28.336	0	71	Rsho	0.000	0.0000
54	28.418	1162	270	VV	0.067	0.0314
55	28.586	55457	12410	VV	0.069	1.4967
56	28.828	8713	1552	VV	0.088	0.2352
57	29.166	13521	1911	VV	0.103	0.3649
58	29.517	0	1016	Fsho	0.000	0.0000
59	29.544	9458	1342	VV	0.100	0.2553
60	29.681	5673	591	VV	0.132	0.1531
61	30.303	0	1559	Fsho	0.000	0.0000
62	30.368	26346	3799	VV	0.096	0.7111
63	30.744	1106	107	VV	0.142	0.0299
64	31.089	10746	1777	VV	0.088	0.2900
65	31.257	24920	6088	VV	0.064	0.6726
66	31.603	34180	7973	VV	0.065	0.9225
67	31.724	59762	14739	VV	0.063	1.6129

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO02953.D
Operator : OB
Instrument : 5890-FID
Sample Name : ProductionTO
Run Time Bar Code:
Acquired on : 25 May 99 11:28 AM
Report Created on: 25 May 99 12:15 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 7
Vial Number : 10
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MTH
Analysis Method : GEORGE1.MTH
Sample Amount : 1
ISTD Amount :

68	31.974	5024	611	VV	0.115	0.1356
69	32.313	1048	135	VV	0.118	0.0283
70	32.675	3717	423	VV	0.132	0.1003
71	32.985	10060	2200	PV	0.069	0.2715
72	33.116	4719	673	VV	0.117	0.1274
73	33.447	1564	245	VV	0.094	0.0422
74	33.707	0	750	Fsho	0.000	0.0000
75	33.737	9761	1143	VV	0.117	0.2634
76	34.188	73979	20550	VV	0.057	1.9966
77	34.420	11773	1889	VV	0.088	0.3177
78	34.609	0	202	Rsho	0.000	0.0000
79	34.749	0	75	Rsho	0.000	0.0000
80	34.891	7121	1860	VV	0.060	0.1922
81	35.131	18191	4226	VV	0.066	0.4910
82	35.329	38023	7325	VV	0.076	1.0262
83	35.475	7746	1688	VV	0.070	0.2091
84	35.619	247196	73145	VV	0.054	6.6716
85	35.821	1294	221	VV	0.097	0.0349
86	35.994	2772	257	VV	0.147	0.0748
87	36.265	2407	210	VV	0.154	0.0650
88	36.501	0	345	Fsho	0.000	0.0000
89	36.562	6831	730	VV	0.133	0.1844
90	36.928	1165	167	VV	0.099	0.0315
91	37.130	0	264	Fsho	0.000	0.0000
92	37.189	4134	396	VV	0.137	0.1116
93	37.479	10678	1928	VV	0.080	0.2882
94	37.745	10169	2270	VV	0.068	0.2744
95	38.080	70708	19631	PV	0.056	1.9083
96	38.335	0	282	Rsho	0.000	0.0000
97	38.433	4926	676	VV	0.105	0.1330
98	38.539	0	205	Rsho	0.000	0.0000
99	38.631	20047	5938	VV	0.054	0.5410
100	38.838	0	418	Fsho	0.000	0.0000
101	38.857	3136	666	VV	0.067	0.0846
102	38.975	47319	13467	VV	0.056	1.2771
103	39.226	0	249	Fsho	0.000	0.0000
104	39.395	975335	218201	VV	0.070	26.3234
105	39.588	3012	491	VV	0.091	0.0813
106	39.693	4326	541	VV	0.111	0.1168
107	39.887	3231	335	VV	0.136	0.0872
108	40.065	2826	291	VV	0.130	0.0763

Data File Name	: C:\HPCHEM\1\DATA\URGENT\EPO02953.D		
Operator	: OB	Page Number	: 8
Instrument	: 5890-FID	Vial Number	: 10
Sample Name	: ProductionTO	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: 25 May 99 11:28 AM	Instrument Method	: GEORGE1.MTH
Report Created on	: 25 May 99 12:15 PM	Analysis Method	: GEORGE1.MTH
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
Multiplier	: 1	ISTD Amount	:

Total amount = 3.70519E+006

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CLOVER RESEARCH CENTRE

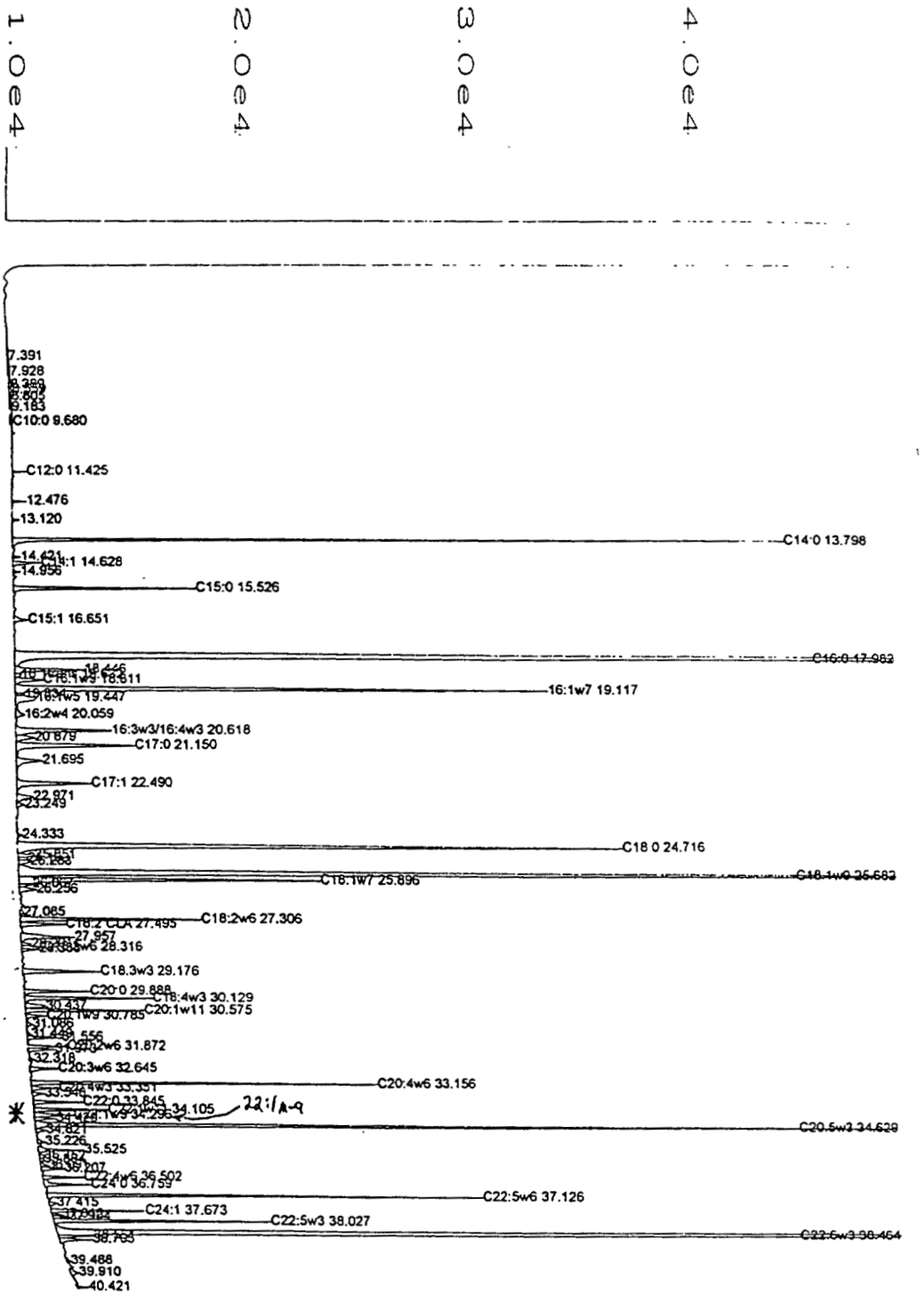
TECHNICAL INFORMATION

- TUNA OIL STANDARDS
- REFERENCE LABORATORIES

Please note:
GC calibrated against standards on a daily basis.

Comparison of G.C. Fatty Acid Composition Determined by Clover and 2 Benchmark Laboratories

Fatty Acid %	Clover Standard	Leatherhead Food R.A.	Mylnefield Research Services
DHA	25.5	26.4	25.3
EPA	5.8	5.8	5.8
Erucic Acid	0.3	0.4	0.1



Data File Name	: C:\HPCHEM\1\DATA\URGENT\EPO03585.D		
Operator	: OB	Page Number	: 1
Instrument	: 5890-FID	Vial Number	: 13
Sample Name	: Standards2000	Injection Number	:
Run Time Bar Code	:	Sequence Line	:
Acquired on	: 01 Feb 00 12:21 PM	Instrument Method	: GEORGE1.MT
Report Created on	: 01 Feb 00 01:06 PM	Analysis Method	: GEORGE1.MT
Last Recalib on	: 17 NOV 93 08:32 AM	Sample Amount	: 1
Multiplier	: 1	ISTD Amount	:
Sample Info	: Signal-38.199 Roche #1 - Standard		

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO03585.D
Operator : OB
Instrument : 5890-FID
Sample Name : Standards2000
Run Time Bar Code:
Acquired on : 01 Feb 00 12:21 PM
Report Created on: 01 Feb 00 01:06 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 5
Vial Number : 13
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.M
Analysis Method : GEORGE1.M
Sample Amount : 1
ISTD Amount :

Not all time reference peaks were found

Area Percent Report

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO03585.D
Operator : OB
Instrument : 5890-FID
Sample Name : Standards2000
Run Time Bar Code:
Acquired on : 01 Feb 00 12:21 PM
Report Created on: 01 Feb 00 01:06 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1
Sample Info : Signal-38.199 Roche #1 - Standard

Page Number : 1
Vial Number : 13
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.M
Analysis Method : GEORGE1.M
Sample Amount : 1
ISTD Amount :

Sig. 1 in C:\HPCHEM\1\DATA\URGENT\EPO03585.D

Pk#	Ret Time	Area	Height	Type	Width	Area %
1	7.391	2273	67	VV	0.410	0.0761
2	7.925	0	94	Fsho	0.000	0.0000
3	7.928	2660	95	VV	0.342	0.0890
4	8.389	1889	87	VV	0.275	0.0632
5	8.559	1013	155	VV	0.087	0.0339
6	8.805	1462	101	VV	0.190	0.0489
7	9.183	1430	92	VV	0.190	0.0479
8	9.628	0	91	Fsho	0.000	0.0000
9	9.680	1943	114	VV	0.211	0.0650
10	11.425	1402	642	BV	0.034	0.0469
11	12.476	1643	666	PV	0.038	0.0550
12	13.120	1149	353	VV	0.049	0.0385
13	13.798	97073	34330	PV	0.044	3.2486
14	13.906	0	418	Rsho	0.000	0.0000
15	14.022	0	95	Rsho	0.000	0.0000
16	14.421	1260	374	VV	0.052	0.0422
17	14.628	5771	1251	VV	0.066	0.1931
18	14.956	1305	345	VV	0.059	0.0437
19	15.526	30161	8227	VV	0.057	1.0094
20	16.581	0	213	Fsho	0.000	0.0000
21	16.651	4085	630	VV	0.092	0.1367
22	17.982	545869	86882	VV	0.095	18.2679

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO03585.D
Operator : OB
Instrument : 5890-FID
Sample Name : Standards2000
Run Time Bar Code:
Acquired on : 01 Feb 00 12:21 PM
Report Created on: 01 Feb 00 01:06 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 6
Vial Number : 13
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.M
Analysis Method : GEORGE1.M
Sample Amount : 1
ISTD Amount :

23	18.229	0	251	Rsho	0.000	0.0000
24	18.446	17930	3161	VV	0.085	0.6000
25	18.632	1798	295	VV	0.089	0.0602
26	18.811	8221	1284	VV	0.095	0.2751
27	19.117	129681	23760	VV	0.085	4.3399
28	19.334	1892	434	VV	0.073	0.0633
29	19.447	11390	963	VV	0.157	0.3812
30	19.560	0	528	Rsho	0.000	0.0000
31	20.018	0	301	Fsho	0.000	0.0000
32	20.059	4287	415	VV	0.144	0.1435
33	20.576	0	2585	Fsho	0.000	0.0000
34	20.618	30091	4315	VV	0.106	1.0070
35	20.879	6705	852	VV	0.120	0.2244
36	21.150	36531	5337	VV	0.105	1.2225
37	21.695	10233	1196	VV	0.122	0.3425
38	22.452	0	2129	Fsho	0.000	0.0000
39	22.490	22675	3350	VV	0.101	0.7588
40	22.683	0	101	Rsho	0.000	0.0000
41	22.971	6675	765	VV	0.126	0.2234
42	23.120	0	153	Rsho	0.000	0.0000
43	23.249	2271	357	VV	0.099	0.0760
44	24.333	1341	238	PV	0.085	0.0449
45	24.716	163767	27006	VV	0.093	5.4806
46	24.876	0	556	Rsho	0.000	0.0000
47	25.051	4416	746	VV	0.089	0.1478
48	25.163	2265	431	VV	0.076	0.0758
49	25.283	3864	539	VV	0.102	0.1293
50	25.482	0	537	Fsho	0.000	0.0000
51	25.682	329199	55579	VV	0.086	11.0169
52	25.896	70889	13440	VV	0.080	2.3724
53	26.067	2926	612	VV	0.080	0.0979
54	26.256	5502	783	VV	0.108	0.1841
55	27.085	1124	159	VV	0.101	0.0376
56	27.306	37385	8154	VV	0.071	1.2511
57	27.495	9773	2041	VV	0.074	0.3271
58	27.883	0	947	Fsho	0.000	0.0000
59	27.957	16209	2421	VV	0.095	0.5425
60	28.201	2090	462	VV	0.069	0.0699
61	28.316	4692	1002	VV	0.069	0.1570
62	28.388	3458	795	VV	0.073	0.1157
63	29.176	18553	3498	VV	0.081	0.6209

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO03585.D
Operator : OB
Instrument : 5890-FID
Sample Name : Standards2000
Run Time Bar Code:
Acquired on : 01 Feb 00 12:21 PM
Report Created on: 01 Feb 00 01:06 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 7
Vial Number : 13
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MT.
Analysis Method : GEORGE1.MT
Sample Amount : 1
ISTD Amount :

64	29.861	0	1777	Fsho	0.000	0.0000
65	29.888	13024	2959	PV	0.068	0.4359
66	30.129	23968	5765	VV	0.065	0.8021
67	30.437	6609	921	VV	0.098	0.2212
68	30.575	21982	5324	VV	0.064	0.7357
69	30.785	4358	914	VV	0.072	0.1458
70	31.086	1717	270	VV	0.096	0.0574
71	31.416	0	124	Fsho	0.000	0.0000
72	31.449	1021	186	PV	0.081	0.0342
73	31.556	6428	1557	VV	0.065	0.2151
74	31.872	6830	1761	VV	0.060	0.2286
75	31.973	5186	1230	VV	0.064	0.1735
76	32.318	1155	229	VV	0.076	0.0387
77	32.645	6152	1276	VV	0.071	0.2059
78	32.733	0	200	Rsho	0.000	0.0000
79	33.156	57601	15436	PV	0.058	1.9277
80	33.351	5324	1226	VV	0.065	0.1782
81	33.546	2940	559	VV	0.077	0.0984
82	33.720	0	38	Rsho	0.000	0.0000
83	33.845	9090	2262	VV	0.062	0.3042
84	34.105	12792	3330	VV	0.060	0.4281
85	34.296	8054	1764	VV	0.068	0.2695 *Erru
86	34.420	3798	986	VV	0.059	0.1271
87	34.629	173720	46843	VV	0.058	5.8137 *EPL
88	34.821	2190	475	VV	0.071	0.0733
89	35.163	0	126	Fsho	0.000	0.0000
90	35.226	1912	354	PV	0.077	0.0640
91	35.525	9107	2083	VV	0.066	0.3048
92	35.752	1798	218	VV	0.117	0.0602
93	35.884	1053	165	VV	0.086	0.0352
94	36.066	0	234	Fsho	0.000	0.0000
95	36.091	1880	328	VV	0.081	0.0629
96	36.207	4511	1054	VV	0.064	0.1510
97	36.502	6852	1848	PV	0.057	0.2293
98	36.735	0	1306	Fsho	0.000	0.0000
99	36.759	9187	2085	VV	0.066	0.3075
100	37.126	69472	19549	PV	0.056	2.3249
101	37.415	2070	364	VV	0.080	0.0693
102	37.673	14984	4227	PV	0.056	0.5015
103	37.812	1372	445	VV	0.049	0.0459
104	37.904	2416	725	VV	0.052	0.0808

Data File Name : C:\HPCHEM\1\DATA\URGENT\EPO03585.D
Operator : OB
Instrument : 5890-FID
Sample Name : Standards2000
Run Time Bar Code:
Acquired on : 01 Feb 00 12:21 PM
Report Created on: 01 Feb 00 01:06 PM
Last Recalib on : 17 NOV 93 08:32 AM
Multiplier : 1

Page Number : 8
Vial Number : 13
Injection Number :
Sequence Line :
Instrument Method: GEORGE1.MTH
Analysis Method : GEORGE1.MTH
Sample Amount : 1
ISTD Amount :

105	38.027	36081	9706	VV	0.059	1.2075	
106	38.292	0	417	Fsho	0.000	0.0000	
107	38.464	762568	168540	VV	0.065	25.5200	*DHA
108	38.561	6063	1596	VV	0.063	0.2029	
109	38.705	7040	1488	VV	0.070	0.2356	
110	39.409	0	67	Fsho	0.000	0.0000	
111	39.488	1215	191	PV	0.089	0.0407	
112	39.910	3095	375	VV	0.112	0.1036	
113	40.000	0	96	Rsho	0.000	0.0000	
114	40.421	1282	524	PBA	0.042	0.0429	

Total amount = 2.98812E+006

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Leatherhead Food RA

Science, Technology
and Information for the
Food Industry Worldwide

Our Ref: MJD/040624/FRA11551-99 F1-5 E\REPORT/11551

1-Mar-00

Structure and Function Test Report

REPORT ON

Received from: Ortwin Bode On 14.02.2000
Clover Corporation
Clover Research Centre
PO. Box 192
Caringbah
NSW 2229
AUSTRALIA

Sample Reference:
F1 = Borage oil #99125A1
* F2 = Tuna oil #R01 10.02.2000
F3 = Borage oil #R03 10.02.2000
F4 = Evening primrose oil #r02 10.02.2000
F5 = Evening primrose oil # E991216 10.02.2000

Date analysis started: 17.02.2000

Method of analysis: Fatty acid profile by BS684 2.34/35 (UKAS FC:4) including internal standard and TBHQ antioxidant.

