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Application for the Approval of DHA-rich Oil

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

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Version No: Draft
Copy Number:

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VOLUME 1

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VOLUME 2 CONFIDENTIAL

INTRODUCTION

Approval is sought under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients*, for the approval of DHA-rich Oil produced from *Schizochytrium* sp., a marine microalgae, for general approval as a **nutritional ingredient in foods**. This submission has been prepared in accordance with *Commission recommendation of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients.* Article 1 (2.) states "This Regulation shall apply to the placing on the market within the Community of foods and food ingredients which have not hither to been used for human consumption to a significant degree within the Community and which fall under the following categories…(d) foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae". DHA-rich oil from *Schizochytrium* sp. is 'novel' as defined, by virtue of its source organism only. Extensive analysis has shown that individual components of the extracted oil are themselves all present to a significant degree in the human food chain in the Community.

The safety of DHA-rich oil is based on the inherent safety of the fatty acid and sterol components of the oil. The safety of these components is based on their presence in food, the small quantities expected to be consumed, extensive knowledge of their metabolism, published safety studies, and the absence of reports of toxicity. The safety is further supported by published studies on a microalgal oil of similar composition, by the historical safe use of fish oils of similar composition and corroborated by a battery of toxicity studies.

An independent panel of experts in the US has concluded that DHA-rich oil from *Schizochytrium* sp., microalgae can be "Generally Regarded as Safe" (GRAS) as a nutritional food ingredient up to a daily intake of 1.5g of DHA (the DHA-rich oil contains 35-45% DHA).

Schizochytrium sp., microalgae itself has previously been "GRAS'd" for use in chicken feed (up to 2.8% for broilers and 4.7% in layers) and has been used in aquaculture for a number of years. The production strain of microalgae has been developed by conventional improvement techniques, no Genetic Modification has been used.

With regard to the statements in paragraph 1 above, under the *Commission recommendation of 29 July 1997,* Section 4. The "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", DHA-rich Oil would be classified as **Class 2.2**, **"Complex Novel Food from non-GM Source", "the source of the NF has no history of use in the Community"**. The requirements for this submission for this class are as follows:

I Specification of the Novel Food.

II Effect of the production process applied to the Novel Food.

III History of the organism used as the source of the Novel Food.

IV Effect of the Genetic Modification on the Properties of the Host Organism. **NOT *APPLICABLE TO DHA-rich oil***

V Genetic Stability of the GMO Used as Novel Food Source.
NOT APPLICABLE TO DHA-rich oil

VI Specificity of Expression of Novel Genetic Material.
NOT APPLICABLE TO DHA-rich oil

VII Transfer of Genetic Material From GMO.
NOT APPLICABLE TO DHA-rich oil

VIII Ability of the GMM to Survive in and Colonise the Human Gut.
NOT APPLICABLE TO DHA-rich oil

IX Anticipated intake/extent of use of the Novel Food.

X Information from previous human exposure to the Novel Food or its source.
Note that in the above mentioned SCF Guidelines this section is omitted, assuming that the source has not been consumed. However in this case ALL components analysed in the DHA-rich Oil are present in the diet, so it is relevant to include this section.

XI Nutritional Information on the Novel Food.

XII Microbiological Information on the Novel Food.

XIII Toxicological Information on the Novel Food.

I. Specification of the Novel Food.

According to the SCF guidelines the following questions must be asked at this stage:

- "Is appropriate analytical information available on the potential toxic inherent constituents, external contaminants and nutrients?"

- "Is the information representative of the Novel Food when produced on a commercial scale?"

- "Is there an appropriate specification (including species, taxonomy etc. for living organisms) to ensure that the Novel Food marketed is the same as that evaluated?"

The answers to these questions are outlined in this Section below:

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I. Description of Substance

A. Common or Usual Name

DHA-rich Oil. DHA-rich oil is derived from the heterotrophically grown marine microalgae, *Schizochytrium* sp. The proposed trade name for DHA-rich Oil Derived from Dried Microalgae is DHA Gold™. Other synonymous names for this product include DHA oil or DHA-rich microalgal oil, when differentiating algal oil from fish oil.

- DHALIPNS (refers to the internal product code for commercial article)
- docosahexaenoic acid (DHA)
- biomass refers to dried, whole-cell microalgae

B. Chemical Abstract Service (CAS) Registry Number

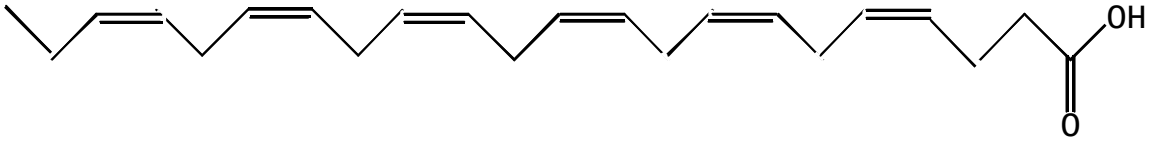
The CAS Number for fatty acids containing 14-22 carbons (C14-C22), and 16-22 carbons (C16-C22) esterified to glycerol is **68424-59-9** (described in the CAS registry as "glycerides", C14-C22 and C16-C22).