Results: Fatty acid profile (% methyl esters, capillary column GC, CP SIL 58 stationary phase on-column injection)

A. Calculated as % m/m without internal standard.

	F1	* F2	F3	F4	F5
C14:0	tr	3.3	tr	tr	tr
C14:1	nd	0.1	nd	nd	nd
C14:2/15:0 br	nd	0.1	nd	nd	nd
C15:0	nd	1.0	nd	nd	nd
C15:1	nd	0.1	nd	nd	nd
C16:0	10.5	19.7	10.1	6.4	6.0
C16:1	0.2	5.4	0.2	0.1	tr
C16:2/17:0 br	nd	1.3	nd	nd	nd
C17:0	nd	1.2	nd	nd	nd

Randalls Road, Leatherhead, Surrey, KT22 7RY, UK Tel: (+44) 01372 376761 Fax: (+44) 01372 386228

Leatherhead Food RA uses every possible care in compiling, preparing and issuing the information herein given but can accept no liability whatsoever in connection with it

THE BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION

NAMAS TESTING

000373

C17:1	tr	1.0	tr	0.1	tr
C18:0	4.3	5.7	3.4	1.9	1.9
C18:1	17.5	14.3	15.6	9.5	7.3
C18:2	36.8	2.4	38.0	70.6	74.1
C18:3(n-6) (GLA)	20.9	0.1	24.4	10.4	9.9
C18:3(n-3) (ALA)	0.3	0.5	0.1	0.2	0.1
C:18:3 total	21.4	0.7	24.7	10.6	10.1
C18:4	nd	0.7	nd	nd	nd
C20:0	0.2	0.4	0.2	0.3	0.3
C20:1	4.4	1.0	4.0	0.3	0.2
C20:2	0.1	tr	tr	tr	tr
C20:3	nd	0.4	nd	nd	nd
C20:4	nd	2.0	nd	nd	nd
* C20:5,N-3 (EPA)	nd	5.8	nd	nd	nd
C22:0	0.2	0.3	0.1	0.1	0.1
* C22:1	2.9	0.4	2.4	0.1	tr
C22:2	nd	tr	nd	nd	nd
C22:3	nd	0.3	nd	nd	nd
C22:4	nd	0.2	nd	nd	nd
C22:5	nd	2.2	nd	nd	nd
* C22:6,n-3 (DHA)	nd	26.4	nd	nd	nd
C24:0	0.1	1.4	tr	tr	nd
C24:1	1.6	0.6	1.4	tr	tr
Unknowns	0.0	1.4	0.0	0.0	0.1

B. Calculated as % m/m using methyl heptadecanoate internal standard.

C14:0	tr	tr	tr	tr
C16:0	9.7	9.8	6.1	5.6
C16:1	0.2	0.2	0.1	tr
C18:0	3.9	3.3	1.8	1.7
C18:1	16.1	15.2	9.06	6.7
C18:2	33.8	37.1	67.3	68.3
C18:3,n-6 (GLA)	19.2	23.8	9.9	9.1
C18:3,N-3(ALA)	0.2	0.1	0.2	0.1
C20:0	0.2	0.2	0.3	0.3
C20:1	4.0	3.9	0.2	0.1
C20:2	0.1	tr	tr	tr
C22:0	0.2	0.1	0.1	0.1
C22:1	2.6	2.3	0.1	nd
C24:0	0.1	tr	tr	nd
C24:1	1.5	1.4	tr	tr
Total eluted material	92.0	97.5	95.3	92.3

nd = not detected

tr = trace (less than 0.05%)

NB. I have been unable to add an internal standard to the fish oil due to the complexity of the chromatogram.

Comments:

Samples will be disposed of one month after report date unless we are instructed to do otherwise.

M.J.Downes
Structure and Function

e Version: 4.1<2F12>
e Name : 11551-2 Time : 25/02/00 14:39
le Number: 1 Study :
erator :

Instrument : NEWTON Channel : A A/D mV Range : 1000
AutoSampler :
Rack/Vial : 0/0

Interface Serial # : 8037252605 Data Acquisition Time: 24/02/00 05:45
Delay Time : 0.00 min.
End Time : 80.00 min.
Sampling Rate : 1.0000 pts/sec

Raw Data File : C:\TC4\NEWTON\F230200M.RAW
Result File : C:\DOS\~RST2F47.RST
Inst Method : C:\TC4\METH_SEQ\FAME from C:\DOS\~RST2F47.RST
Proc Method : C:\TC4\METH_SEQ\FAME.MTH
Calib Method : C:\TC4\METH_SEQ\FAME.MTH
Sec ence File : C:\TC4\METH_SEQ\FAME.SEQ

Sample Volume : 1.0000 ul Area Reject : 0.000000
Sample Amount : 1.0000 Dilution Factor : 1.00

FATTY ACID METHYL ESTERS

Fame Report

Tim [min]	Area [µV·s]	Area [%]	Norm Area [%]	BL	Component Name	Amount [Norm. %]
9.6	1404	0.01	0.01	BB		0.01
11.8	4427	0.03	0.03	BB	C12:0	0.03
14.7	5206	0.03	0.03	BB		0.03
16.4	2808	0.02	0.02	BB		0.02
18.1	530520	3.25	3.29	BV		3.29
18.5	1279	0.01	0.01	VV	C14:0	0.01
18.7	3249	0.02	0.02	VB		0.02
19.1	3914	[illegible]	[illegible]	[illegible]		[illegible]
19.5	4977	0.03	0.03	VB		0.03
20.0	16179	0.10	0.10	BB		0.10
20.5	3787	0.02	0.02	BB		0.02
21.1	2054	0.01	0.01	BB		0.01
21.7	158014	0.98	0.98	BV		0.98
22.1	1289	0.01	0.01	VB	C15:0	0.01
22.7	1403	0.01	0.01	BB		0.01
23.8	11577	0.07	0.07	BB		0.07
25.9	3181321	19.73	19.73	BB	C16:0	19.73
26.3	10210	[illegible]	0.06	BV		[illegible]
26.6	110292	[illegible]	0.68	VV		[illegible]
27.0	719171	4.45	4.45	VV	[illegible]	[illegible]
27.3	14041	0.09	0.09	VB		[illegible]
27.7	21296	0.13	0.13	BB		[illegible]
28.2	1320	0.01	0.01	BB		0.01
28.3	6435	0.04	0.04	BB		0.04
29.0	3059	0.02	0.02	BV		0.02
29.2	207844	1.29	1.29	VB		1.29
29.6	192498	1.19	1.19	BV	C17.0	1.19
30.0	33924	0.21	0.21	VV		0.21
30.4	9713	0.06	0.06	VV		0.06

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Area (µV·s)	Area [%]	Norm Area [%]	BL	Component Name	Amount [Norm %]
.7 111295	0.69	0.69	VB	C14:1	0.69
1.1 1048	0.01	0.01	BV		0.01
31.3 4430	0.03	0.03	VV		0.03
31.6 25454	0.16	0.16	VB		0.16
32.2 4675	0.03	0.03	BV		0.03
32.4 2553	0.02	0.02	VB		0.02
33.8 923735	5.73	5.73	BV	C18:0	5.73
34.1 27233	0.17	0.17	VV		0.17
34.8 1907693	11.83	11.83	VV		11.83
35.0 352729	2.19	2.19	VV		2.19
35.4 17943	0.11	0.11	VB	C18:1	0.11
35.7 2625	0.02	0.02	BV		0.02
35.9 3878	0.02	0.02	VB		0.02
36.5 209555	1.30	1.30	BB		1.30
37.3 107132	0.66	0.66	BV	C18:2	0.66
37.5 71649	0.44	0.44	VB		0.44
38.2 18040	0.11	0.11	BV	~~GLA(C18:3~~	0.11
38.4 15219	0.09	0.09	VV		0.09
38.6 75067	0.47	0.47	VB	C18:3(n-3)	0.47
39.4 116987	0.73	0.73	BB	~~C18:4~~	0.73
39.8 1059	0.01	0.01	BB		0.01
40.4 65564	0.41	0.41	BB	C20:0	0.41
41.0 27747	0.17	0.17	BV		0.17
41.2 120550	0.75	0.75	VV		0.75
41.4 14611	0.09	0.09	VB	C20:1	0.09
41.8 2043	0.01	0.01	BB	~~C20:2~~	0.01
42.1 1827	0.01	0.01	BB		0.01
4 28760	0.18	0.18	BV	C20:2	0.18
42.7 41275	0.26	0.26	VB	C20:3	0.26
43.5 27911	0.17	0.17	BV		0.17
43.6 14632	0.09	0.09	VB		0.09
44.3 317038	1.97	1.97	BB	C20:4	1.97
44.9 15813	0.10	0.10	BB		0.10
46.0 61701	0.38	0.38	BV		0.38
46.8 934590	5.80	5.80	VB	C20:5	5.80
47.3 43835	0.27	0.27	BB	C22:0	0.27
47.9 3427	0.02	0.02	BV		0.02
48.2 35608	0.22	0.22	VV		0.22
48.5 16272	0.10	0.10	VV		0.10
49.0 11223	0.07	0.07	VB	C22:1	0.07
49.6 1688	0.01	0.01	BB		0.01
50.9 3768	0.02	0.02	BB	~~C20:6~~	0.02
52.1 48440	0.30	0.30	BB	C22:3	0.30
53.4 4764	0.03	0.03	BV		0.03
54.3 37718	0.23	0.23	VB	C22:4	0.23
55.3 351866	2.18	2.18	BB	C22:5	2.18
56.7 3525	0.02	0.02	BB		0.02
58.7 226710	1.41	1.41	BB	C24:0	1.41
60.5 4258428	26.41	26.41	-BE	C22:6	26.41
60.9 91511	0.57	0.57	EV	C24:1	0.57
61.7 6284	0.04	0.04	VV		0.04
62.2 10515	0.07	0.07	VV		0.07
62.8 15959	0.10	0.10	VB		0.10
5 3970	0.02	0.02	BB		0.02
2 2332	0.01	0.01	BB		0.01
74.0 5318	0.03	0.03	BB		0.03
78.4 6882	0.04	0.04	BB		0.04
2e+07	100.00	#####			100.00

Tuna 0.1

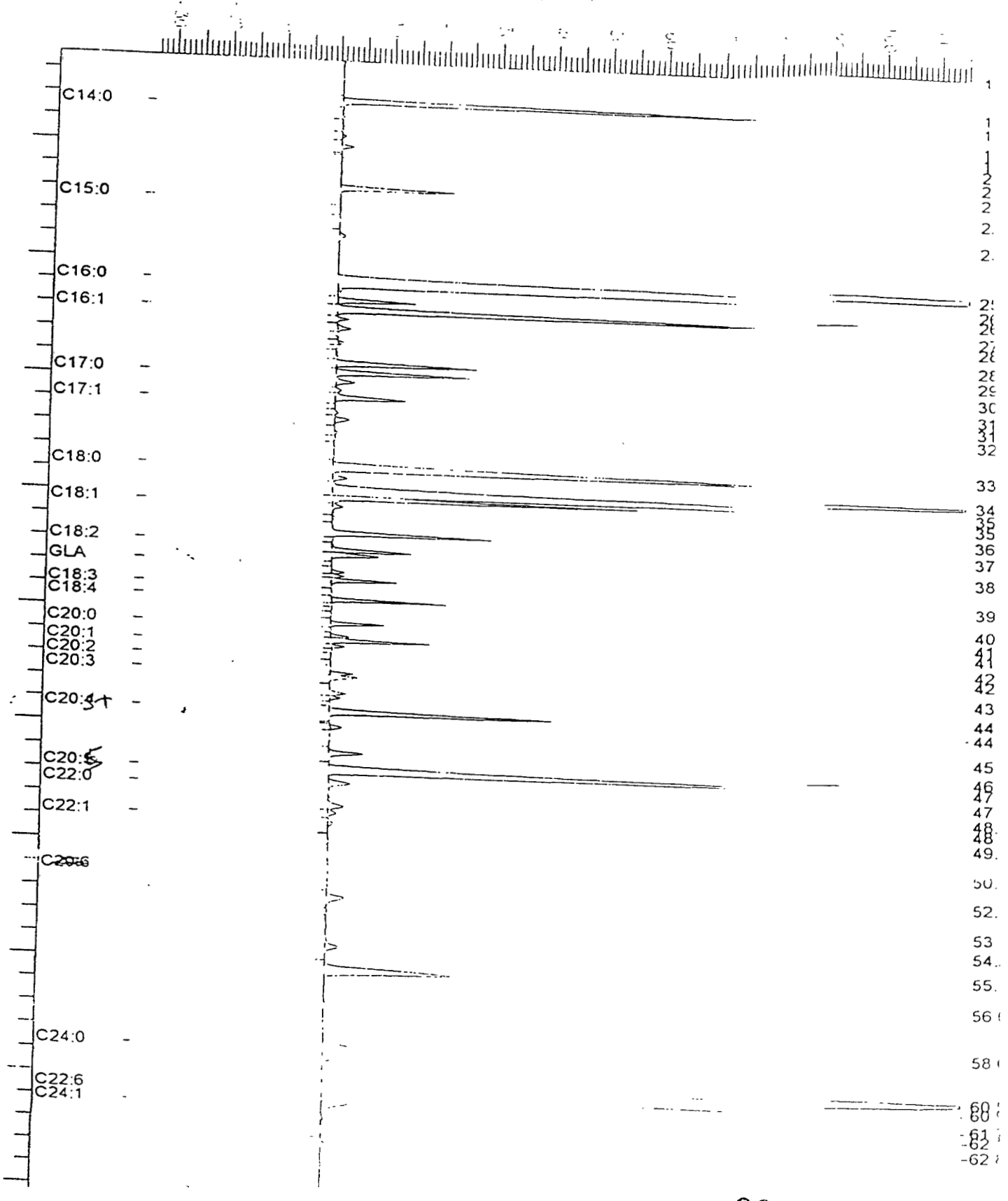
Chromatogram

Sample Name : 11551-2
FileName : C:\TC4\NEWTON\F230200M.+AW
Method : FAME.MTH
Start Time : 15.33 min
Scale Factor: 0.0

End Time : 65.57 min
Plot Offset: -33 mV

Sample # : 1
Date : 24/02/00 10:00
Time of Injection: 24/02/00
Low Point -33.46 mV
Plot Scale 149.0 mV

Response [mV]



000378

Mylnefield Research Services Ltd.

The Lipid Analysis Unit,
Mylnefield, Invergowrie, Dundee DD2 5DA, Scotland

MRSLU\2000\197 (Continued)

Concentration of AA as Free Fatty Acid	15 mg/g Oil
Concentration of EPA as Free Fatty Acid	43 mg/g Oil
Concentration of DHA as Free Fatty Acid	185 mg/g Oil

Duncan Adam

Laboratory Technician 13/3/00

C. Reid C.Chem MRSC

Senior Analytical Chemist 13/3/00

National : 01382 568568 International : +44 1382 568568

Mylnefield Research Services Ltd.

The Lipid Analysis Unit,
Mylnefield, Invergowrie, Dundee DD2 5DA, Scotland

Designation of sample - Marine Oil
Batch No. R01

Submitted by - Clover Corporation

Our number - MRS LU/2000/197

Your Order number - 3105

Fatty acid profile (%)

14:0	myristic	3.3
15:0	pentadecanoic	1.1
16:0	palmitic	18.9
16:1	hexadecenoic	5.2
16:2	hexadecadienoic	0.3
18:0	stearic	5.6
18:1	oleic	13.9
18:2	linoleic	1.3
18:3	γ-linolenic	0.4
18:3	α-linolenic	0.5
18:4(n-3)	octadecatetraenoic	0.8
20:0	icosanoic	0.4
20:1	icosenoic	1.1
20:2(n-6)	icosadienoic	0.2
20:3(n-6)	dihomo-γ-linolenic	0.1
20:3(n-3)	icosatrienoic	0.1
20:4(n-6)	arachidonic	2.0
20:4(n-3)	icosatetraenoic	0.4
20:5(n-3)	icosapentaenoic	5.8
22:0	docosanoic	0.3
22:1	docosenoic	0.1
22:5(n-3)	docosapentaenoic	1.2
22:6(n-3)	docosahexaenoic	25.3
24:0	tetracosanoic	0.2
24:1	tetracosenoic	0.5
	minor components	11.0

Analysis carried out on a capillary column

Material Safety Data Sheet

Product Name: **Hi-DHA Oil** Issued Date: 17th November 1999

Not Classified as Hazardous according to criteria of Worksafe Australia

COMPANY DETAILS

Company Name	Clover Corporation Limited/ Nu-Mega Lipids Pty Ltd
Address	31 Pinnacle Road, Altona North Victoria 3025 Australia PO Box 1111, Altona Gate Victoria 3025 Australia
Emergency Tel.	0418 994744
Tel/Fax	Ph: 61 3 9369 4774 Fax: 61 3 9369 8900

IDENTIFICATION

Product Name	Tuna Oil with a high content of Long Chain Polyunsaturated Fatty Acids (LCPs or LCPUFAs) including DHA (DocosaHexaenoic Acid) Edible Oil refined from selected species of the tuna fish family. Product Codes 7001 to 9099
Hazardous Ingredients	None
Product Use	Nutritional supplement or functional food ingredient
UN Number	None allocated
HAZCHEM Number	Not applicable
Dangerous Goods Class/Subsidiary Risk	Not applicable
Poison Schedule Number	Not applicable
CAS Number	None allocated

Physical Data

Appearance	A pale yellow liquid with faint fish odour
Vapour Pressure	Not applicable
Specific Gravity	0.92 – 0.94
Flamm. Limit LEL/UEL	Not applicable
Flash point	approx.260°C
Solubility	Not soluble in water, soluble in acetone/hexane

HEALTH HAZARD INFORMATION

Health Effects

The product might be an irritant to the eyes and may be absorbed through the skin.