C. Empirical Formula

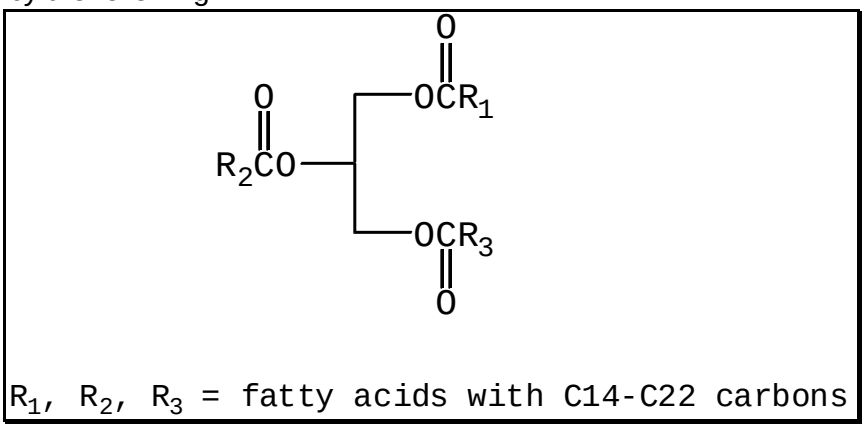
Docosahexaenoic acid (DHA) is a long chain, polyunsaturated fatty acid, with empirical formula $C_{22}H_{32}O_2$. The complete name is 4,7,10,13,16,19-docosahexaenoic acid. The short-hand nomenclature used in this review is 22:6n-3. The numbers indicate the number of carbon atoms in the molecule (22), the number of double bonds (6) and the number of carbon atoms from the methyl terminus to the first double bond (3).

D. Structural Formula

The structural formula for docosahexaenoic acid (DHA), is represented by the following:



The structural formula for triglycerides, described by CAS number 68424-59-9, is represented by the following:



E. Specifications and Methods

1. Quality Control Specifications and Methods

DHA-rich oil is described as a yellow to light orange-colored oil derived from the heterotrophically grown marine microalgae, *Schizochytrium* sp., intended for use as a nutritional food ingredient. The oil is winterized, refined, bleached and deodorized. Vitamin E is added for nutritional supplementation. Antioxidants and stabilisers are added in accordance with Directive 95/2/EC. Specifications for the nutritional food ingredient are given in Table I-1.

Five lots of DHA-rich oil were produced in a campaign designed to demonstrate a reproducible and representative process capable of meeting proposed product specifications outlined in Table I-1. Results of quality control testing on the five demonstration lots of DHA-rich oil (referred to as DHALIPNS lots 97398, 97399, 97400, 97401, and 97402) are shown in Table I-2.

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Table I-1. Product specifications for DHA-rich oil derived from dried microalgae

PHYSICAL AND CHEMICAL TESTS		
Test	**Specification**	**Test Method**
Color (Lovibond)	Passes test	AOCS Method Cc 13b-45
Acid Value	Not more than 0.5 mg KOH/g	AOCS Method Cd 3d-63
Peroxide Value (PV)	Not more 5.0 meq/kg oil	AOCS Method Cd 8-53
Moisture and Volatiles	Not more 0.05%	AOCS Method Ca 2d-25
Unsaponifiables	Not more than 4.5%	AOCS Method Ca 6b-53
Trans-fatty acids	Not more than 2%	AOCS Method Cd 14-61
DHA content	Not less than 32.0%	POS AS.SOP-104
Hexane	Not more than 10 mg/kg	AOCS Method Ca 3b-87
ELEMENTAL ANALYSIS		
Test	**Specification**	**Test Method**
Arsenic	Not more than 0.20 mg/kg	POS AS.SOP-103
Copper	Not more than 0.05 mg/kg	POS AS.SOP-103
Iron	Not more than 0.20 mg/kg	POS AS.SOP-103
Mercury	Not more than 0.20 mg/kg	POS AS.SOP-103
Lead	Not more than 0.20 mg/kg	POS AS.SOP-103

KOH : potassium hydroxide
Meq: milliequivalents

Table I-2. Quality control test results on DHA-rich oil derived from dried microalgae

	DHALIPNS Lot Numbers				
	97398	***97399***	***97400***	***97401***	***97402***
Color (Lovibond)	70.0Y 6.3R	70.0Y 2.6R	70.0Y 8.1R	70.0Y 2.5R	70.0Y 3.5R
Acid Value (mg KOH/g)	0.3	0.1	0.1	0.1	0.1
Peroxide Value (meq/kg)	2.8	3.0	2.1	1.9	2.9
Moisture and Volatiles (%)	0.00	0.00	0.00	0.00	0.00
Unsaponifiables (%)	2.8	2.1	4.1	2.1	4.2
Trans-fatty Acids (%)	1.7	1.8	1.2	1.0	1.1
DHA Content (% FAME)	35.5	39.3	34.2	39.2	34.7
Hexane (mg/kg)	<3	<3	<3	<3	13
Arsenic (mg/kg)	<0.20	<0.20	<0.20	<0.20	<0.20
Copper (mg/kg)	<0.05	<0.05	<0.05	<0.05	<0.05
Iron (mg/kg)	0.12	<0.02	<0.02	<0.02	<0.02
Mercury (mg/kg)	<0.20	<0.20	<0.20	<0.20	<0.20
Lead (mg/kg)	<0.20	<0.20	<0.20	<0.20	<0.20

FAME: Fatty Acid Methyl Ester

The results of this demonstration campaign indicate that the process is both reproducible and capable of making product that meets product specifications.

F. Chemical Composition

Five lots of DHA-rich oil, produced under the product code DHALIPNS (lots 97398, 97399, 97400, 97401, and 97402), were analysed for fatty acid, unsaponifiable and sterol composition, proximate analysis, metals by ICP, heavy metals and physical properties. Fatty acids were determined following analytical procedure SD-AN-014-V0 “Fatty Acid Composition Analysis of DHA-rich Oil by Gas-Liquid Chromatography - SOP. Unsaponifiable content in DHA-rich oil was determined using analytical method SD-AN-016-V0 “Unsaponifiable Matter in DHA-rich Oil - SOP”. The unsaponifiable fraction was further analyzed by gas-liquid chromatography using mass selective detection as per SD-AN-017-V0 “Sterol Components in Unsaponifiable Fraction by Gas-Liquid Chromatography-Mass Spectrometry - SOP”. Proximate analyses, fat, protein, ash, moisture, and carbohydrate were performed by Covance Laboratories (Madison, Wisconsin) following Covance test procedures, FANW (MP-FANW-MA), PGEN (MP-PGEN-MA), ASHM (MP-ASHM-MA), M100 (MP-M100-MA), and CHO (MP-CHO-MA), respectively. Inorganic determinations based on ICP Scan and Atomic Absorption were performed by Covance procedures ICPL (MP-ICPL-MA), ASA (MP-ASA-MA), PBHL (MP-PBHL-MA), CDA (MP-CDA-MA), and HGAS (MP-HGAS-MA). Color, odor, freezing point determination, specific gravity and flash point were determined as part of physical chemical assessment.

1. Fatty Acids

Integration of peaks and quantitation based on six-point calibration curves constructed from individual FAME reference standards gave results, reported as mg FAME per gram oil, as shown in Table I-3.

All five lots of DHA-rich oil (DHALIPNS) products are shown to contain the following FA greater than 4 mg/g oil: laurate, myristate, palmitate, palmitoleate, stearate, dihomo-gamma-linolenate (20:3n-6) + methyl eicosatetraenoate (20:4n-7), arachidonate (20:4n-6), eicosatetraenoate (20:4n-3), eicosapentaenoate (20:5n-3), docosatetraenoate (22:4n-9), docosapentaenoate (22:5n-6), and docosahexaenoate (22:6n-3).

As is evident in Table I-3, acceptable reproducibility is shown in the fatty acid composition data for the major fatty acid components in DHA-rich oil lots with CV's (std. dev. ÷ average x 100) for myristate, palmitate, DPA (n-6) and DHA of 8.5, 4.0, 11.1, and 7.0%.

Table I-3. Fatty acid profile of DHA-rich oil lots

FA NAME	DHA-rich oil (mg FAME/g oil) DHALIPNS lot numbers 97398	97399	97400	97401	97402	Average[8]	Std. Dev.
Laurate	4.0	4.0	4.0	4.0	4.1	4.0	<0.1
Myristate	112.1	105.4	92.8	103.1	91.9	101.1	8.6
Tetradecatrienoate[1]	Tr	tr	4.5	tr	4.1	tr-4.5	range
Palmitate	232.7	227.2	242.3	231.3	250.3	236.8	9.4
Palmitoleate	32.4	9.8	21.9	8.1	16.0	17.6	9.9
Hexadecatrienoate[2]	Tr	tr	5.0	tr	4.1	tr-5.0	range
Stearate	4.5	4.0	5.0	4.0	5.0	4.5	0.5
Vaccenate	13.6	tr	9.7	tr	7.5	tr-13.6	range
Octadecatetraenoate[3]	4.0	4.0	8.5	tr	7.5	tr-8.5	range
Dihomo-gamma-linolenate & Eicosatetraenoate (n-7)[4]	20.1	20.0	25.3	21.2	24.0	22.1	2.4
Arachidonate	8.8	9.0	10.9	7.1	11.1	9.4	1.7
Eicosatetraenoate (n-3)[5]	8.0	9.0	9.0	8.8	8.8	8.7	0.4
EPA	23.1	23.8	33.2	18.6	32.6	26.3	6.4
Docosatraenoatenoate(n-9)[6]	4.8	4.0	7.2	4.8	6.3	5.4	1.3
DPA (n-6)[7]	135.4	150.0	117.6	149.4	122.4	135.0	15.0
DHA	344.3	372.0	323.9	379.1	330.6	350.0	24.6
Total FA (mg/g oil) =	947.8	942.2	924.8	939.5	926.3	936.1	10.1