Threshold Limit Value	Not applicable
Toxicity	Food Grade

First Aid

Skin	Wash with soap and water
Eyes	Flush with Water
Ingestion	Edible, Food Grade
Inhalation	Minimise risks through ventilation (oils/fats at high temperatures may produce gaseous products of acrolein variety)

Material Safety Data Sheet

Product Name: **Hi-DHA Oil** Issued Date: 17th November 1999

Not Classified as Hazardous according to criteria of Worksafe Australia

Other Health Hazard Information

PRECAUTIONS FOR USE

Hazardous Reactions	Polymerisation not likely to occur under normal conditions
Engineering Controls	On prolonged exposure to air and if in contact with absorbent combustible material, the product may self-ignite
Conditions to Avoid	Prolonged exposure to air/oxygen High temperatures near flash point
Incompatability (Materials to avoid)	Strong oxidising agents
Hazardous Decomposition Products	Secondary oxidation decomposition products eg. acrolein

Personal Protection

Respiratory Protection	In presence of fish oil mists, use respiratory mask
Skin Protection	For hot oil, use neoprene gloves
Eye Protection	Use goggles for hot oil
Others	Rubber boots and apron for hot oils

SAFE HANDLING INFORMATION

Storage and Transport

Precautions to be taken in Handling and Storing	Store cool, dry and away from products with a strong or foreign odour. If stored in tanks the use of nitrogen blanketing is advised; incorporation and contact with iron should be avoided. We recommend storing in sealed containers ideally plastic or epon coated drums.
Other precautions	Store below ambient temperature. Protect from direct sunlight. Keep away from heat, sparks and fire. At times oil-soaked materials may spontaneously combust. Do not allow absorbent material to accumulate in closed conditions when exposed to air.

Spills and Disposal

Minor	Wipe or mop up spilled oil. Contain rags and dispose of in fire-proof bins to prevent self-ignition.
Major	Absorb or contain with clay or sand. Destroy by incineration with off-gas scrubbers. Contaminated absorbent may be landfilled.

Material Safety Data Sheet

Product Name: **Hi-DHA Oil** Issued Date: 17th November 1999

Not Classified as Hazardous according to criteria of Worksafe Australia

Spillage on Water	Since the density of oil is less than water, it will float on the surface. The oil can then be taken off and disposed according to regulations.
Disposal	Disposal of this material should be undertaken by a registered chemical disposal company.

Fire/Explosion Hazard

Extinguishing Media	Foams, Carbon Dioxide, Inert Powder
Extinguishing Media to Avoid	Water
Special Exposure Hazards	Fine oil mists may be hazardous on prolonged heating at high temperature. Gaseous decomposition components of acrolein variety may occur.
Protection Against Fire	Standard, the oil floats and spreads on the surface of water

OTHER INFORMATION

Environ. Protection	Avoid contaminating waterways. Fully biodegradable.
Packaging	As required by the National Code of Practice for the Labeling of Workplace Substances. As required by the Standard for the Uniform Scheduling of Drugs and Poisons
Contact	Business Hours: Mr. Robert Anderson, Tel: 61 3 9369 8899 After Hours: Tel: 61 3 9809 0075

IMPORTANT ADVICE:

This MSDS summarises our best knowledge of the health and safety hazard information of the product and how to safely handle and use the product in the workplace. Each user should read this MSDS and consider the information in the context of how the product will be handled and used in the workplace including its use in conjunction with other products. If clarification or further information is needed to ensure that an appropriate risk assessment can be made, the user should contact Clover Corporation Limited. Our responsibility for products sold are subject to our standard terms and conditions, a copy of which is available on request.

The information has been obtained from sources believed to be reliable and accurate but has not been independently verified by Clover Corporation Limited. **Accordingly, no representation or warranty, express or implied, with respect to merchantability and fitness for a particular purpose is made with respect to the information contained herein.**

. . . End of Report. . .

000383

Material Safety Data Sheet

Product Name: **Driphorm Hi-DHA Bake** Issued Date: 17th November 99

Product code: **D101**

Not Classified as Hazardous according to criteria of Worksafe Australia

COMPANY DETAILS

Company Name	Clover Corporation Limited
Address	31 Pinnacle Road, Altona North Victoria 3025 Australia PO Box 1111, Altona Gate Victoria 3025 Australia
Emergency Tel.	0418 994744
Tel/Fax	Ph: 61 3 9369 8899 Fax: 61 3 9369 8900

IDENTIFICATION

Product Name	Driphorm Hi-DHA Bake Product Code- D101 Spray dried powder containing 25% Hi-DHA Tuna Oil, microencapsulated in a matrix of Sodium Caseinate and Modified Starch and natural food grade antioxidants.
Hazardous Ingredients	None
Product Use	Nutritional supplement, Functional Food ingredient
UN Number	None allocated
HAZCHEM Number	Not applicable
Dangerous Goods Class/Subsidiary Risk	Not applicable
Poison Schedule Number	Not applicable
CAS Number	None allocated

Physical Data

Appearance	Free flowing light tan coloured powder with bland odour and flavour.
Vapour Pressure	Not applicable
Specific Gravity	Not applicable
Flamm. Limit LEL/UEL	Not applicable
Flash point	Not applicable

HEALTH HAZARD INFORMATION

Health Effects

The product might be an irritant to the eyes and may be absorbed through the skin.

Threshold Limit Value	Not applicable
Toxicity	Food Grade

First Aid

Skin	Wash with soap and water
Eyes	Flush with Water-particulates may scratch eye surfaces
Ingestion	Edible, Food Grade
Inhalation	Minimise risks through ventilation , may cause irritation. If there are breathing difficulties, seek medical advice

Continued on next page

Material Safety Data Sheet

Product Name: Driphorm Hi-DHA Bake Issued Date: 17th November 99

Product code: D101

Not Classified as Hazardous according to criteria of Worksafe Australia

Other Health Hazard Information

PRECAUTIONS FOR USE

Hazardous Reactions	Powder and dust clouds are susceptible to fire and possible explosion in confined spaces.
Engineering Controls	Ventilation/ dust extraction
Conditions to Avoid	High temperatures , sources of ignition and water To maximise shelf life avoid prolonged exposure to air/oxygen
Incompatability (Materials to avoid)	Not applicable
Hazardous Decomposition Products	Not applicable

Personal Protection

Respiratory Protection	If ventilation is inadequate or powdered product is handled for long periods, use dust mask
Skin Protection	Not applicable
Eye Protection	Safety glasses if product handling causes dusting.

SAFE HANDLING INFORMATION

Storage and Transport

Precautions to be taken in Handling and Storing	Store cool, dry and away from products with a strong or foreign odour. We recommend storing in sealed containers Avoid contact with moisture.
Other precautions	Store below ambient temperature. Protect from direct sunlight. Keep away from heat, sparks and fire.

Spills and Disposal

Spills	Vacuum or scoop into suitable container, then sweep area and wash with water. Recovered product is not suitable for human consumption.
Disposal	Comply with applicable waste disposal regulations.

Material Safety Data Sheet

Product Name: **Driphorm Hi-DHA Bake** Issued Date: 17th November 99

Product code: **D101**

Not Classified as Hazardous according to criteria of Worksafe Australia

Fire/Explosion Hazard

Extinguishing Media	Water, Foams, Carbon Dioxide, Dry chemical
Protection Against Fire	Typical combustion products are carbon dioxide and carbon monoxide

OTHER INFORMATION

Environ. Protection	Avoid contaminating waterways. Fully biodegradable.
Packaging	As required by the National Code of Practice for the Labeling of Workplace Substances. As required by the Standard for the Uniform Scheduling of Drugs and Poisons
Contact	Business Hours: Mr. Robert Anderson, Tel: 61 3 9369 8899 After Hours: Tel: 61 3 9809 0075

IMPORTANT ADVICE:

This MSDS summarises our best knowledge of the health and safety hazard information of the product and how to safely handle and use the product in the workplace. Each user should read this MSDS and consider the information in the context of how the product will be handled and used in the workplace including its use in conjunction with other products. If clarification or further information is needed to ensure that an appropriate risk assessment can be made, the user should contact Clover Corporation Limited. Our responsibility for products sold are subject to our standard terms and conditions, a copy of which is available on request.

The information has been obtained from sources believed to be reliable and accurate but has not been independently verified by Clover Corporation Limited. **Accordingly, no representation or warranty, express or implied, with respect to merchantability and fitness for a particular purpose is made with respect to the information contained herein.**

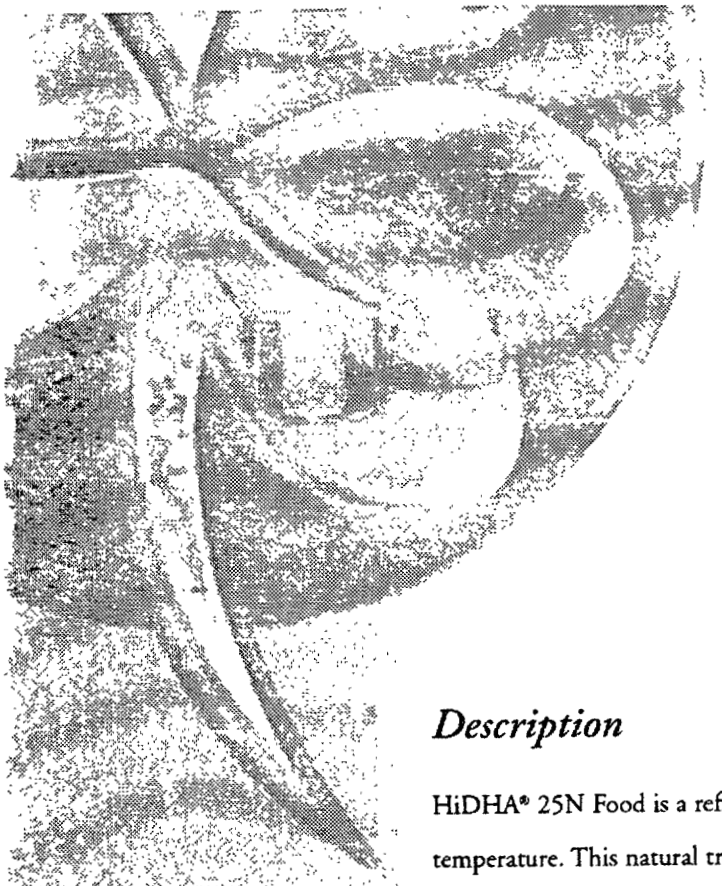
. . . End of Report. . .

Appendix II.

Product Data Sheets

CLOVER

Leaders in Innovative Functional Lipids



PRODUCT DATA

HiDHA® 25N Food 7003

Uses

For Food enrichment

For Infant food enrichment

Description

HiDHA® 25N Food is a refined non-winterised tuna oil. It is a cloudy oil at room temperature. This natural triglyceride contains approx. 25% DHA and 34% total omega-3 long chain polyunsaturated fatty acids present in the natural and bioactive all-cis form.

PHYSICAL-CHEMICAL PROPERTIES	TYPICAL VALUE
Docosahexaenoic acid (DHA)*	25 %
Eicosapentaenoic acid (EPA)*	6 %
Total Omega-3 Fatty Acid Content*	34 %
Acid Value	0.5 mg KOH/g
Peroxide Value	1.5 meq O_2/kg
p-Anisidine Value	10
Colour	3 Gardner
Unsaponifiable Matter	2 %
HEAVY METALS	
Lead	< 0.1 ppm
Mercury	< 0.1 ppm
Cadmium	< 0.1 ppm
Arsenic	< 0.1 ppm
Total Heavy Metals as Pb	< 2 ppm

* GC analysis: Fatty acid composition expressed as percentage of fatty acid methyl esters.

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Shelf Life & Storage

HiDHA® oil is sensitive to air, light and extended periods at elevated temperatures. The product may be stored for 2 years from date of manufacture in the unopened original drum, in cool (10-25°C) dry conditions (relative humidity less than 75%). After opening contents should be used within a short period of time. To protect the oil from oxidation, after opening the original container the oil should be thoroughly flushed with an inert gas (nitrogen, argon, etc) and resealed under inert gas.

Packaging

Standard 194 kg net drums.

HiDHA® 25N Food is a non-winterised tuna oil and at room temperature the solid material may settle out on the bottom of the drum. These solids will melt and become homogeneous on heating above 40°C.

General Handling

Since the HiDHA® oil is sensitive to exposure to air and pro-oxidant materials, special handling instructions must be observed in addition to the standard health, safety and hygiene practices normally observed in the food and pharmaceutical industry. Ideally when opening the drum, the oil should be transferred in a closed system under inert gas. Only the following are suitable materials for equipment coming into contact with the oils: stainless steel, aluminium, glass, enamel or food-approved plastic.

Recommended Omega-3 Intake

The role that DHA plays in both health and disease has become the focus of considerable research and is considered to be beneficial for the brain and eyes, heart and reducing inflammation.

DHA is a vital nutrient with particularly important roles in the structure and function of the brain, the eye and the nervous system and is considered essential for the optimum neurological development of the foetus and infant. Many national and international bodies strongly recommend that DHA should be added to infant formula and to infant weaning foods. There is also increasing support for the addition of DHA to the maternal diet.

A NIH working group recommended daily intakes of

- 650 mg per day of DHA plus EPA for adults
- 300mg of DHA for pregnant and breastfeeding women.

Further information is available upon request or visit www.hi-dha.com

Quality Assurance

An intensive analytical program has established that the HiDHA® oil is substantially free of environmental contaminants including pesticide residues, PCBs/Dioxins, PAHs, heavy metals and microbial contaminants. Monitoring of the oils continues to ensure continued compliance with international standards such as those of Codex Alimentarius.

Analytical data to illustrate the high quality and purity of HiDHA® oil can be obtained upon request.

Manufacturing compliance

The HiDHA® oil is produced in Australia in premises licenced by the Therapeutic Goods Administration in accordance with pharmaceutical standard GMP. The premises and the manufacturing processes have qualified for ISO 9002 and AQIS certification and operate under a strict EPA code.

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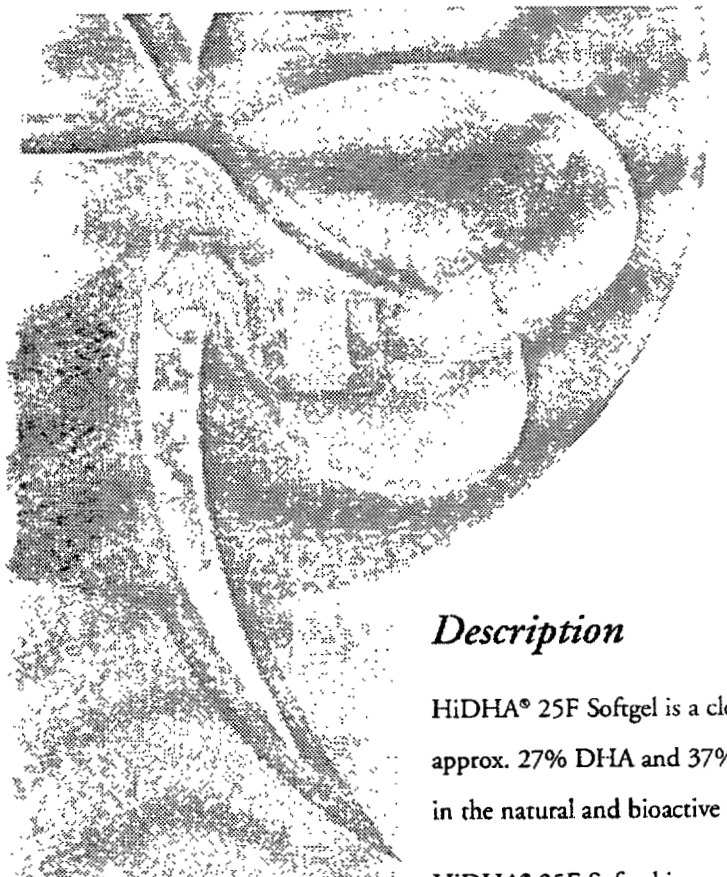

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PRODUCT DATA

HiDHA® 25F SOFTGEL 9004

Uses

For softgel encapsulation

Nutritional Supplement

Description

HiDHA® 25F Softgel is a clear pale yellow tuna oil. This natural triglyceride contains approx. 27% DHA and 37% total omega-3 long chain polyunsaturated fatty acids present in the natural and bioactive all-cis form.

HiDHA® 25F Softgel is a refined tuna oil which has been clarified and enriched by full winterisation at 0°C to produce a bright, free-flowing, light yellow oil.

PHYSICAL-CHEMICAL PROPERTIES	TYPICAL VALUE
Docosahexaenoic acid (DHA)*	27 %
Eicosapentaenoic acid (EPA)*	6 %
Total Omega-3 Fatty Acid Content*	37 %
Acid Value	0.7 mg KOH/g
Peroxide Value	2.0 meq O_2/kg
p-Anisidine Value	10
Colour	3 Gardner
Unsaponifiable Matter	2 %
Cold Test	passes, no haze at 3hrs at 0°C
HEAVY METALS	
Lead	< 0.1 ppm
Mercury	< 0.1 ppm
Cadmium	< 0.1 ppm
Arsenic	< 0.1 ppm
Total Heavy Metals as Pb	< 2 ppm

*GC analysis: Fatty acid composition expressed as percentage of fatty acid methyl esters.

Revision 4 June 2001

Shelf Life & Storage

HiDHA® oil is sensitive to air, light and extended periods at elevated temperatures. The product may be stored for 2 years from date of manufacture in the unopened original drum, in cool (10-25°C) dry conditions (relative humidity less than 75%). After opening contents should be used within a short period of time. To protect the oil from oxidation, after opening the original container the oil should be thoroughly flushed with an inert gas (nitrogen, argon, etc) and resealed under inert gas.

Packaging

Standard 194 kg net drums.

When the oil is exposed to very low temperatures during transportation or storage, a slight turbidity may form. This is a natural phase transition phenomenon and is completely reversed when heated to temperatures above 20°C.

General Handling

Since the HiDHA® oil is sensitive to exposure to air and pro-oxidant materials, special handling instructions must be observed in addition to the standard health, safety and hygiene practices normally observed in the food and pharmaceutical industry. Ideally when opening the drum, the oil should be transferred in a closed system under inert gas. Only the following are suitable materials for equipment coming into contact with the oils: stainless steel, aluminium, glass, enamel or food-approved plastic.

Recommended Omega-3 Intake

The role that DHA plays in both health and disease has become the focus of considerable research and is considered to be beneficial for the brain and eyes, heart and reducing inflammation.

DHA is a vital nutrient with particularly important roles in the structure and function of the brain, the eye and the nervous system and is considered essential for the optimum neurological development of the foetus and infant. Many national and international bodies strongly recommend that DHA should be added to infant formula and to infant weaning foods. There is also increasing support for the addition of DHA to the maternal diet.

A NIH working group recommended daily intakes of

- 650 mg per day of DHA plus EPA for adults
- 300mg of DHA for pregnant and breastfeeding women.

Further information is available upon request or visit www.hi-dha.com

Quality Assurance

An intensive analytical program has established that the HiDHA® oil is substantially free of environmental contaminants including pesticide residues, PCBs/Dioxins, PAHs, heavy metals and microbial contaminants. Monitoring of the oils continues to ensure continued compliance with international standards such as those of Codex Alimentarius.

Analytical data to illustrate the high quality and purity of HiDHA® oil can be obtained upon request.