1 Tetradecatrienoate assigned as 5,8,11-tetradecatrienoate based on GC-MS of DMOX derivative and quantified using myristate response factor

2 Hexadecatrienoate assigned as 4,7,10-hexadecatrienoate based on GC-MS of DMOX derivative and quantified using palmitoleate response factor

3 Octadecatetraenoate assigned as 6,9,12,15-octadecatetraenoate based on GC-MS of DMOX derivative and quantified using linoleate response factor

4 Dihomo-gamma-linolenate, 20:3 n-6, and eicosatetraenoate, 20:4 n-7,were found to co-elute, eicosatetraenoate, 20:4 n-7 assignment based on GC-MS of DMOX derivative and quantified using arachidonate response factor

5 Eicosatetraenoate assigned as eicosatetraenoate, 20:4 n-3, based on GC-MS of DMOX derivative and quantified using arachidonate response factor

6 Docosatetraenoate assigned as 4,7,10,13-docosatetraenoate based on GC-MS of DMOX derivative and quantified using DHA response factor

7 DPA assigned as 4,7,10,13,16-docosapentaenoate based on GC-MS as DMOX derivative and quantified using DHA response factor

8 Average of all values reported except when a lot contained a FAME below the lowest calibration curve concentration, in which cases, ranges are reported

tr = present but below the lowest calibration curve concentration (4 mg/g oil) and therefore not quantified

Note:Original assignment of homogammalinolenate corrected to homogammalinolenate and eicosatetraenoate n-7.

Original assignment of eicosatatraenoate n-7 corrected to eicosatatraenoate n-3.

2. Sterols

Table I-4. Unsaponifiable content in DHA-rich oil lots

DHALIPNS Lot Numbers	% Unsaponifiable
97398	2.8
97399	2.1
97400	4.1
97401	2.1
97402	4.2
	3.1 ± 1.0[1]

[1] reported as average value ± std. dev.

%Unsaponifiable reported on weight basis

Integration of all peaks using total ion current peak area in the MS detector are shown in Table I-5. Average results (± standard deviation) for the five lots of DHA-rich oil analysed document cholesterol, brassicasterol, stigmasterol, and stigmasta-5,23-dien-3-ol content in oil of 25 ± 3%, 15 ± 3%, 19 ± 2%, and 8 ± 1% (reported as % total peak area), respectively.

Table I-5. Sterol profile of DHA-rich oil lots

	% Peak Area[1] DHALIPNS Lot Numbers						
STEROL NAME	**97398**	**97399**	**97400**	**97401**	**97402**	**Average**	**Std. Dev.**
Cholesta-5-en-3-ol (Cholesterol)	29	25	26	23	20	25	3
Ergosta-5,22-dien-3-ol (Brassicasterol)	13	12	20	12	16	15	3
Ergosta-7,22-dien-3-ol	<5[2]	<5	7	<5	<5	<5-7	range
Ergosta-7,24-dien-3-ol	5	<5	6	<5	6	<5-6	range
Stigmasta-5,22-dien-3-ol (Stigmasterol)	22	20	18	19	16	19	2
Stigmasta-5,23-dien-3-ol	8	8	7	8	7	8	1

1 *All peaks greater than 5% total peak area are reported individually and as the average ± std. dev. of n=5, except in cases when a lot contained a sterol at <5% of total peak area, in which cases, ranges are reported*

2 *Peak present but less than 5% total peak area*

3. Proximate Analysis

Moisture, ash, protein, and fat were measured using standard methods and carbohydrate content was estimated by difference (Table I-6).

Table I-6. Proximate analysis of DHA-rich oil lots

	DHALIPNS Lot Numbers				
	97398	***97399***	***97400***	***97401***	***97402***
Moisture (%)	<0.1	<0.1	<0.1	<0.1	<0.1
Ash (%)	<0.1	<0.1	<0.1	<0.1	<0.1
Protein (%)	<0.1	<0.1	<0.1	<0.1	<0.1
Fat (%)	100.4	100.8	100.5	100.6	100.3
Carbohydrate (%)	<0.1	<0.1	<0.1	<0.1	<0.1

Results, shown in Table I-6, indicate >99.9% fat content in all samples analyzed. There is no carbohydrate or protein present in the DHA-rich oil.

4. Elemental Analysis

The five lots of DHA-rich oil were subjected to elemental analysis. All elements tested were below the sensitivity of the methods (see Table I-7).