Manufacturing compliance

The HiDHA® oil is produced in Australia in premises licenced by the Therapeutic Goods Administration in accordance with pharmaceutical standard GMP. The premises and the manufacturing processes have qualified for ISO 9002 and AQIS certification and operate under a strict EPA code.

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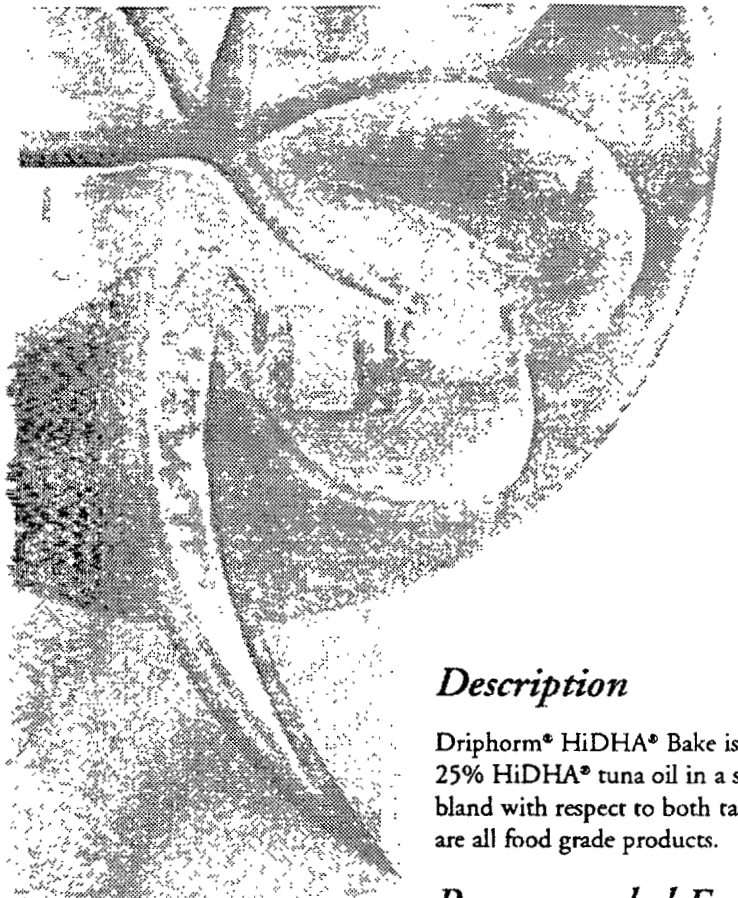

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PRODUCT DATA

DRIPHORM® HiDHA® Bake D101

Description

Driphorm® HiDHA® Bake is a fine, light beige, free-flowing, spray-dried powder containing 25% HiDHA® tuna oil in a stable and protected microencapsulated form. The powder is bland with respect to both taste and odour and the matrix materials and antioxidants used are all food grade products.

Recommended Enrichment Levels

Up to 2.0% in dry foods

DHA + EPA content in final product: 30-110 mg per serve

Uses

Recommended for DHA enrichment of bread premixes, bakery products, cereals, muesli, pasta, dehydrated products (soups, cooking sauces, instant potato powder, infant teething rusks, etc).

Details of how to use Driphorm® HiDHA® Bake can be obtained upon request.

PHYSICAL-CHEMICAL PROPERTIES	TYPICAL
Total Fat Content	27 %
Typical Fatty Acid Content per 1g of powder	
Docosahexaenoic acid (DHA)	56mg
Eicosapentaenoic acid (EPA)	13mg
Total Omega-3 Fatty Acid Content	74mg
Carbohydrate	approx. 54%
Protein	approx. 10%
Loss on Drying	max. 5%
Bulk density (100 taps)	0.45-0.65 g/ml
Particle Size	100-200 microns 100% through 20 mesh USP (850 µm) 95% through 40 mesh USP (425 µm) 4% through 325 mesh USP (45 µm)

MICROBIAL LIMITS	
Standard Aerobic Plate Count	max 1,000 cfu/g
Coliforms (37° C)	Not detected in 0.1g
Coagulase positive Staphylococci	Not detected in 0.01g
Salmonella spp.	Not detected in 25 g
Bacillus Cereus	Not detected in 0.01g

Stability

Driphorm® HiDHA® Bake is sensitive to air, light and extended periods at elevated temperatures. Stability data in specific applications is available upon request.

Shelf Life & Storage

The product may be stored for 18 months from date of manufacture in the unopened original package, in cool (10-25°C) dry conditions (relative humidity less than 75%). After opening contents should be used within a very short time period.

Packaging

Laminated foil bag (sealed under nitrogen) in a cardboard box.
Standard 1kg net.

General Handling

Since the powder is sensitive to exposure to air and pro-oxidant materials, special handling instructions must be observed in addition to the standard health, safety and hygiene practices normally observed in the food industry. Transfer and weighing operations should be done as quickly as possible to minimise exposure to air. Only the following are suitable materials for equipment coming into contact with the powder: stainless steel, aluminium, glass, enamel or food-approved plastic.

Recommended Omega-3 Intake

The role that DHA plays in both health and disease has become the focus of considerable research and is considered to be beneficial for the brain and eyes, heart and reducing inflammation.

DHA is a vital nutrient with particularly important roles in the structure and function of the brain, the eye and the nervous system and is considered essential for the optimum neurological development of the foetus and infant. Many national and international bodies strongly recommend that DHA should be added to infant formula and to infant weaning foods. There is also increasing support for the addition of DHA to the maternal diet.

A NIH working group recommended daily intakes of

- 650 mg per day of DHA plus EPA for adults
- 300mg of DHA for pregnant and breastfeeding women.

Further information is available upon request or visit www.hi-dha.com

Quality Assurance

An intensive analytical program has established that the HiDHA® oil used in the manufacture of Driphorm® Bake is substantially free of environmental contaminants including pesticide residues, PCBs/Dioxins, PAHs, heavy metals and microbial contaminants. Monitoring of the oils continues to ensure continued compliance with international standards such as those of Codex Alimentarius.

Analytical data to illustrate the high quality and purity of Driphorm® HiDHA® Bake and HiDHA® oil can be obtained upon request.

Manufacturing compliance

The HiDHA® oil used in Driphorm® HiDHA® Bake is produced in Australia in premises licenced by the Therapeutic Goods Administration in accordance with pharmaceutical standard GMP. The premises and the manufacturing processes have qualified for ISO 9002 and AQIS certification and operate under a strict EPA code. ■

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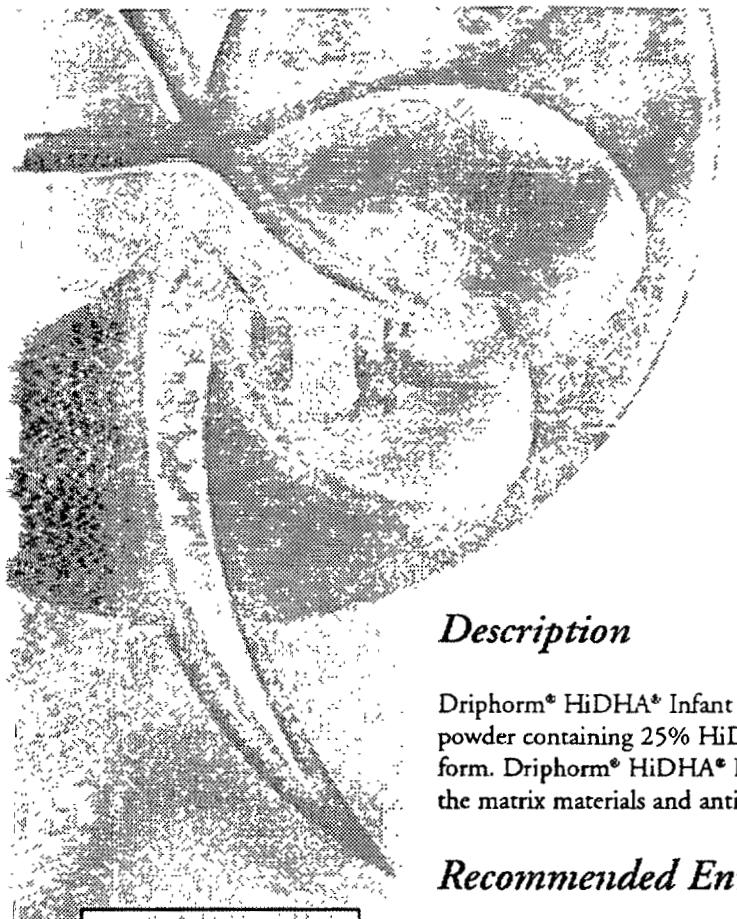

PRODUCT DATA

DRIPHORM® HiDHA® Infant D103

Description

Driphorm® HiDHA® Infant is a fine white to light beige, free-flowing, spray-dried powder containing 25% HiDHA® tuna oil in a stable and protected microencapsulated form. Driphorm® HiDHA® Infant is bland with respect to both taste and odour and the matrix materials and antioxidants used are all food grade products.

Recommended Enrichment Levels

Up to 2.4 % in infant formula.

Uses

For addition to infant formula, weaning products and milk powder preparations for pregnant and lactating women.

Also for use in specialised nutritional products for the elderly and the infirm; slimming products and other products for those on restrictive diets; sports nutrition; and enteral clinical support nutrition.

Details of how to use Driphorm® can be obtained upon request.

PHYSICAL-CHEMICAL PROPERTIES	TYPICAL
Total Fat Content	26 %
Typical Fatty Acid Content per 1g of powder	
Docosahexaenoic acid (DHA)	54mg
Eicosapentaenoic acid (EPA)	12mg
Total Omega-3 Fatty Acid Content	70mg
Carbohydrate	approx. 47%
Protein	approx. 18%
Loss on Drying	max. 5%
Bulk density (100 taps)	0.55-0.70 g/ml
Particle Size	80-160 microns
	100% through 20 mesh USP (850 µm)
	95% through 40 mesh USP (425 µm)
	3% through 325 mesh USP (45 µm)

MICROBIAL LIMITS	
Standard Aerobic Plate Count	max 1,000 cfu/g
Yeasts and Moulds	max. 50 cfu/g
Coliforms (37° C)	Not detected in 1.0 g
Coagulase positive Staphylococci	Not detected in 0.01g
Salmonella spp.	Not detected in 30x10 g
Bacillus Cereus	Not detected in 0.01g
Clostridium perfringens	Not detected in 0.01g
E. coli	Not detected in 25 g
Listeria spp.	Not detected in 2x25 g

Stability

Driphorm® HiDHA® Infant is sensitive to air, light and extended periods at elevated temperatures. Stability data in specific applications is available upon request.

Shelf Life & Storage

The product may be stored for 18 months from date of manufacture in the unopened original package, in cool (10-25°C) dry conditions (relative humidity less than 75%). After opening contents should be used within a very short time period.

Packaging

Laminated foil bag (sealed under nitrogen) in a cardboard box. Standard 1kg net.

General Handling

Since the powder is sensitive to exposure to air and pro-oxidant materials, special handling instructions must be observed in addition to the standard health, safety and hygiene practices normally observed in the food industry. Transfer and weighing operations should be done as quickly as possible to minimise exposure to air. Only the following are suitable materials for equipment coming into contact with the powder: stainless steel, aluminium, glass, enamel or food-approved plastic.

Recommended Omega-3 Intake

The role that DHA plays in both health and disease has become the focus of considerable research and is considered to be beneficial for the brain and eyes, heart and reducing inflammation.

DHA is a vital nutrient with particularly important roles in the structure and function of the brain, the eye and the nervous system and is considered essential for the optimum neurological development of the foetus and infant. Many national and international bodies strongly recommend that DHA should be added to infant formula and to infant weaning foods. There is also increasing support for the addition of DHA to the maternal diet.

A NIH working group recommended daily intakes of

- 650 mg per day of DHA plus EPA for adults
- 300mg of DHA for pregnant and breastfeeding women.

Further information is available upon request or visit www.hi-dha.com

Quality Assurance

An intensive analytical program has established that the HiDHA® oil used in the manufacture of Driphorm® HiDHA® Infant is substantially free of environmental contaminants including pesticide residues, PCBs/Dioxins, PAHs, heavy metals and microbial contaminants. Monitoring of the oils continues to ensure continued compliance with international standards such as those of Codex Alimentarius.

Analytical data to illustrate the high quality and purity of Driphorm® HiDHA® Infant powder and HiDHA® oil can be obtained upon request.

Manufacturing compliance

The HiDHA® oil used in Driphorm® Infant is produced in Australia in premises licenced by the Therapeutic Goods Administration in accordance with pharmaceutical standard GMP. The premises and the manufacturing processes have qualified for ISO 9002 and AQIS certification and operate under a strict EPA code. ■

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PRODUCT DATA

GELPHORM® HiDHA® WPI G001

Description

Gelphorm® HiDHA® WPI is a stable liquid oil-in-water emulsion, which can be used to enhance the nutritional value of food products. The emulsion typically contains 25% of tuna fish oil and appears as an attractive smooth off-white liquid with excellent flow and dispersing properties. The emulsion is well stabilised in terms of aggregation, creaming and clarification reaction. Oil droplet size is below 0.5 micron in a very narrow particle size distribution. The product has been UHT treated. All the ingredients used in Gelphorm products comply with the Australian New Zealand Food Authorities (ANZFA) standards. Gelphorm® HiDHA® WPI is based on Whey Protein Isolate.

Uses

For Food enrichment

Suitable for adding to acidic food products such as yoghurts and mayonnaise

Recommended Enrichment Levels

Up to 1.2% in liquid foods

PHYSICAL-CHEMICAL PROPERTIES	TYPICAL
Total Fat Content	25 %
Protein	5 %
Carbohydrate	10 %
Typical Fatty Acid Content per 1g of emulsion	
Docosahexaenoic acid (DHA)	60mg
Eicosahexaenoic acid (EPA)	14mg
Total Omega-3 Fatty Acid Content	83mg

STABILITY

Gelphorm® HiDHA® WPI is sensitive to microbial contamination, air, light and extended periods at high temperatures. It has been UHT treated.

000396

Storage

Store in unopened original container. Treat as a UHT dairy product and refrigerate at +4°C after opening.

Packaging

Laminated foil bag in a cardboard box. Standard 5 kg net.

Recommended Omega-3 Intake

The role that DHA plays in both health and disease has become the focus of considerable research and is considered to be beneficial for the brain and eyes, heart and reducing inflammation.

DHA is a vital nutrient with particularly important roles in the structure and function of the brain, the eye and the nervous system and is considered essential for the optimum neurological development of the foetus and infant. Many national and international bodies strongly recommend that DHA should be added to infant formula and to infant weaning foods. There is also increasing support for the addition of DHA to the maternal diet.

A NIH working group recommended daily intakes of

- 650 mg per day of DHA plus EPA for adults
- 300mg of DHA for pregnant and breastfeeding women.

Further information is available upon request or visit www.hi-dha.com

Gelphorm® HiDHA® WPI is intended for use in product incorporation trials only. ■

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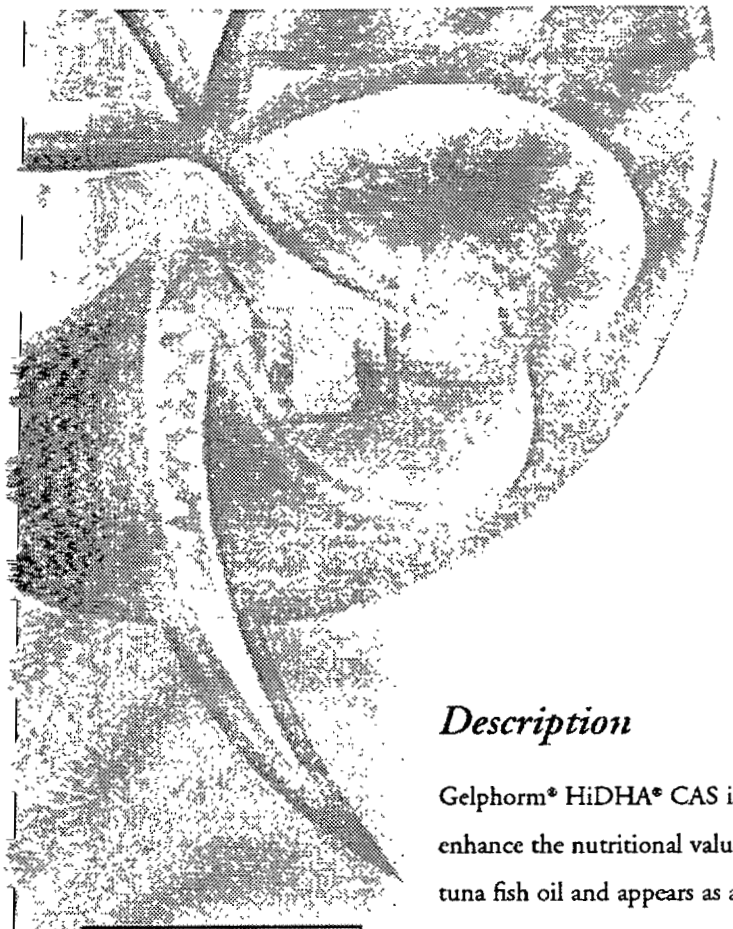

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Leaders in Innovative Functional Lipids

PRODUCT DATA

GELPHORM® HiDHA® CAS G002

Uses

For enrichment of dairy products

Description

Gelphorm® HiDHA® CAS is a stable liquid oil-in-water emulsion, which can be used to enhance the nutritional value of food products. The emulsion typically contains 25% of tuna fish oil and appears as an attractive smooth off-white liquid with excellent flow and dispersing properties. The emulsion is well stabilised in terms of aggregation, creaming and clarification reaction. Oil droplet size is below 0.5 micron in a very narrow particle size distribution. The product has been UHT treated. All the ingredients used in Gelphorm products comply with the Australian New Zealand Food Authorities (ANZFA) standards. Gelphorm® HiDHA® CAS is based on Sodium Caseinate.

Recommended Enrichment Levels

Up to 1.2% in liquid foods

PHYSICAL-CHEMICAL PROPERTIES	TYPICAL
Total Fat Content	25 %
Protein	5 %
Carbohydrate	12 %
Typical Fatty Acid Content per 1g of emulsion	
Docosahexaenoic acid (DHA)	60mg
Eicosahexaenoic acid (EPA)	14mg
Total Omega-3 Fatty Acid Content	83mg

STABILITY

Gelphorm® HiDHA® CAS is sensitive to microbial contamination, air, light and extended periods at high temperatures. It has been UHT treated.

Storage

Store in unopened original container. Treat as a UHT dairy product and refrigerate at +4°C after opening.

Packaging

Laminated foil bag in a cardboard box. Standard 5 kg net.

Recommended Omega-3 Intake

The role that DHA plays in both health and disease has become the focus of considerable research and is considered to be beneficial for the brain and eyes, heart and reducing inflammation.