Table I-7. ICP scan determinations in DHA-rich oil lots

	Concentration (ppm) DHALIPNS Lot Numbers				
Analyte	***97398***	***97399***	***97400***	***97401***	***97402***
Calcium	<5.00	<5.00	<5.00	<5.00	<5.00
Copper	<0.13	<0.13	<0.13	<0.13	<0.13
Iron	<0.50	<0.50	<0.50	<0.50	<0.50
Magnesium	<5.00	<5.00	<5.00	<5.00	<5.00
Manganese	<0.08	<0.08	<0.08	<0.08	<0.08
Phosphorous	<5.00	<5.00	<5.00	<5.00	<5.00
Potassium	<25.0	<25.0	<25.0	<25.0	<25.0
Sodium	<25.0	<25.0	<25.0	<25.0	<25.0
Zinc	<0.10	<0.10	<0.10	<0.10	<0.10

Additional tests were performed to determine the presence of heavy metals. Results of analysis for the heavy metals, arsenic, lead, cadmium and mercury are shown in Table I-8.

Table I-8. Heavy metal determination in DHA-rich oil lots

	Concentration (ppm) DHALIPNS Lot Numbers				
Analyte	***97398***	***97399***	***97400***	***97401***	***97402***
Arsenic	<0.5	<0.5	<0.5	<0.5	<0.5
Lead	<0.05	<0.05	<0.05	<0.05	<0.05
Cadmium	<0.04	<0.04	<0.04	<0.04	<0.04
Mercury	<0.025	<0.025	<0.025	<0.025	<0.025

Heavy metals in the DHA-rich oil obtained from the process demonstration campaign were shown to be below the sensitivity of the methods.

5. Physical properties

Results of the physical properties tested on the five lots of DHA-rich oil, color, odor, freezing point, flash point and specific gravity, are shown in Table I-9.

Table I-9. Physical properties of DHA-rich oil lots (DHALIPNS)

	DHALIPNS Lot Numbers				
	97398	***97399***	***97400***	***97401***	***97402***
Color[1]	5Y (8/12)	7.5Y (8.5/10)	1.25Y (8/14)	7.5Y (8/8)	5Y (8/8)
Odor[2]	50	10	25	40	10
Freezing Point (°C)	2.0	1.5	1.0	0.5	0.5
Flash Point (°C)	162	168	167	174	215
Specific Gravity	0.9377	0.9381	0.9374	0.9379	0.9377

[1] Color results are in the format of H(V/C) where: H specifies the Munsell hue; V specifies the Munsell value; and C specifies the Munsell chroma

[2] Odor intensity is based upon a scale of 1 to 100 with 100 being most pungent

II. Effect of the production process applied to the Novel Food.

According to the SCF guidelines the following questions must be asked at this stage:

- "Does the Novel Food undergo a production process?"
- "Is there a history of use of the production process for the food?"
- "Does the process result in a significant change in the composition or structure of the Novel Food compared to its traditional counterpart?"
- "Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from the use of the process?"
- "Are the means identified for controlling the process to ensure that the Novel Food complies with its specification?"
- "Has the process the potential to alter the levels of Substances with an adverse effect on public health, in the Novel Food?"
- "After processing is the Novel Food likely to contain micro-organisms of adverse public health significance?"

The answers to these questions are outlined in this Section below:

II. Manufacturing Process Description

A. Overview of Process

DHA-rich oil is produced *via* an algal fermentation process using a microalgae from the genus *Schizochytrium.* The algae are grown *via* a pure culture heterotrophic fed-batch fermentation process. The organism used is an improved strain of the original wild-type culture, *Schizochytrium* sp. ATCC 20888. The improved strain was derived using a classical mutagenesis/screening program, which employed well-accepted techniques commonly used in industrial microbial strain improvement programs. The intermediate dried microalgae product is fermented, recovered and dried. The resulting dried microalgae cells are extracted to produce a crude oil product, which is further refined into the finished product using process operations commonly employed in the vegetable oil industry.

DHA-rich oil is manufactured under the general guidelines of food chemical Good Manufacturing Practices (Food Chemical Codex pp xxvii, 4th edition). The incorporation or growth of typical food borne microbes is inhibited by a combination of a heat treatment applied to the cultured microalgal cells, the environmental conditions of the oil extraction and processing, and the extremely low water activity of the finished oil product.

1. Fermentation

Frozen cultures are used to inoculate a shake flask, which is then used to inoculate a seed tank. The seed stage is allowed to grow for approximately 1 day prior to being used to inoculate the final fermentor. The microalgal cells are aseptically cultured in a medium consisting of a carbon source, nitrogen source, bulk nutrients, trace minerals and vitamins. Critical operating parameters such as temperature, pH and aeration are carefully controlled to ensure reproducible process performance and final product composition.

2. Intermediate Product (Dried Microalgae) Recovery

The algae cells are separated from the broth and dried using a double drum dryer.
The intermediate dried microalgal biomass is tested to ensure that it meets certain compositional criteria prior to release for final processing.

3. Oil Recovery

The dried microalgae are suspended in commercial-grade n-hexane and wet milled to extract the oil from the microalgal biomass. Filtration is employed to separate the

spent biomass from the oil-rich miscella. The resultant mixture of oil and solvent, referred to as miscella, is filtered to remove any cellular debris. The miscella is chilled and held for a period of time, to crystallize any saturated fats, or high melting point components (winterised). The miscella is then filtered to remove the crystallized stearine phase. Hexane is removed from the miscella, leaving behind the winterised oil.

The winterised oil is processed in a manner very similar to established processes edible vegetable oil manufacture. The oil is first refined to remove water-soluble gums, then bleached to remove any peroxides or residual gums, and deodorized to remove any residual undesired volatile components.

Safe and suitable antioxidants are added in accordance with EU Directive 95/2/EC. The stabilised oil is packaged in a clear, phenolic-lined metal container under a nitrogen atmosphere to prevent oxidation. The finished product is analyzed to ensure it meets all product specifications prior to release for sale.
for sale.

III History of the organism used as the source of the Novel Food.

According to the SCF guidelines the following questions must be asked at this stage:

- "Is the Novel Food obtained from a biological source. i.e. a plant, animal or microorganism?"
- "Has the organism used as the source of the Novel Food been derived using GM?
- "Is the source organism categorised?"
- "Is there evidence to show that the source organism and/or the foods obtained from it are not detrimental to human health?"

The answers to these questions are outlined in this Section below:

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III. History of the Source Organism

A. Taxonomic Review

1. Taxonomic Classification of *Schizochytrium* sp.: The Organism

DHA-rich oil is extracted from dried algae produced via fermentation using *Schizochytrium* sp., a member of the kingdom Chromista (also called Stramenopilia) which includes the golden algae, diatoms yellow-green algae, haptophyte and cryptophyte algae, oomycetes and thraustochytrids. *Schizochytrium* sp. is a thraustochytrid. The current taxonomic placement of the thraustochytrids, and *Schizochytrium* sp. specifically, is summarized below:

Kingdom:	Chromista (Stramenopilia)
Phylum:	Heterokonta
Class:	Thraustochytridae
Order:	Thraustochytriales
Family:	Thraustochytriaceae
Genus:	Schizochytrium
Species:	ATCC 20888

Thraustochytrids are microalgae or microalgae-like microorganisms. The earliest research of thraustochytrids placed them in the fungi because of their heterotrophic nature and superficial resemblance to chytrids (Sparrow, 1936). Current analyses using molecular biology techniques have demonstrated that thraustochytrids are not fungi, and they are related to the heterokont algae (Cavalier-Smith, 1994). There is still controversy on whether thraustochytrids are Heterokonta that have lost their chloroplasts, or as some of the most recent analyses suggest, the thraustochytrids may be the earliest member of the Heterokonta representing the form prior to acquisition of chloroplasts (Leipe et al., 1994).

Dinoflagellate species of microalgae produce the most commonly known microalgal toxins. These toxins cause paralytic shellfish poisoning and diarrhetic shellfish poisoning. They are produced in the dinoflagellates, accumulated in filter-feeding shellfish which feed on the algae, and then passed on to human or other invertebrate consumers. Note that the *Schizochytrium* sp. organism is not related to Dinophyta (dinoflagellates) which are in a a completely separate kingdom.

2. Microalgae as food: Occurrence of *Schizochytrium* sp. in the Human Food Chain

Thraustochytrids are found throughout the world in estuarine and marine habitats. Their nutritional mode is primarily saprotrophic (obtain food by absorbing dissolved organic matter) and as such are generally found associated with organic detritus (Findlay et al., 1980; Raghukumar & Balasubramanian, 1991), decomposing algal and plant material (Bremer, 1995; Sathe-Pathak et al., 1993) and in sediments (Bahnweg & Sparrow, 1974). Because they are very lightly pigmented, thraustochytrids are generally under-reported in phytoplankton samples. More recent analyses using an epifluorescence microscopic technique, developed specifically to detect thraustochytrids, indicate that thraustochytrids can comprise a significant portion of the phytoplankton community (e.g., 5.4×10^6 cells per gram dry weight phytoplankton, Raghukumar & Schaumann, 1993). Thraustochytrids have also been reported to compose up to 30% of the microbial community on detritus derived from brown algae (Sathe-Pathak et al., 1993).

Thraustochytrids can utilize a wide range of dissolved organic carbon and nitrogen compounds for growth (Bahnweg, 1979a, b). Most studies to date have focused on their distribution,

taxonomy, ultrastructure and physiology. Alderman (1982) has noted that *Schizochytrium* sp. is frequently found associated with marine animals but without any specific evidence of pathogenicity.