DHA is a vital nutrient with particularly important roles in the structure and function of the brain, the eye and the nervous system and is considered essential for the optimum neurological development of the foetus and infant. Many national and international bodies strongly recommend that DHA should be added to infant formula and to infant weaning foods. There is also increasing support for the addition of DHA to the maternal diet.

A NIH working group recommended daily intakes of

- 650 mg per day of DHA plus EPA for adults
- 300mg of DHA for pregnant and breastfeeding women.

Further information is available upon request or visit www.hi-dha.com

Gelphorm® HiDHA® CAS is intended for use in product incorporation trials only. ■

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Appendix III.

Karicare First Infant Formula

NUTRICIA
AUSTRALIA

12th June 2001

To whom it may concern

Nutricia Australia has marketed and sold Karicare First Infant Formula[1] containing LCPUFAs since 2.12.97. For the 8 months prior to this the product belonged to Douglas Pharmaceuticals. The data presented here is only relevant to the period that Nutricia has been responsible for the Karicare brand.

From its launch, in February 1997, the product was presented as a unit of standard infant formula with 7 capsules of tuna oil and GLA attached under the can lid. (Caregivers would add 7 drops of the oil mixture to prepared formula). From December 1998 onwards 0.2% DHA & 0.4% AA were encapsulated into the final product.

Since the end of 1997, 584,963 units[2] of Karicare formula containing LCPUFAs have been distributed from this facility to retail pharmacy and grocery. Each unit is reconstituted to 6.25 litres. If it assumed that the average feed is 125mls the total sales represent 3.65 million feeds.

In that time, 204 complaints have been registered with Nutricia with 24 complaints referring to an baby ill. The other complaints related to missing scoops, can not full, foreign body, poor miscibility.

In all cases, Nutricia attempts to retrieve the product from the consumer and have it tested by our manufacturer. To date the results have all meant with specifications. It is not Nutricia policy to refer infants to a medical practitioner though in some cases parents do get a medical opinion. Nutricia is unaware of the outcome of these visits.

In reference to the implication of fish oil in SIDS cases I can only refer you to the SIDS website at www.sidsaustralia.org.au. There are comprehensive requirements for the pathology reporting on these cases.

Catherine McNamara
National Marketing Manager

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[1] As of January 2001, Karicare First Infant Formula has been trading as Karicare Gold 1. An additional LCPUFA product, Karicare Gold 2 was launched into the marketplace at the same time The sales figures incorporate Karicare Gold 2.

[2] Of the 584,963 units of Karicare LCPUFA product sold by Nutricia only 16,060 were the first generation product.

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PO Box 6745, Baulkham Hills Business Centre NSW 2153
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Appendix IV.

Bioavailability of HiDHA® tuna oil (1)

Yep YL, Li D, Mann NJ and Sinclair AJ. Bread enriched with microencapsulated tuna oil increases omega 3 fatty acids in humans. RMIT

Bread enriched with microencapsulated tuna oil increases plasma omega 3 fatty acids in humans

Yolande L Yep, Duo Li, Neil J Mann and Andrew J Sinclair*

Department of Food Science, RMIT University

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Contributors: Andrew J Sinclair supervised the project and secured the funding. Yolande L Yep initiated this paper, conducted the experiments and collected data. Duo Li prepared the draft of paper and did statistics. All authors contributed to the preparation of the paper.

Guarantor: Professor Andrew J Sinclair

Short running title: MTO bread and plasma LC n-3 PUFA

1 **ABSTRACT**

2 **Objective**: The aim of this study was to determine the acute and chronic effects of low doses
3 of long chain (LC) n-3 polyunsaturated fatty acids (PUFA)/(<100mg per day) on plasma LC
4 n-3 PUFA levels, using a novel delivery form, bread containing microencapsulated tuna oil
5 (MTO).

6 **Design**: Supplementation study with a randomised design.

7 **Setting**: RMIT University, Melbourne, Australia.

8 **Subjects**: Sixteen subjects aged 21-64 were recruited by advertisement at RMIT and
9 Vegetarian Society of Victoria newsletters.

10 **Interventions**: Six omnivores (3 males, 3 females) participated in the acute study, which
11 involved ingesting a prototype MTO bread containing ~ 80 mg of LC n-3 PUFA/4 slices.
12 Plasma triacylglycerol fatty acid compositions were measured after an overnight fast and
13 postprandially at 2 and 4 hours. Ten vegetarian subjects (9 males, 1 female) consumed MTO
14 bread/6-8 slices/day containing ~ 60 mg of LC n-3 PUFA for three weeks in the chronic
15 study. Fasting plasma total and phospholipid fatty acid compositions were measured at
16 baseline and endpoint.

17 **Results**: In the acute study, proportion of 22:6n-3 and total n-3 PUFA in plasma
18 triacylglycerol were significantly increased ($p<0.05$). In the chronic study, the proportions of
19 20:5n-3, 22:5n-3, 22:6n-6, total n-3 PUFA in plasma, and 22:6n-6 and total n-3 PUFA in
20 plasma phospholipid fraction were significantly increased ($p<0.05$) at the endpoint compared
21 with the baseline. Ratio of n-6/n-3 in plasma and plasma phospholipid fraction were
22 significantly decreased ($p<0.05$) at the endpoint compared with the baseline.

23 **Conclusions**: This study showed that low dose LC n-3 PUFA, consumed as MTO-enriched
24 bread, increased the LC n-3 PUFA levels in plasma of human subjects.

25

INTRODUCTION

The relationship between fish/fish oil intakes and human tissue long chain (LC) n-3 polyunsaturated fatty acid (PUFA) accretion and subsequent manifestation of numerous health benefits is well documented in the literature. The Australian diet, typical of Western diets, contains low levels of LC n-3 PUFA which have been estimated to be between 100 to 190 mg/day for adults (Sinclair et al, 1994). It is much lower than International Society for the Study of Fatty Acid and Lipids (ISSFAL) recommended dietary intakes 650 mg/day (Simopoulos et al. 1999).

The richest food sources of 20- & 22-carbon LC n-3 PUFA are fish and seafood. Minor amounts are also present in meats especially lean meat, offal, eggs, milk and dairy products. High consumption of these food sources elevates LC n-3 PUFA tissue levels (Bonaa et al, 1992; Anttolainen et al, 1996; Mann et al, 1997). However, these conventional food sources may not be suitable or convenient for certain individuals or communities for various religious, ethical or personal (palatability, allergenicity) reasons. Ovolacto vegetarians obtain a limited amount of LC n-3 PUFA from eggs, milk and dairy products. Vegans must rely entirely on *in vivo* biosynthesis of these nutrients from the precursor alpha linolenic acid (18:3n-3), and the rate relies on the level of 18:3n-3 and its ratio to linoleic acid (18:2n-6) in the diet (Li et al, 1999a). However, using dietary 18:3n-3 is not as effective as direct consumption of LC n-3 PUFA from fish/fish oil for increasing tissue LC n-3 PUFA levels (Sanders and Roshanai 1983). Vegetarians have been demonstrated to have lower levels of LC n-3 PUFA in their platelets and plasma, which is associated with increased platelet activity and plasma 11-dehydro-thromboxane B_2 production (Li et al. 1999b).

50 One approach to improving the LC n-3 PUFA status of populations is to incorporate these
51 important nutrients into frequently consumed processed foods. There have been a number of
52 studies which have examined the effects of omega-3 enriched eggs (Jiang et al, 1993; Farrel,
53 1998), milk (Visioli et al, 2000) and omega-3 enriched processed foods (Roche & Gibney,
54 1994; Lovegrove et al, 1997; Sorensen et al, 1998) as means of improving tissue LC n-3
55 PUFA levels. In general, these studies have used foods containing 290 mg to 600 mg LC n-3
56 PUFA per serving, however there have been no studies looking at regular consumption of
57 smaller doses of n-3 PUFA which if added to a variety of staple food may offer a more
58 practical option without extensive change to habitual diets. Bread enriched with low level of
59 microencapsulated tuna oil (MTO) (10 mg LC n-3 PUFA per slice) is currently available to
60 Australian consumers. However, there is no data on the effect of low dose LC n-3 PUFA in
61 processed foods on human n-3 PUFA status.
62
63 The aims of this study were: (1) to investigate the acute effects of a single dose of low level
64 LC n-3 PUFA (~ 80 mg), as MTO-enriched bread on postprandial plasma LC n-3 PUFA
65 levels; (2) to investigate the chronic effects of a daily dose of a lower level of LC n-3 PUFA
66 (~ 60 mg), as MTO-enriched bread for three weeks on fasted plasma LC n-3 PUFA status.
67 We hypothesized that low dose MTO-enriched bread would improve plasma LC n-3 PUFA
68 status.
69
70 **METHODS**
71 *Subjects*: Ethics approval was granted by the Human Research Ethics Committee of RMIT
72 University, and all subjects gave their written consent before participating. Free-living and
73 healthy volunteers, 12 males and 4 females (6 omnivores, 9 ovolacto vegetarians, 1 vegan)
74 aged 21- 64 years were recruited from RMIT University and through advertisements in the

75 Vegetarian Society of Victoria newsletter. An omnivore was defined as someone who ate
76 meat at least 5 times a week, an ovolacto vegetarian was defined as someone who ate no
77 meat, not more than one fish meal and 3 eggs a week, and a vegan was defined as someone
78 who ate meat, eggs and dairy products less than six times per year and had been following
79 this diet for at least six months. The exclusion criteria were major medical illness, cigarette
80 smoking, excessive alcohol intake and chronic use of aspirin or other anti-inflammatory
81 drugs.
82
83 *Study design and diet*: The project consisted of two intervention studies; the acute and
84 chronic effects of MTO-enriched bread on plasma LC n-3 PUFA status. In the acute study, 6
85 omnivores (3males, 3 females) were advised to consume their usual foods for one week prior
86 to the experiment day except one day before the study, when fish meals were not permitted.
87 Subjects were given a single dose of LC n-3 PUFA (~ 80 mg) to observe changes in
88 postprandial plasma LC n-3 PUFA levels at 2 and 4 hours. LC n-3 PUFA was delivered
89 through 4 slices of prototype MTO-enriched bread (Bunge Cereal Foods Pty Ltd, Melbourne,
90 Australia) together with 15 g of margarine (monounsaturated sunflower oil, Flora, Unilever
91 Foods, Marrickville, NSW, Australia). The bread was eaten either lightly toasted or
92 untoasted. Table spreads (jam, vegemite) and beverages (coffee, tea, orange juice) were also
93 made available. After this meal, subjects were asked to refrain from consuming food except 1
94 apple and water or zero calorie drinks, and to avoid any strenuous physical activity for the
95 next 4 hours.
96
97 In the chronic study, 9 ovolacto vegetarians (8 males, 1 female) and 1 vegan (male) were
98 instructed to consume 6 slices of commercially available MTO-enriched bread (containing 60
99 mg of LC n-3 PUFA) daily either untoasted or toasted lightly with their habitual diet for three

100 weeks to investigate the changes in fasting plasma LC n-3 PUFA status. Foods rich in n-3
101 PUFA, such as fish and all seafood, fish oil capsules, oat germ, wheat germ/germ oils,
102 walnuts, linseeds and soy/linseed breads were excluded during the intervention period. The
103 first loaf of enriched bread (toast sliced) was provided for subjects on their first blood
104 sampling day, thereafter they purchased their own as required from local supermarkets. Each
105 subject was required to fill out a semi-quantitative food frequency questionnaire recording his
106 or her daily intake of fat-containing foods consumed during the 3-week intervention period.
107 The questionnaire was primarily designed to estimate the habitual fat intake of each subject,
108 specifically the LC n-3 PUFA and to check for compliance. Subjects were asked to avoid
109 foods listed on the exclusion list, however, when it was not possible to adhere, details of food
110 items and portion sizes of the 'prohibited foods' were to be declared. Subjects were advised
111 to record the number of slices of bread consumed daily and to specify whether the slices were
112 toasted or untoasted. Compliance for bread consumption was monitored by requesting
113 subjects to present used bread packets and receipts at the end of the study period. All
114 subjects were contacted once a week during the study period to check on their progress with
115 bread consumption, to remind them to avoid exclusion foods and to generally maintain their
116 interest and compliance.
117
118 *Blood specimen collections*: In the acute study, venous blood was drawn into a 9 mL EDTA
119 vacuum tube from subjects between 0830 - 0900 hours after an overnight fast (baseline) and
120 postprandially 2 and 4 hours following the ingestion 4 slices of prototype MTO-enriched
121 bread. The sampling times were selected to coincide with peak plasma triacylglycerol (TAG)
122 absorption (between 2-4 hrs postprandially) based on data from Agren et al (1996) and
123 Dubois et al (1998). In the chronic study, subject's height, weight, percentage body fat, pulse
124 and blood pressure were measured before bleeding. In the chronic study, venous blood was

125 collected into a 9 mL EDTA vacuum tube from subjects before (week 0) and after (week 3)
126 the intervention period following a 12 hr overnight fast. Plasma was isolated by
127 centrifugation at 3000 rpm for 10 mins at 4°C and stored at –80^{0}C frozen in portions until
128 analyses.
129
130 *Dietary assessment:* The diet records were analysed for total fat intake using Diet Version 4
131 software (Xyris Software Pty Ltd, Highgate Hill, QLD, Australia) with NUTTAB 95
132 database based on Composition of Food Australia (COFA). Estimation of the individual LC
133 n-3 PUFA intake was based on published values of omega 3–containing food fatty acid
134 concentrations (Sinclair et al, 1992; Mann et al, 1995; Quigley et al, 1995; Nichols et al, 1998
135 and Sinclair et al, 1998).
136
137 *Plasma fatty acids:* Total lipid of plasma was extracted with chloroform : methanol (1 : 1,
138 v/v), the plasma phospholipid (PL) and triacylglycerol (TAG) fractions were separated by
139 thin-layer chromatography and the methyl esters of the fatty acids of total plasma, plasma PL
140 and TAG fractions were prepared, fatty acid methyl ester were separated and quantified by
141 gas-liquid chromatography as described previously (10).
142
143 *Statistical analyses:* The data analyses were performed using a STATVIEW software
144 program (Abacus Concepts Inc, Berkeley, CA, USA). ANOVA with repeated measures was
145 used to determine the effect of MTO on plasma LC n-3 PUFA status. The values were
146 reported as mean ± SD in all the results tables and mean ± SEM in all the graphs unless
147 otherwise specified. P values <0.05 were considered as significant.
148
149

150 **RESULTS**

151 Table 1 reports the fatty acid contents of prototype and commercial MTO-enriched breads
152 and monounsaturated sunflower oil margarine. The total fat was found to be 2.9 g/100g
153 bread. Total LC n-3 PUFA was 56 mg/100g and 24 mg/100g for prototype and commercial
154 MTO-enriched breads, respectively. The prototype MTO-enriched bread contained 44 mg of
155 22:6n-3, 8.9 mg of 20:5n-3 and 2.8 mg of 22:5n-3, and also 3.5 mg of AA per 100g. The
156 commercial MTO-enriched bread contained 18 mg of 22:6n-3, 4.5 mg of 20:5n-3 and 1.5 mg
157 of 22:5n-3, and also 1.9 mg of AA per 100g. There were no LC n-3 PUFA in
158 monounsaturated sunflower oil margarine.

159

160 Table 2 shows the intake of major fatty acids intake in acute study. The mean intake of total
161 fat was 19.1 g; 4.1 g from prototype MTO-enriched bread and 15 g from monounsaturated
162 sunflower oil margarine. The mean intake of total LC n-3 PUFA was 79 mg; 22:6n-3 62 mg,
163 20:5n-3 13 mg and 22:5n-3 4 mg.

164

165 In the acute study, the proportion of 22:6n-3 and total n-3 PUFA of plasma TAG increased in
166 all the six subjects between 5% to 121% for total n-3 PUFA (p=0.0163) and 22% to 153% for
167 22:6n-3 (p=0.0147) from baseline to 2 hours postprandial, respectively (Figures 1A and 1B).
168 The 20:5n-3 proportion of plasma TAG was also increased in all the six subjects between
169 25% to 288% (p=0.14) (Figure 1C).