There are no reports in the literature of human consumption of thraustochytrids or of *Schizochytrium* sp. in particular. This in due to the fact that prior to the late 1980's, *Schizochytrium* sp. had never been cultured on a scale larger than a shake flask. Barclay (1992) and Bajpai et al. (1991a, b) were the first to successfully cultivate *Schizochytrium* sp. and *Thraustochytrium* sp., respectively, in fermentors.

Microalgae and other microscopic organisms are primarily consumed by filter feeding invertebrates in the marine ecosystem. Filter feeding organisms accumulate the particulate material they filter from water in the foregut before passing the material into their digestive system. By examining foregut contents, one can determine what the organism is feeding on. Barclay (unpublished data) has examined the foregut contents (both microscopically and on streak plates) of a variety of filter feeding invertebrates in the southern California coastal region. The data demonstrate that thraustochytrids are consumed by a wide variety of filter feeding organisms including mussels that are consumed directly by humans. Examples of thraustochytrids in the human food chain (as documented from foregut samples of filter feeding invertebrates in the San Diego Bay area) are illustrated in Figure III-1. Published data were used to determine some of the types of fish that regularly feed on the filter feeding invertebrates.

In summary, the literature indicates that thraustochytrids, especially those of the genus *Schizochytrium*, have a widespread distribution, and that they are regularly consumed as food by a wide range of invertebrates. Field tests by OmegaTech confirm the widespread occurrence of the thraustochytrids in a typical marine food chain, including the potential for direct consumption by man (when consuming mussels and most likely clams).

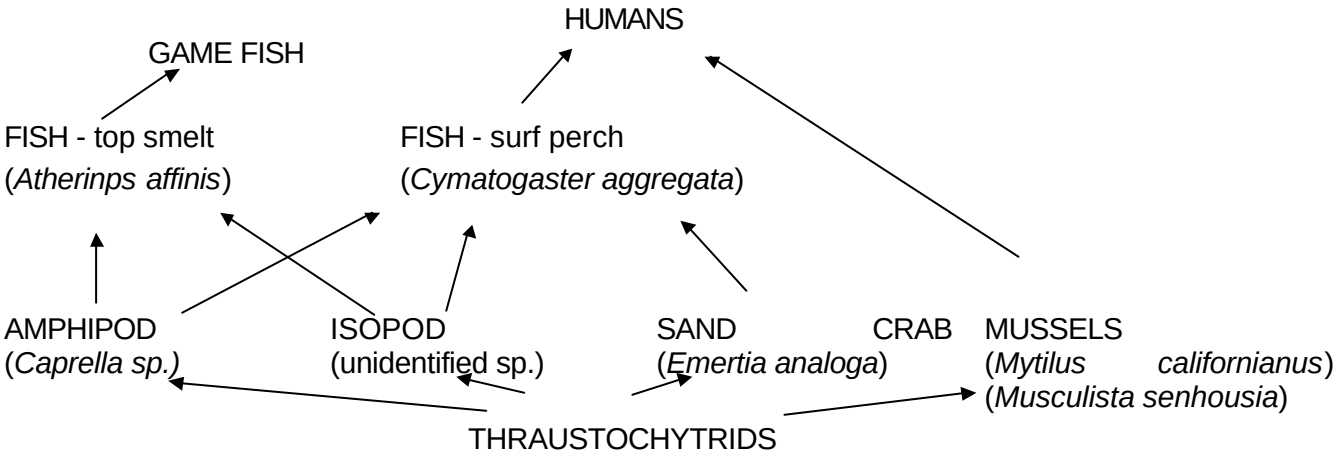

Figure III-1. Role of thraustochytrids in the marine food chain in the San Diego Bay region

3. Relationship of Thraustochytrids to Known Toxic Species of Microalgae

It has long been known that some species of marine and freshwater microalgae produce toxic substances. The occurrence of these toxins in microalgae has been reviewed extensively (Carmichael, 1981; Graneli et al., 1990; Tu, 1988). All of the species known to produce toxins are found in just six of the approximate 76 known orders of microalgae and algae-like microorganisms (Table III-1 and Table III-2). The specific species of microalgae that are known to produce toxins are summarized in Table III-1 along with the types of toxins they produce.

As can be seen, the majority of toxins produced in microalgae occur in the species of dinoflagellates (kingdom Protozoa, phylum Dinophyta) and blue-green algae (kingdom Eubacteria, phylum Cyanobacteria).

The most commonly known microalgal toxins are the toxins produced by the dinoflagellate species of microalgae. These toxins cause paralytic shellfish poisoning and diarrhetic shellfish poisoning. They are produced in the dinoflagellates, accumulated in filter-feeding shellfish which feed on the algae, and then passed on to human or other invertebrate consumers. Dinoflagellate toxins are heat stable and cause paralysis by blocking sodium channels in nerves and muscles. They are water soluble compounds, slightly soluble in methanol and ethanol, but insoluble in lipid solvents.