170

171 Daily fat intakes of habitual diets and during the 3 weeks commercial MTO-enriched bread
172 intervention period (experimental diet) in the chronic study are shown in Table 3. The mean
173 daily intakes of total fat were 52 g/day and 55 g/day, PUFA were 20% and 23%, MUFA were
174 42% and 40%, and SFA were 38% and 37% of total fat for habitual diet and experimental

175 diet, respectively. The daily intakes of total LC n-3 PUFA were 1 mg and 64 mg for habitual
176 diet and experimental diet, respectively.
177
178 Physiological characteristics of the subjects in the chronic study are reported in the Table 4.
179 There were no significant changes in any measured physiological characteristics in the
180 subjects during 3 weeks study period.
181
182 The proportion of total n-3 PUFA in the plasma increased between 1% to 42 % (p=0.001),
183 while there was an increase in the proportion of 22:6n-3 for nine of the ten subjects
184 (p=0.006), eight of ten for 20:5n-3 (p=0.0059) and six of ten for 22:5n-3 (p=0.034) at day 21
185 compared with day 0 (Figures 2A, 2B, 2C & 2D), respectively. Total plasma ratio of n-6 to n-
186 3 decreased in nine of ten subjects at day 21 compared with day 0 (Figure 2E).
187
188 In the plasma PL fraction, the proportions of total n-3 PUFA and 22:6n-3 increased in seven
189 and eight of ten subjects at day 21 compared with day 0, respectively (*p*-value was 0.03 and
190 0.0137) (Figure 3A & 3B). The 20:5n-3 proportion was raised in seven of ten subjects at day
191 21 compared with day 0 (p=0.119) (Figure 3C). The ratio of n-6 to n-3 in plasma PL fraction
192 decreased in all the ten subjects after 3 weeks intervention (p=0.006) (Figure 3D).
193
194 **DISCUSSION**
195 The aim of the present study was to determine whether the consumption of bread enriched
196 with a low dose MTO would improve LC n–3 PUFA status in healthy individuals. To achieve
197 this, an acute study (single dose ingestion with 4 hr follow up) was conducted to establish the
198 feasibility of the use of a low intake of LC n–3 PUFA, delivered as MTO-enriched bread.
199 This was followed by a 3-weeks chronic study using commercial MTO bread as the LC n-3

200 PUFA source. We found that low dose MTO-enriched bread did improve plasma LC n-3
201 PUFA status in both the acute and the chronic study.
202
203 Several biological markers have been employed as a measure of LC ω-3 PUFA status in
204 humans. Commonly in use are accessible tissues such as blood (plasma, platelets,
205 erythrocytes, neutrophils), adipose tissue, breast milk and cheek cells. All have been reported
206 to correlate reasonably well with dietary intake in prospective as well as dietary intervention
207 trials (Brown et al, 1991; Hjartaker et al, 1997; Nelson et al, 1997; Vidgren et al, 1997 and
208 Garland et al, 1998). The selection criteria for their use would then ultimately depend on the
209 objectives and duration of the study. In the present study, where the main objective was to
210 determine extent of dietary LC n-3 PUFA incorporation in general as opposed to specific
211 organ function or pharmacological effects, plasma was selected because of its relative ease of
212 sample collection and preparation. In the acute study, we monitored the plasma TAG fatty
213 acid, not PL, since the plasma TAG can be acutely influenced by a single meal (Riboli et al
214 1987).
215
216 In the acute experiments where early postprandial plasma was investigated, the level of LC n-
217 3 PUFA incorporation was quantified in the nascent TAG fractions (location of newly
218 absorbed LC n-3 PUFA following a meal). The acute study was primarily designed to
219 ascertain that ingestion of low dose of LC n-3 PUFA in microencapsulated oil was
220 bioavailable.
221
222 Dietary lipids are digested and absorbed rapidly after ingestion, following which, the longer
223 chain fatty acids and monoacylglycerols are packaged into chylomicrons. These transport the
224 absorbed TAG and other dietary lipids via the lymphatic system to the blood (Nelson &

225 Ackman, 1988). In this experiment, the plasma TAG at 2 and 4 hrs following the acute low
226 dose of LC n-3 PUFA in MTO-enriched bread was used to represent the TAG in the
227 chylomicrons since peak absorption generally occurs in the first 2-4 hrs after ingestion
228 (Agren et al, 1996; Sanders et al, 1997). Additionally, it was also used to determine the
229 feasibility of a novel delivery form, prototype bread enriched with the functional ingredient in
230 the MTO form (80 mg LC n-3 PUFA). Twenty-grams of margarine was administered with
231 the meal to boost chylomicron formation to attain adequate TAG for LC n-3 PUFA
232 quantitation. The amount of fat intake selected was based on data from Dubois et al (1998).
233 In that study, a 30 g fat meal increased serum TAG markedly within first 2 hrs and peaking 2-
234 3 hrs following ingestion, while only a modest, though still significant rise, was observed in
235 the 15 g fat meal, and no change occurred in the fat free meal during the 7 hr follow-up.
236
237 Having verified that a single low dose of LC n-3 PUFA (80 mg) in a novel delivery form
238 (bread containing MTO) led to a significant increase in postprandial plasma LC n-3 PUFA
239 levels, it was logical to follow on to a chronic feeding study involving a larger group of
240 subjects. Hence the aim of the chronic study was to determine whether daily consumption of
241 lower dose LC n-3 PUFA (approx. 60 mg) in 6 slices of enriched bread would elevate the
242 fasting plasma LC n-3 PUFA content of healthy subjects.
243
244 Vegetarians (9 males and 1 female) were selected for this study because they do not normally
245 consume LC n-3 PUFA-rich foods namely fish, seafood or other meat products. Therefore,
246 any increase in LC n-3 PUFA status after the intervention period could be accurately
247 attributed to the enriched bread without the need reliance on comprehensive diet records and
248 LC n-3 PUFA database which is not available at the present time. This 3-week study
249 demonstrated that daily consumption of low dose MTO-enriched bread (60 mg LC n-3

250 PUFA) enhanced the LC n-3 PUFA content of plasma lipids. The increase was significant in
251 the plasma total lipids (18 % rise) as well as the PL fraction (12 % rise). All of the subjects
252 had elevated total plasma LC n-3 PUFA levels after the test period. While this is a modest
253 rise, the intake dose of 60mg LC n-3 PUFA is low by comparison with other studies (Gibney
254 and Daly, 1994; Lovegrove et al, 1997; Wallace et al, 2000).
255
256 Saldeen et al (1998) more recently reported 50 % rise in plasma PL LC ω-3 PUFA content in
257 omnivorous subjects (n=9) after 2 weeks of consuming bread enriched with fish oil (non-
258 encapsulated). The average LC n-3 PUFA intake of approx. 319 mg/day was five times the
259 amount administered for the present study. Both studies, therefore, show that bread is a
260 suitable matrix for delivery of LC n-3 PUFA to humans.
261
262 Microencapsulation technology is one of few strategies utilised by the food industry to
263 protect sensitive PUFA against oxidation, thus preserving the LC n-3 PUFA concentration
264 during processing and storage. In addition, microencapsulation masks the undesirable fishy
265 odour and taste in the final product, and facilitates easy handling and storage. The
266 bioavailability of LC n-3 PUFA in the MTO-enriched foods has been reported to be the same
267 as n-3 PUFA in a capsule (Wallace et al, 2000). They found that there was no significant
268 difference on platelet AA, 20:5n-3 and 22:6n-3 composition when 13 female subjects aged
269 20-26 years consumed MTO-enriched foods (soup, biscuits and bread) containing 900 mg LC
270 n-3 PUFA per day, equivalent to 3 g of tuna oil compared with age and sex matched 12
271 subjects ingested three 1-gram tuna oil capsules per day. A similar dietary enrichment study
272 (22 days) using microencapsulated fish oil in processed foods (as LC n-3 PUFA delivery
273 vehicles) demonstrated elevated plasma 22:6n-3 and 20:5n-3 levels (greater than 11-fold and
274 3-fold compared with habitual and control diets, respectively) in omnivores (n=9) (Lovegrove

et al, 1997). The level of LC n-3 PUFA consumption in their study however, was markedly higher than the present study by 10-fold at 1.4 g/day. Some of the food items were enriched with fish oil directly as opposed to the encapsulated form. The resulting dosage of LC n-3 PUFA was achieved with 9 exchanges of control foods for the identical enriched variety, including 113 g mackeral pate.

Current Australian intake of dietary LC n-3 PUFA is approximately 100 to 190 mg/day, and recommendations for LC n-3 PUFA from 214 mg/day by British Nutrition Foundation (2000) to higher value 600 mg/day by ISSFAL (Simopoulos et al,1999). In order to bridge the gap between current intake at say 100 – 200 and recommended levels of 600 mg/day, the food industry may choose to enrich a variety of foods, all of which could then make a contribution. MTO facilitates the incorporation of LC n-3 PUFA into many food matrices. Since high levels of enrichment may raise the cost of the end products, a suitable minimal level is sought to meet consumer affordability without compromise on bioavailability and consequently health benefits. This study showed that as little as 60 mg/day was bioavailable, thus demonstrating the validly of MTO in bread as a delivery vehicle.

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Table 1. Fatty acid contents of prototype and commercial MTO-enriched bread, and monounsaturated sunflower oil margarine.

Fatty acid	Prototype MTO-enriched bread (mg/100g)[a]	Commercial MTO-enriched bread (mg/100g)[a]	Monounsaturated sunflower oil Margarine (g/100g)[a]
14:0	33	5	1.4
16:0	575	343	7.7
18:0	226	145	6.6
18:1	501	572	44.8
18:2n-6	1004	752	7.3
18:3n-3	41	107	1.7
20:4n-6	3.5	1.9	nd
20:5n-3	8.9	4.5	nd
22:5n-3	2.8	1.5	nd
22:6n-3	44	18	nd

[a]n=2, nd=not detected.

Table 2. Amount of major fatty acids ingested from prototype MTO-enriched meals in the acute study.

Fatty acid	Prototype MTO-enriched bread + Margarine[a]
16:0	2.1 g
18:0	1.6 g
18:1	10.3 g
18:2n-6	3.0 g
18:3n-3	0.6 g
20:5n-3	13 mg
22:5n-3	4 mg
22:6n-3	62 mg
Total LC n-3 PUFA	79 mg
Total fat	19.1 g[a]

[a]Analysed in duplicate, [b]4.1 g from prototype MTO-enriched bread and 15 g from monounsaturated sunflower oil margarine.

Table 3. Daily fat and LC n-3 PUFA intake of habitual diet and during the 3 weeks commercial MTO-enriched bread intervention period in vegetarians.

	Habitual diet (n=10)	Experimental diet (n=10)
Total fat (g)	52 ± 25	55 ± 26
SFA (% of total fat)	38 ± 8	37 ± 7
MUFA (% of total fat)	42 ± 5	40 ± 5
PUFA (% of total fat)	20 ± 6	23 ± 5
Total LC n-3 PUFA (mg/day)	1 ± 0	64 ± 8*

SFA=saturated fat, MUFA=monounsaturated fat, PUFA=polyunsaturated fat.

*$p<0.05$

Table 4. Physiological characteristics of vegetarian subjects in chronic study.

	Day 0 (n=10)	Day 21 (n=10)
BMI (kg/m^2)	22.9 ± 2.4	22.8 ± 0.9
Waist/hip ratio	0.84 ± 0.06	0.84 ± 0.02
Systolic BP (mmHg)	110 ± 7	117 ± 4
Diastolic BP (mmHg)	71 ± 5	71 ± 2
Pulse (bpm)	63 ± 7	66 ± 11
% Body fat	17.6 ± 5.0	17.2 ± 1.7

BMI=body mass index, BP=blood pressure.

Legend

Figure 1. Changes in composition of plasma TAG fraction 22:6n-3 (1A), total n-3 PUFA (1B) and 20:5n-3 (1C) after 6 subjects consumed a prototype MTO-enriched meal.

Pre = pre meal, Post 2 = postprandial 2 hours, Post 4 = postprandial 4 hours.

Figure 2. Changes in composition of total plasma n-3 PUFA (2A), 22:6n-3 (2B), 20:5n-3 (2C), 22:5n-3 (2D) and ratio of n-6/n-3 (2E) after 10 vegetarian subjects consumed commercial MTO-enriched bread for three weeks.

Figure 3. Changes in composition of plasma phospholipid total n-3 PUFA (3A), 22:6n-3 (3B), 20:5n-3 (3C) and ratio of n-6/n-3 (3D) after 10 vegetarian subjects consumed commercial MTO-enriched bread for three weeks.

Appendix V.

Bioavailability of HiDHA® tuna oil (2)

Childhood Asthma Prevention Study, Children's Hospital at Westmead, NSW

12 JUL 2001

Childhood Asthma Prevention Study

CAPS

The New Children's Hospital
Cnr Hawkesbury Rd & Hainsworth St
Westmead NSW 2145

Institute of Respiratory Medicine
The University of Sydney
NSW 2006

Liverpool Hospital
Elizabeth St
Liverpool NSW 2170

Westmead Hospital
Darcy Road
Westmead NSW 2145

Clover Corporation Limited
PO Box 192 Caringbah NSW 2229
Research & Development
Attn: Mr Ortwin Bode
June 20th, 2001

Childhood Asthma Prevention Study
Childrens Hospital at Westmead
Locked Bag 4001,
Westmead NSW 2145 Australia
phone: (02) 9845 1323

Dear Mr Bode

RE: The use of tuna fish oil capsules in the Childhood Asthma Prevention Study.

The Childhood Asthma Prevention Study (CAPS) is a primary prevention randomised control trial (RCT) that is being conducted to investigate whether we can prevent the development of asthma in young children. To date, a cohort of approximately 600 infants who are at high risk of developing asthma and who live in the western Sydney area have been enrolled in this trial. The youngest child is now 18 months old and the oldest is 3 years of age.

This study was conducted following the evidence that increases in the prevalence of asthma during the 1980's and 1990's has been significant with the result that asthma is now a common chronic illness in children. (1) The reasons for the increase must be environmental and there is evidence that the most important environmental factors that increase symptoms in children are related to diet and allergen exposure. (2) The research evidence suggests that a diet which is deficient in anti-oxidant nutrients may increase susceptibility to adverse exposures to allergens or industrial pollutants and lead to increased airway inflammation. However a healthy diet with a favourable balance of omega –3 and omega-6 fatty acids and adequate anti-oxidants together with house dust mite avoidance may protect against the development of asthma or symptoms of wheeze in children who are prone to this illness. (3)

The CAPS study has a factorial design in that all combinations of an active and placebo diet intervention and active and non-active house dust mite allergen avoidance interventions are being tested.(3)

A principal component of the dietary intervention involves the use of tuna fish oil soft gel capsules supplied by Clover Corporation. The capsules have been used by approximately half of the 600 infants who have been enrolled in the CAPS study.

Clover supplies CAPS with 600 capsules per day or, over five years with 1 056 000 individual capsules. To date Clover regularly test our capsules when they are near their use-by dates to ensure that they have not oxidised and are still safe for consumption.

The dose of tuna fish oil provided in the active capsules constitutes 33mg (6.7%) eicosapentaenoic acid (EPA) and 129mg (25%) docosahexaenoic Acid (DHA), 240mg total polyunsaturated fatty acids (PUFA), 7mg (1.4%) linoleic Acid and 2500ppm vitamin E. Thus the ratio DHA to EPA in the active capsules is about 4:1 . (3)

Because vitamin A, D and E in the fish oil are reduced in the refining process, a small amount of vitamin E is added to protect the oil from oxidation. Vitamin E is approved for use in edible oils and infant formula by the Australian New Zealand Food Authority and other authorities world wide. (3)

Recent studies show that the dietary supplementation of formula fed infants with DHA at 0.36% of total fatty acids maintains cellular fatty acid composition at a level equivalent to that of breastfeeding.(4)
There have been concerns about possible growth problems in infants supplemented with EPA due to interference of EPA and DHA with arachidonic acid metabolism. However, small doses of up to 500mg have been used safely in many trials of healthy term infants. (5)

The plasma phospholipid levels of babies in the CAPS trial show that the tuna oil capsules have altered red cell plasma fatty acid membrane composition and are an effective measurable intervention for our purpose of investigating risk factors associated with the development of asthma.

The CAPS study is now in its 3rd year with parents being asked to continue to give their child one capsule per day. A minority of parents choose to give 2 capsules per day. To date, no children have had a negative effect from taking Clover manufactured tuna oil capsules.

Frequent comments by parents include that the smell is pungent. The research team at CAPS have put together a fact sheet called "Are you cornered by capsules?" which suggests ways to add the capsule to foods with minimal fuss to the taste sensitive child. (see attached) This has helped many of our parents use the capsules everyday in creative ways.

Our working relationship with Clover has been a supportive, interested and satisfactory one.
We look forward to ongoing research in the benefits of dietary interventions and to good industry relations with Clover Corporation in coming years.

Yours sincerely

Natalia Knezevic Research Dietitian	Jennifer K Peat Principal Investigator	Seema Mihrshahi CAPS Coordinator

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1. Peat JK, van den Berg RH, Green WF, Mellis CM, Leeder SR, Woolcock AJ. Changing prevalence of asthma in Australian children. Br Med J 1994; 308; 1591-1596.
2. Peat JK, Li J. Reversing the trend: reducing the prevalence of asthma. J allergy Clin Immunol 1999; 103; 1-10.

3. Peat J., Mihrshahi S., Webb K. The Childhood Asthma Prevention Study (CAPS) Study Handbook. The Childrens Hospital at Westmead PO Box 3515 Parramatta NSW 2124.

4. Makrides M., Neumann MA., Simmer K., Gibson R. Erythrocyte fatty acids of term infants fed either breast milk, standard formula, or formula supplemented with long chain polyunsaturates. Lipids 1995; 30:941-948.

5. Makrides M., Neumann M, Simmer K., Pater J., Gibson R. Are long chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 1995; 345:1463-1468.

Are you cornered by Capsules??

As you know, the CAPS intervention for diet includes giving the daily oil capsule. Some children happily chew it like a lolly and others take it without noticing, but a few hate it! Because it is so important for the study for your children to take the capsules, we'd like to help you find ways to make capsule-giving, and feeding, in general, an easier daily experience..
Tips on how to address food refusal and CAPS capsules are listed below. Feel free to call the CAPS team if you have trouble with giving the capsule and need some assistance with more ideas.

Points to remember with food refusal and capsule intake;

- By six months of age, a gradual change from milk to a variety of foods is necessary for good health and adequate growth. But children don't always accept these new foods with good humour! Often they refuse them the first few times.

- Infants and children who refuse to try a "new food" are either becoming "independent" or reacting to a 'learned caution' response. Research has shown with 2-5 year old children that as many as 8 or 10 exposures to a new food may be needed to reduce food refusal and increase acceptance.

- Just because they've never eaten a particular food before...don't be afraid of offering it again. Soon enough, they will try the food and usually, accept it.

- So, it is not always related to the study capsules that your child refuses to eat what you provide. Often the child is exerting their independence or they may be cautious about a new food.

- Try providing the capsule when the appetite is likely to be great; eg. with breakfast or dinner.

- Don't forget appetites ebb and flow like the tide each day. It is inevitable that some food will be wasted.

- Your child may dawdle and toy with food from time to time.. try to take it in your stride (as frustrating as it can be!) Try to develop a "take it or leave it" attitude towards the food being consumed. From the childs viewpoint, it takes off the pressure!

- Capsule refusal may happen when they see the bottle of capsules. Try to add it out of sight of the child.

- As children grow, their tastes also develop and they can become sensitive to some flavours . So, perhaps the best way to add a capsule to food is in a strongly flavoured food in small quantity. For eg; homemade pizza; where a combination of flavours disguise the taste and texture of the oil or an omelette that includes cheese and tomato. Alternatively, mix the oil in their BBQ or tomato sauce to go with the dinner or snack!

A BIG THANK YOU FOR THE EFFORT YOU ARE PUTTING IN TO MAKE THE STUDY A SUCCESS.

Appendix VI.

Comparison of DHA rich fish oil to EPA rich fish oil

Howe P. Cardiovascular trial to evaluate cardiovascular health benefits of docosahexaenoic acid rich fish oil supplement. Research Report for Clover Corporation, 1997.

NU-MEGA LIPIDS TRIAL

FINAL REPORT

to

CLOVER CORPORATION Pty Ltd

prepared by

Peter R. C. Howe
CSIRO DIVISION OF HUMAN NUTRITION
ADELAIDE

27th March, 1997

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Conclusions and recommendations	12
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Appendices

Patient information sheet

Clinical data files

Laboratory data files

Specifications/analyses of supplements

ABBREVIATIONS

BMI	body mass index
BP	blood pressure
DBP	diastolic blood pressure
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
HDL	high density lipoprotein
LDL	low density lipoprotein
PUFA	polyunsaturated fatty acids
SBP	systolic blood pressure
TG	triglycerides

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EXECUTIVE SUMMARY

A clinical intervention trial was conducted by the Clinical Research Unit, CSIRO Division of Human Nutrition to compare the potential cardiovascular and anti-inflammatory benefits of the client's DHA-rich tuna fish oil (Nu-Mega) with those of a commercial MaxEPA-type fish oil.

A double-blind, crossover protocol was used. Adult male volunteers were randomised to take either supplement (eight 1g capsules/day) for 6 weeks then, after a 4 week washout period, to take the alternate supplement for a further 6 weeks. Initially and at the end of each intervention phase, blood pressure was measured in the clinic and by 24 hour ambulatory monitoring and a blood sample was taken for assessment of plasma lipids, platelet thromboxane production and monocyte cytokine production.

Thirty subjects completed the trial. With one exception, the supplements were well-tolerated, with fewer unfavourable comments about Nu-Mega oil than the comparator. They resulted in high circulating levels of ω-3 fatty acids, with anticipated differential effects on EPA and DHA.

The average initial values for blood pressure and blood lipids were marginally elevated. Neither oil supplement affected total or HDL cholesterol and there were no clinically relevant changes in blood pressure. However, each supplement produced comparable reductions of both serum thromboxane production (40%) and plasma triglycerides (26%), with a modest (6%) elevation of LDL cholesterol. Effects of the supplements on cytokine production are still being analysed.

These findings demonstrate that a DHA-rich oil derived from Australian fish is as efficacious as imported EPA-rich fish oil in counteracting two major risk factors for cardiovascular disease, viz. hypertriglyceridaemia and thrombotic tendency, and they indicate that DHA can mediate these benefits. They warrant further evaluation of the appropriate doses for achieving optimal therapeutic benefits with minimal adverse effects in at risk individuals.

INITIAL PROPOSAL - ANNEXURE A OF RESEARCH AGREEMENT

CLINICAL TRIAL TO EVALUATE CARDIOVASCULAR HEALTH BENEFITS OF A DOCOSAHEXAENOIC ACID-RICH FISH OIL SUPPLEMENT

Sponsor: Mr. Hamish Drummond, Clover Corporation Pty Ltd

Investigator: A/Prof PRC Howe, CSIRO Division of Human Nutrition

Brief title: NU-MEGA LIPIDS TRIAL

Objective: To compare, in healthy volunteers, the bioavailability and cardiovascular health attributes of a DHA-rich tuna oil (Nu-Mega) and an EPA-rich oil (MaxEPA), taken as supplementary capsules.

Rationale: The cardioprotective effects of regular consumption of fish or fish oil are well established. These include reductions of blood pressure (BP), plasma triglycerides (TG), platelet aggregation and prevention of fatal arrhythmias. It is widely accepted that the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), present in varying proportions in all fish oils, are the mediators of these beneficial effects. EPA, which predominates in fish oils obtained from the Northern hemisphere, has a key role as a substrate competing with arachidonic acid for the production of eicosanoids with a wide range of cardiovascular and inflammatory functions. However, recent studies in our Division suggest that DHA rather than EPA may be the principal active omega-3 fatty acid mediating a wide range of cardiovascular health benefits.

Typical doses of omega-3 fatty acids required to show benefits in short-term clinical trials have ranged upward from 2g/day for anti-thrombotic and TG-lowering effects or 3-4 g/day for blood-pressure reduction. This amounts to at least six 1g capsules/day of imported commercial fish oil supplements such as MaxEPA, containing 18% EPA and 12% DHA. On the other hand, consumption of as little as two meals of fish per week is thought to convey similar benefits. This may be attributable to the higher proportion of DHA to EPA in fish. Australian fish tend to be less oily than those of the Northern hemisphere. However, as the DHA/EPA ratio in their oil tends to be higher, it may yield comparable cardiovascular benefits at lower doses, thus reducing cost and increasing consumer acceptability.

Approach: We will compare the effects of taking MaxEPA or the sponsor's DHA-rich tuna oil, Nu-Mega (5%EPA and 25%DHA), on cardiovascular risk factors (BP, cholesterol, TG and thromboxane production) and markers of inflammation, interleukin 1b (IL1b) and tumor necrosis factor (TNFa), in normal healthy subjects. Based on the outcome, subsequent trials may be conducted to test indications for specific pathological conditions, such as hypertension, diabetes, arthritis or inflammatory bowel disease.

Subjects: Thirtytwo adult male volunteers will be recruited by advertisment. They will be fully informed of the nature of the study and screened by questionnaire with respect to the following exclusion criteria.

Exclusions: Uncontrolled hypertension (DBP >105 mmHg or SBP >160 mmHg); regular use of aspirin or NSAID; likelihood of commencing or requiring change of antihypertensive medication during the trial; risk of excessive bleeding; history of acute cardiac or renal disease, unstable angina or arrhythmia, diabetes, stroke, transient ischaemic attack; history of substance abuse.

Location: The study will be conducted in the Nutrition Research Clinic of the CSIRO Division of Human Nutrition at Kintore Avenue, Adelaide.

Protocol: A placebo-controlled, double-blind crossover design will be used. Subjects will visit the clinic on 3 occasions: initially and at the end of each treatment phase. At each visit, BP will be recorded seated and standing (average of 3 Dinamap readings taken at 1 minute intervals), a blood sample will be taken and a Spacelabs 90207 monitor will be fitted to record ABP every 20 min (day) or 40 min (night) for 24 hrs. Subjects will be randomly assigned to take eight 1g capsules/day of either Nu-Mega or MaxEPA for 6 wks, followed by a 4 wk washout period. They will then take the alternate oil for a further 6 wks. Oils conforming to NFA standards will be supplied by the sponsor in identical 1g soft gelatin capsules.

Assays: At the end of each treatment phase, platelet thromboxane production will be measured in serum as an index of anti-thrombotic potential. Total, HDL and LDL cholesterol and TG will also be measured in serum. Monocytes derived from blood samples initially and after 6 wks will be used to measure IL1b and TNFa production. Compliance will be assessed by counting returned capsules and from changes in serum fatty acid profiles.

Outcomes: The primary efficacy variables will be the within-individual differences in the 24-hr averaged SBP and/or DBP between the end of each intervention phase, serum TG and production of thromboxane. Inhibition of IL1b and TNFa production by isolated monocytes, an indicator of anti-inflammatory potential, is a secondary outcome measure.

Statistics: Data will be analysed on an intention-to-treat basis, with stratification of the variables used for subject blocking. Based on previous experience, we assume 80% power to detect a significant ($p<0.05$) within-individual change of at least 5 mmHg in the 24 hour averaged value for SBP.

Reporting: Data obtained for each subject will be maintained in confidential case report forms. Subjects will receive summaries of their individual results at the end of the trial. A report of the trial with detailed analyses of overall outcomes will be forwarded to the sponsor, with recommendations for publication.

Approval: Approval will be obtained from the CSIRO Human Ethics Committee. The National Food Authority will be notified of the study.

Timetable:

August -	patient recruitment, capsules supplied by Clover
October -	randomisation of subjects
December -	1st treatment period completed
February, 97 -	2nd treatment period completed

March - data analysed, report submitted to Clover

PROGRESS

- The research agreement with Clover Corporation was effected on 16th August, 1996.

- The CSIRO Human Ethics Committee was notified on 5th August, 1996 of modifications contained in the research proposal and approved the appended patient information sheet. The Australia New Zealand Food Authority was notified of the trial.

- Patients were recruited by advertisment in the Sunday Mail, 8th September, 1996. Screening visits were conducted in the following month with enrolment and randomisation completed by 15th October, when the first intervention phase commenced. This phase ended as scheduled on 20th December, 96. The second intervention phase finished as scheduled on 28th February, 97.

- CSIRO-based laboratory analyses were completed by 21st March, 97.

In addition to the proposed analyses at the end of each treatment phase, pre-intervention blood samples taken at the first visit were also used for measurements of plasma lipids and platelet thromboxane production.

- Cytokine production by monocytes in blood samples obtained during the first intervention phase is being undertaken by Dr. M. James of Royal Adelaide Hospital. The cytokine assays have been temporarily delayed whilst quality control is being improved.

- With the exception of the cytokine data, which will be presented in a supplementary report, this report is a final presentation of the outcomes of this trial.

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RESULTS

Subjects

A total of 32 adult males were recruited and randomised to commence on either treatment. Their screening data appears below. They were predominantly middle-aged and overweight, with casual BP readings ranging from high normal to moderate hypertension. None, however, were currently taking antihypertensive or hypolipidaemic medication.

Group A (commencing on supplement A)					Group B (commencing on supplement B)				
ID	**BMI** (kg/m^2)	**age** (years)	**SBP** (mmHg)	**DBP** (mmHg)	**ID**	**BMI** (kg/m^2)	**age** (years)	**SBP** (mmHg)	**DBP** (mmHg)
C01	34.4	45	168	109	C03	31.8	55	145	104
CO2	27.1	48	147	101	C04	29.2	57	142	96
CO5	29.4	50	146	93	C06	29.9	62	166	98
C07	27.8	60	158	106	C08	22.6	51	141	86
C09	23.9	57	155	88	C11	25.6	33	161	88
C10	34.4	68	159	94	C12	25.6	60	146	80
C13	28.7	58	166	86	C14	27.0	47	143	92
C15	32.4	47	136	77	C18	29.2	52	179	101
C16	28.3	66	157	93	C19	26.7	49	144	101
C17	26.1	49	155	93	C20	23.2	40	141	94
C21	24.9	40	134	92	C23	31.2	72	149	90
C22	23.6	46	152	97	C24	29.1	53	130	87
C25	26.0	39	137	86	C27	26.2	40	147	80
C26	30.8	62	156	80	C28	26.0	35	140	82
C30	30.6	33	152	75	C29	26.5	55	137	86
C32	28.5	43	140	88	C31	28.6	60	181	94
mean:	**28.5**	**50.7**	**151.1**	**91.1**	**mean:**	**27.4**	**51.3**	**149.5**	**91.2**

One subject (C10) withdrew due to ill health during the first intervention phase. Another subject who was initially randomised to supplement B (C11) withdrew prior to the final study visit due to an unexpected interstate transfer. Hence 15 subjects in each supplement group completed the study.

Acceptability/tolerability of supplements

Subject C10 experienced stomach cramps and constipation whilst taking supplement A which necessitated his withdrawal from the trial. Numerous comments were made about taste and gastrointestinal responses, esp. reflux, particularly during the first intervention phase (see clinic data in appendix). The comments referred predominantly to supplement A (16 versus 7 for B).

Compliance

Patients were issued with 3 containers (*nominally* containing 120 capsules each) for each intervention phase, thus providing 24 spare capsules. With the exception of known causes, capsule returns varied from 8 to 95, suggesting that the number of capsules actually packaged had only been approximated. However, the average return was 34, indicating a high overall level of compliance.

Effects of supplementation on body weight

Mean BMI measurements at visit 1 for Groups A and B were 28.2 ± 0.8 and 27.5 ± 0.7 kg/m^2 respectively. There were no significant changes of body weight with either supplement.

Effects of supplements on blood pressure

Treatment effects on BP were assessed from clinic measurements (average of four Dinamap readings), taken both seated and standing, and from 24 hour ambulatory monitoring, with readings taken every 20 minutes during the day or 40 minutes at night (11pm to 6 am).

Figs. 1-4 show means ± SEM of BP measurements initially (pre) and whilst taking supplement A or B. Sitting clinic BP had fallen approx. 5/3 mmHg from screening values. Standing measurements reflect the postural rise in DBP. Both overestimate the 24 hour average obtained by ambulatory monitoring, due to the anticipated decline of BP whilst sleeping (nocturnal dipping).

Figure 1: SITTING DINAMAP BP

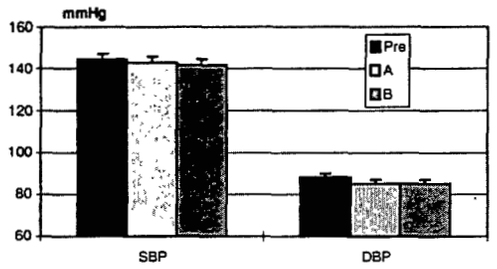


Figure 2: STANDING DINAMAP B.P

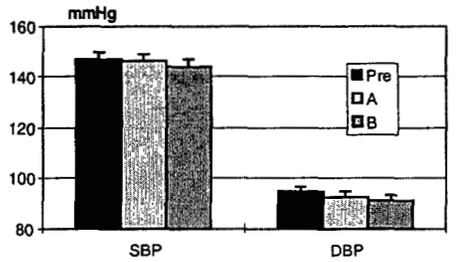


Figure 3: 24hr AMBULATORY BP

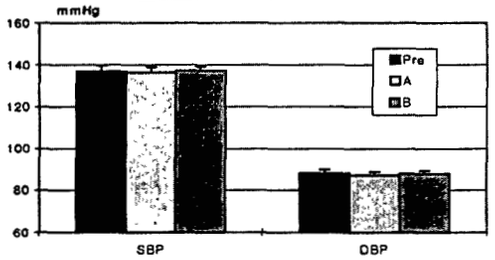
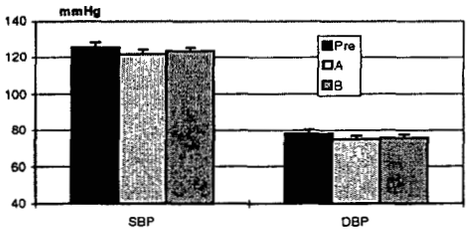


Figure 4: OVERNIGHT AMBULATORY BP



The changes in BP tabulated below were derived by comparing measurements, within individuals, taken on each treatment phase either with each other (crossover) or with the respective pretreatment value.

BP change (mmHg)	**pretreatment → A**	**pretreatment → B**	**A → B (crossover)**
seated SBP	-1.5±1.4	-2.9±1.6	-1.3±1.5
DBP	-3.3±1.3*	-3.2±1.2*	0.1±1.5
standing SBP	-0.8±1.6	-2.9±1.8	-2.1±2.0
DBP	-2.3±1.0*	-3.7±1.2**	-1.4±1.5
24 hr ambulatory SBP	-1.0±1.8	0.1±1.5	0.6±1.5
DBP	-1.4±1.2	-0.4±1.0	0.7±1.0
overnight SBP	-4.1±2.3	-2.6±1.8	0.4±2.1
DBP	-3.4±2.0	-2.5±1.7	0.1±1.5
*p<0.05, **p<0.01			

There were significant reductions in clinic DBP with both supplements. However, these are probably temporal trends, which are commonly observed with repeated clinic measurements, reflecting progressive familiarity/comfort with the procedure. For this reason, comparisons are usually made with a simultaneous control (placebo-treated) group. The 24 hour ambulatory measurements are less susceptible to this artefact. Notably, neither the average 24 hour BP nor the average overnight BP were significantly affected by supplement. The primary outcome measure was the within-individual difference between supplements (crossover). Clearly, there was no difference between effects of supplements A and B on BP.

Effects of supplement on plasma lipids

Total, HDL and LDL cholesterol and total TG were measured in plasma samples taken from each subject initially and after each intervention phase. Means ± SEM of the values before and after each treatment appear in figs. 5- 8.Pretreatment cholesterol was mildly elevated (5.8 mM), with moderately high TG (1.96 mM).

Figure 5: PLASMA CHOLESTEROL (mM)

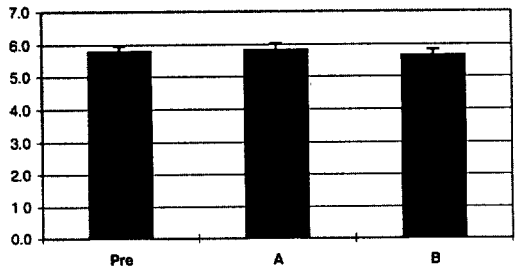


Figure 6: HDL CHOLESTEROL (mM)

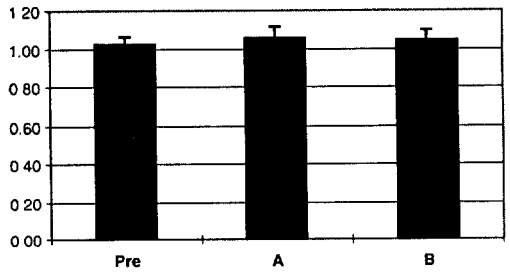


Figure 7: LDL CHOLESTEROL (mM)

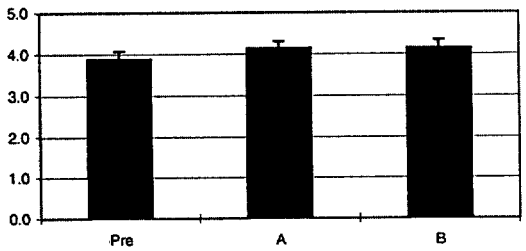
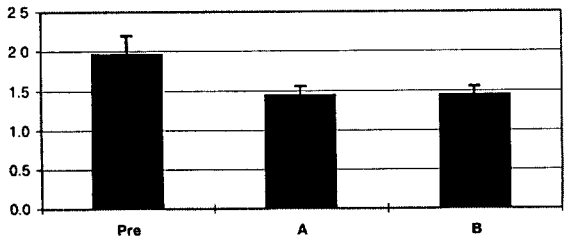


Figure 8: PLASMA TRIGLYCERIDES (mM)



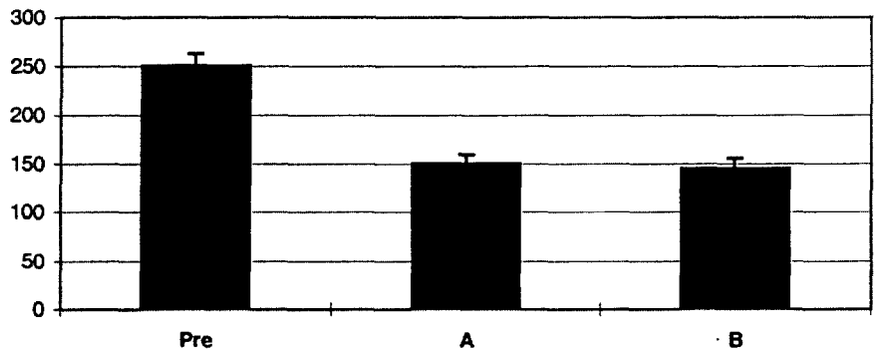
The within-individual changes in lipid concentrations are tabulated below. Both supplements caused a highly significant decrease in TG (-26%) accompanied by a significant elevation of LDL cholesterol (6%), the magnitude being almost identical for each supplement. There were no significant changes in total or HDL cholesterol.

Change in lipids (mM)	pretreatment → A	pretreatment → B	A → B (crossover)
total cholesterol	0.05±0.12	-0.14±0.13	-0.01±0.12
HDL cholesterol	0.03±0.04	0.02±0.04	-0.02±0.04
LDL cholesterol	0.25±0.10*	0.25±0.08**	0.01±0.10
TG	-0.51±0.15**	-0.51±0.17**	0.01±0.06
*p<0.05, **p<0.01			

Effects of supplements on platelet thromboxane production

Maximally stimulated platelet thromboxane production was assessed by enzyme immunoassay of the stable product, thromboxane B_2, in serum obtained from whole blood incubated for 1 hr at 37°C. As shown in Fig. 9, both supplements caused highly significant ($p<0.001$) reductions of thromboxane production: 40% and 42% for A and B respectively. Thus there was no difference in the crossover comparison between supplements A and B.

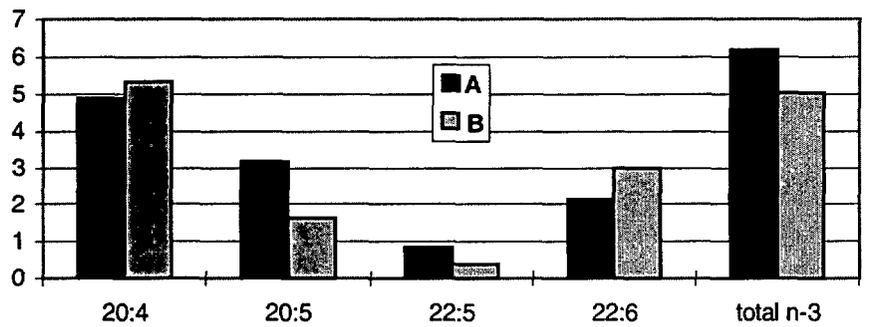
Figure 9: SERUM THROMBOXANE B2 (ng/ml)



Effects of supplements on plasma fatty acid profiles

Fig. 10 shows relative proportions of long chain PUFA present in plasma during treatment with A and B. The values are means for group A and B of plasmas taken at visit 2. The total for ω-3 approximates that of the ω-6 PUFA, arachidonic acid. As expected, the relative proportion of DHA/EPA was much greater with supplement B (1.9±0.1) than with supplement A (0.7±0.04).

Figure 10: PLASMA FATTY ACIDS (% of total)



Identification/composition of supplements

At the conclusion of the trial, supplements A and B were identified as Epanoil (a commercial MaxEPA type of EPA-rich oil) and Nu-Mega tuna oil (the client's DHA-rich oil) respectively. The appendix contains information supplied by the client on the fatty acid, antioxidant and microbiological analyses for these oils, together with fatty acid analyses conducted in our laboratory on randomly sampled capsules. The relative proportion of DHA appears to be lower in our analysis of the capsules. However, the DHA/EPA ratio was essentially reversed between the two oils, i.e. 1/2.1 for supplement A (MaxEPA) and 3.2/1 for supplement B (Nu-Mega tuna oil), at least a sixfold difference.

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DISCUSSION

The purpose of this study was to compare the relative efficacy of the client's new DHA-rich tuna oil product (supplement B) with that of a typical commercial EPA-rich product, with respect to selected indices of clinical cardiovascular and anti-inflammatory benefits.

Recognition of the central role of eicosanoid mechanisms in the anti-inflammatory, anti-thrombotic and possibly antihypertensive effects of fish oil has led to the view that EPA is likely to be the primary mediator of these effects, by competing directly with arachidonic acid, the immediate precursor of eicosanoid synthesis[1-3]. For this reason, commercial interests have tended to focus on sources of EPA-rich fish oil. However, our research on dietary administration of purified fatty acids to experimental animals indicates that DHA may be more efficacious than EPA in reducing cardiovascular risk factors[4]. This is consistent with early observations on the relative cardiovascular benefits of consuming DHA-rich fish[5-7]. The recent introduction of DHA supplements during infant development has raised interest in sources of DHA-rich oil such as tuna. Studies are now being undertaken to evaluate other human health benefits of supplemental DHA in various forms, e.g. fish, fish oil, microalgal oil. However, there have been few reports of direct comparisons between DHA and EPA-rich supplements.

A primary objective of this study was to test the BP lowering potential of the DHA-rich oil. The most sensitive and reliable approach was to use 24 hour ambulatory BP monitoring[8]. This was supplemented with clinic BP measurements, taken both seated and standing. The clinic measurements of DBP were reduced by both fish oil supplements. However, this may simply reflect a commonly observed temporal regression of clinic BP readings. Indeed, the ambulatory measurements, which are less susceptible to this artefact, showed no significant change with supplement, although there was a trend for reduction of night-time BP. Meta-analyses indicate that the effective antihypertensive dose of ω-3 fatty acids is >3g/day[9,10], slightly higher than the dose used (approx 2.5g/day). However, as 3g of ω-3 fatty acids from MaxEPA represents only 1g of DHA, the tuna oil supplement (B), yielding 2g of DHA/day, would have been expected to be efficacious were DHA the principal mediator of the antihypertensive effect of ω-3 fatty acids. Clearly this was not the case. There was no indication of a differential effect between the EPA- and DHA-rich oils. The lack of effect of either oil can be attributed to the marginal dose. This dose may nevertheless be effective when combined with a low salt diet and/or in subjects on selected antihypertensive drug therapy[11].

Plasma lipids, on the other hand, were affected by both supplements. Moreover, their effects were almost identical. Consistent with the established role of ω-3 fatty acids in mildly hyperlipidaemic patients[12], there was a substantial reduction of plasma TG and a small rise in LDL cholesterol with no change in total cholesterol. Average pretreatment plasma lipids were marginally elevated. In two subjects, however, plasma TG exceeded 4 mM; these were markedly reduced by both supplements.

The effects of DHA on plasma TG are controversial[13-15]. In a recent trial, a microalgal oil containing DHA (1.6 g/day) without EPA reduced TG by up to 22%[13] but in another trial, DHA-rich fish oil (1.5-1.8 g of DHA/day) did not affect plasma TG levels[14]. In yet another trial[15], a DHA-rich fish diet was compared with EPA-rich fish oil and the microalgal DHA oil. The maximal reductions of fasting plasma TG were 27%, 26% and 17% respectively. Unlike the other supplements, the DHA oil failed to reduce postprandial TG but the dose of DHA

(1.7 g/day) was quite modest. Nevertheless, the fish diet, which was the most efficacious, provided only 0.4g of EPA and 0.7 g of DHA/day.
The current study appears to be unique in directly contrasting the effects of two fish oil preparations with similar total ω-3 content but markedly different proportions of EPA and DHA. The equivalent efficacy suggests that DHA-rich tuna oil would be a suitable therapeutic option for the treatment of hypertriglyceridaemia. However, there are concerns about possible hyperglycaemic effects of fish oil supplementation in the most prevalent hypertriglyceridaemic condition, non-insulin dependent diabetes[16,17]. It will be important to establish that a minimum effective dose of the DHA-rich tuna oil will not adversely affect glycaemic control, as has been shown for Omacor, an EPA-rich oil now registered in Europe as a hypotriglyceridaemic agent[18].

One of the first properties of fish oil postulated to contribute to prevention of cardiovascular mortality was the inhibition of platelet aggregation[19]. The significance of this antithrombotic effect was offset by concerns about increased risk of bleeding during fish oil supplementation, although concern has gradually declined with the use of moderate doses of fish oil[17]. However, we still need to determine the threshold antithrombotic dose and the relative efficacy of EPA and DHA. EPA competes directly with arachidonic acid as a substrate for thromboxane production, yielding the relatively inactive thromboxane A_3 whilst maintaining prostacyclin levels[1-3]. DHA can also inhibit thromboxane production by displacing arachidonic acid from precursor pools, inhibiting cyclooxygenase and possibly by blocking thromboxane receptors[3].

The equal suppression of thromboxane production in the present study following consumption of equivalent amounts of EPA-rich and DHA-rich fish oil, resulting in a threefold difference in the plasma EPA/DHA ratio, strongly suggests that DHA can also counteract thromboxane production. It also implies that, like the consumption of DHA-rich fish[5,6], the DHA-rich oil may help to reduce the risk of thrombosis. However, the dose of microalgal DHA oil which was found to lower plasma TG had no effect on thromboxane production[13], emphasising the need to determine an adequate dose for each indication.

The effects of the fish oil supplements on cytokine production by monocytes are yet to be analysed. The cytokines being examined, viz. IL1β and TNFα, are mediators of inflammatory responses and their production is known to be inhibited by ω-3 fatty acids[20]. Moreover, there is evidence that DHA may be more effective than EPA in suppressing inflammatory mechanisms[21,22]. The results should give an indication of the relative potential of EPA- and DHA-rich fish oils to suppress inflammation in chronic conditions such as psoriasis and rheumatoid arthritis.

CONCLUSIONS AND RECOMMENDATIONS

The results indicate that neither Nu-Mega tuna oil nor the comparator oil are likely to improve blood pressure at the dose level used in this study. However, the DHA-rich Nu-Mega oil was equally effective as the EPA-rich oil in reducing plasma triglycerides and thrombotic potential, two significant cardiovascular risk factors.

Considering that triglyceride reduction is a registered therapeutic indication for EPA-rich oil, a dose/response relationship and safety profile (with particular reference to management of non-insulin dependent diabetes) should be established for Nu-Mega.

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April 1, 1997

Mr Hamish Drummond
Clover Corporation Pty Ltd
PO Box 1133
MONA VALE, NSW 2103

Dear Hamish,

I have pleasure in enclosing our report on the Nu-Mega Lipids Trial recently conducted in our Nutrition Research Clinic. As you are aware, the cytokine data from Mick James is still forthcoming but I was keen to provide you with our data at this stage.

The main findings are the reduction of triglycerides and inhibition of thromboxane production, in which the DHA-rich tuna oil was equally efficacious as the comparator.

I would be pleased to discuss any aspects of our findings or their interpretation with you.

With best wishes,

Yours sincerely,

Peter R. C. Howe
Senior Principal Research Scientist

NU-MEGA LIPIDS TRIAL

ADDENDUM TO FINAL REPORT

from

CSIRO DIVISION OF HUMAN NUTRITION

to

CLOVER CORPORATION LIMITED

prepared by

Peter R. C. Howe

1st July, 1997

(to be read in conjunction with the report dated 27th March 1997)

Overall Study Objective

To compare, in healthy volunteers, the bioavailability and cardiovascular health attributes of a DHA-rich tuna oil (Nu-Mega) and an EPA-rich oil (MaxEPA), taken as supplementary capsules.

Specific objective

As a secondary outcome measure, to assess effects of the above supplements on production of pro-inflammatory cytokines, viz. interleukin 1b (IL1b) and tumor necrosis factor (TNFa). This objective was undertaken in collaboration with Dr Michael James, Royal Adelaide Hospital.

Rationale

Purported health benefits of ω-3 fatty acids extend from their role in early infant development through to the chronic cardiovascular and inflammatory disorders of middle age[1]. There is increasing evidence that ω-3 supplementation can help alleviate inflammatory disorders such as arthritis, psoriasis, asthma, ulceratice colitis, autoimmune nephropathy and transplant rejection, but it is difficult to quantify the benefit. These disorders are characterised by excessive or inappropriate production of the pro-inflammatory cytokines, IL1b and TNFa, which accordingly provide an index for assessing anti-inflammatory activity[2]. Their production by monocytes in both healthy and affected subjects can be inhibited by ω-3 supplementation[3]. Although the extent of inhibition has been correlated with monocyte EPA content[4], the relative efficacy of EPA and DHA has not been established. This trial offered an opportunity for such a comparison.

Experimental

Monocytes were isolated from fresh 20ml blood samples taken from 15 patients in each of the treatment groups at the 1st (pre-intervention) visit and again after 6 weeks (2nd visit). The IL1b and TNFa produced during lipopolysaccharide stimulation of the monocytes was measured using ELISA kits obtained from Cayman Chemical and Genzyme Duoset respectively. The assays had been delayed to enable validation of these kits.

Results

Figs 1 & 2 show mean values ± SEM for monocyte production of IL1b and TNFa respectively in blood taken at each visit. Supplementation with both MaxEPA and NuMega caused significant reductions of Il1b; the respective within-subject reductions over 6 weeks of supplementation were 4.8 ± 1.5 and 2.6 ± 1.0 ng/ml. Statistical analysis (repeated measures ANOVA; SPSS) indicates that the effects of the two supplements were not significantly different. Thus the overall effect of ω-3 supplementation was to reduce IL1b by 21% ($P<0.001$), an effect which was unrelated to within-individual changes in the ratio of EPA/DHA in plasma (see table). Although there was a strong correlation between IL1b and TNFa production at the pre-intervention visit, there was no consistent effect of ω-3 supplementation on TNFa.

Fig 1

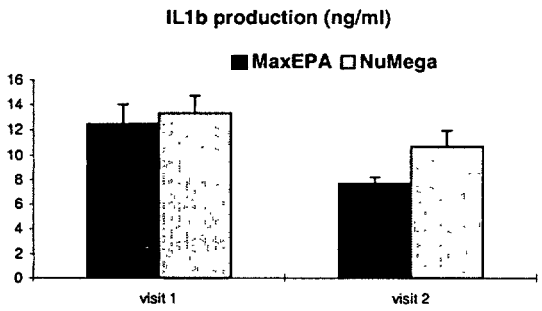
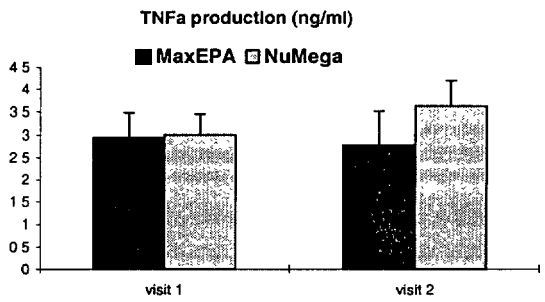


Fig 2



		IL-1b (ng/ml)			TNFa (ng/ml)			plasma f.a. at visit 2	
ID	treatment	visit 1	visit 2	change	visit 1	visit 2	change	total n-3%	DHA/EPA
C01	A-B	7.2	8.7	1.5	2.77	2.03	-0.74	7	0.676
C02	A-B	22.3	7	-15.3	8.83	2.27	-6.56	4.7	0.615
C05	A-B	9.03	7.9	-1.13	1.73	0.77	-0.96	4.7	0.593
C07	A-B	9.07	5.27	-3.8	3.23	1.47	-1.76	4.9	0.630
C09	A-B	7.73	11.13	3.4	1.1	4.07	2.97	7.2	0.465
C13	A-B	12.13	9	-3.13	2.8	3.7	0.9	5.1	0.704
C15	A-B	7.17	5.4	-1.77	1.4	3.23	1.83	4	1.059
C16	A-B	9.5	6.87	-2.63	1.4	1.8	0.4	8.4	0.600
C17	A-B	20	9.83	-10.17	5.33	3.7	-1.63	6.3	0.436
C21	A-B	15.12	8	-7.12	3.87	2.23	-1.64	6.1	0.958
C22	A-B	6.27	3.63	-2.64	1.57	0.43	-1.14	7.6	0.590
C25	A-B	16.1	9.77	-6.33	3.03	0.87	-2.16	6.7	0.788
C26	A-B	11.8	7.77	-4.03	1.17	1.2	0.03	6.3	0.647
C30	A-B	8.37	6.7	-1.67	2.03	1.77	-0.26	6.2	0.714
C32	A-B	25.1	8.3	-16.8	4.1	12.2	8.1	7.3	0.909
	MaxEPA	12.46	7.68	-4.77	2.96	2.78	-0.17	6.17	0.69
		1.54	0.51	1.45	0.53	0.73	0.81	0.32	0.04
		15	15	15	15	15	15	15	15
C03	B-A	14.4	9.9	-4.5	2.37	1.03	-1.34	4.5	1.150
C04	B-A	6.13	6.93	0.8	1.23	3.4	2.17	4.1	1.714
C06	B-A	16.4	13.5	-2.9	4.77	3.53	-1.24	5	2.615
C08	B-A	5.7	3.57	-2.13	0.63	4.13	3.5	3.7	1.833
C12	B-A	18.7	15	-3.7	2.13	1.63	-0.5	5.1	1.474
C14	B-A	17.1	18.9	1.8	2.9	4.13	1.23	4.1	1.714
C18	B-A	14.7	17.7	3	2.4	3.53	1.13	4.3	2.455
C19	B-A	3.8	7.13	3.33	0.97	4.83	3.86	5.4	2.063
C20	B-A	13.6	12.5	-1.1	3.97	7.37	3.4	5.4	1.632
C23	B-A	11.3	4.17	-7.13	3.8	0.87	-2.93	5.7	2.400
C24	B-A	19.4	9.37	-10.03	3.73	2.6	-1.13	5	1.611
C27	B-A	8.33	6.33	-2	1.3	1.9	0.6	4.7	2.000
C28	B-A	14.6	10.6	-4	5.93	6.2	0.27	6.9	1.864
C29	B-A	12.77	9.67	-3.1	2.63	1.4	-1.23	5.5	1.833
C31	B-A	22.5	15.2	-7.3	6.33	7.9	1.57	6.5	1.727
	NuMega	13.30	10.70	-2.60	3.01	3.63	0.62	5.06	1.87
		1.39	1.21	0.99	0.45	0.57	0.53	0.23	0.10
		15	15	15	15	15	15	15	15

Discussion

The reduction of IL1b production by the ω-3 supplements is consistent with their recognised anti- inflammatory effects. However, the results fail to show a predominant effect of either DHA or EPA. The extent of reduction was less than that reported in other studies, even in healthy subjects, where 40-85% reductions of IL1 have been reported[3,4]. Effects on TNF appear to be more variable, with no change seen in 3/6 studies[3,4]. Comparison with other studies indicates that both the dose and duration of treatment were adequate to demonstrate cytokine inhibition.

Our collaborators have obtained evidence that thromboxane A_2 facilitates cytokine production[5]. It is of interest, therefore, that MaxEPA and NuMega treatments reduced platelet thromboxane production by the same extent in this study. While the inhibition of IL1b could not be attributed specifically to either EPA or DHA, these fatty acids may have differential effects on other inflammatory mechanisms. For example, DHA has been shown to counteract cytokine-induced expression of cell adhesion molecules by human endothelial cells, a mechanism with major implications for the prevention of atherosclerosis[6]. Thus, in comparing the anti-inflammatory effects of EPA- and DHA-rich supplements, evaluation of additional indices may be appropriate.

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END SUBMISSION

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MEMO

AM

TO: **Linda Kahl** 00\( ) **202 418 3131**

FROM: **HAMISH DRUMMOND, CLOVERCORPORATION LIMITED**

DATE: **24 January 2002**

RE: **FDA Statement**

Dear Linda

Per your email of 19 January 2002, we advise that "the data and information that are the basis for the GRAS determination are available for FDA review and copying and will be sent upon FDA request" by contacting Mr. Andrew Fairfull. His contact details are below.

Mr. Andrew Fairfull
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Mob: +61 403 601 116
E: andrewf@clovercorp.com.au

Best regards

ee

Hamish Drummond
Chairman