Toxic cyanobacteria (bluegreen algae) can produce neurotoxic, hepatoxic, and dermatotoxic compounds. These toxic compounds are highly polar, dissolve readily in water, and as such, pose a direct threat to human and animal water supplies where blooms of these algae occur. Acute lethal toxicity can occur from ingestion of toxic cells or water containing toxins from certain freshwater/brackish water species of *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nodularia* and *Oscillatoria*. These lethal toxins consist of a family of hepatotoxic cyclic hepta- and penta-peptides called microcystins or cyanoginosins. The compounds contain D- and L-amino acids plus two novel amino acids. Strains of *Anabaena* and *Aphanizomenon* also produce neurotoxins called anatoxin and saxotoxin, respectively. Additionally, forms of dermatitis (e.g., swimmers itch) can be caused by skin contact with marine species of *Lyngbya*, *Oscillatoria* and *Schizothrix*.

Table III-1. List of the major divisions[1] containing microalgae and microalgae-like organisms. As noted, toxin producing species of microalgae are reported from only six (6) of the approximate 76 orders within these divisions

KINGDOM	**Phylum**	**Class**	Order
EUBACTERIA	**Cyanobacteria** (bluegreen algae)		
	5 orders, all toxin producing species in one order Nostocales		
PLANTAE	**Chlorophyta** (green algae)		
	33 orders, no toxin producing species		
	Rhodophyta (red algae)		
	4 orders, no toxin producing species		
PROTOZOA	**Euglenophyta**		
	2 orders, no toxin producing species		
	Dinophyta (dinoflagellates)		
	9 orders, all toxin producing species in three orders: Peridiniales, Gymnodiniales, Prorocentrales		
CHROMISTA (Stramenopilia)	**Heterokonta**		
		Bacillariophycea (diatoms)	
		2 orders, all toxin producing species in one order: Pennales	
		Chrysophycea (golden algae)	
		4 orders, no toxin producing species	
		Eustigmatophycea	
		1 order, no toxin producing species	
		Oomycetidae (oomycetes)	
		4 orders, no toxin producing species	
		Thraustochytridae (thraustochytrids)	
		1 order, no toxin producing species	
		Xanthophycea (yellow-green algae)	
		6 orders, no toxin producing species	
	Cryptophyta (cryptomonads)		
	1 order, no toxin producing species		
	Prymnesiophyta (haptophytes)		
	4 orders, all toxin producing species in one order: Prymnesiales		

[1] Based on taxonomic classification of Cavalier-Smith (1981, 1993, 1994) and the molecular genetic analyses of Van de Peer et al., 1996.

Table III-2. List of the species of microalgae that are known to produce toxins

Organism	Toxin	Chemical Form	Human Effect
Dinoflagellates (Dinophyta)			
Gambierdiscus toxicus	*ciguatoxin*	*polyether compound*	*diarrhetic*
Prorocentrum spp. *(concavum*, *lima, mexicanum)*	*maitotoxin, scaritoxin*		*diarrhetic*
Amphidinium spp.	*okadaic acid*	*polyether compound*	*diarrhetic*
Ostreopsis spp. *(siamensis, lenticularis, ovata)*	" "		
Gonyaulax spp. *(excavata, catenella, tamarensis, acatenella)*	*saxitoxins, gonyautoxins*		*neurotoxic*
Protogonyaulax spp. *(catenella, bahamense)*	" "		"
Pyrodinium bahamense	" "		"
Cochlodinium sp.	" "		"
Gymnodinium catenatum	"	"	"
Prorocentrum spp. (*lima, minimum*)	yessotoxin	polyether	diarrhetic
Ptychodiscus brevis	brevetoxin	polycyclic polyethers	neurotoxin
Bluegreen algae (Cyanobacteria)			
Aphanizomenon flos*-aquae*		*saxitoxins, gonyantoxins*	
Anabena spp. *(*flos-aquae, circinalis, planctonica*)*	*anatoxins, microcystins*	*cyclic heptapeptide*	*hepatotoxin*
Microcystis spp. *(*aeruginosa*,* virdis*)*	*microcystin*	*cyclic heptapeptide*	
Nodularia spumigena	microcystin	cyclic pentapeptide	
Oscillatoria agardhii	*oscillatoria toxin*	*peptide*	
Oscillatoria nigrovirdis	*oscillatoxin*	*phenolic bislactone*	
Lyngbya majuscula	*lyngbyatoxin*	*alkaloid*	*skin irritant*
Schizothrix calcicola	*debromoaplysiatoxin*	*phenolic bislactone*	
Scytonema spp. (*hofmanni*, *pseudohofmanni*)	*scytophycin, cyanobacterin*	*methylformanide*	
Diatoms (Bacillariophcae)			
Pseudonitzschia spp. (pungens, australis, delicatissima, pseudodelicaissima, seriata)	*domoic acid*	*amino acid*	*neurotoxin*
Haptophytes (Prymnesiophyta)			
Prymnesium spp.*(parvum, patelliferum)*	*prymnesin*	*proteolipid*	*neurotoxin*
Chrysochromulina spp. *(polylepis)*	*prymnesin (unconfirmed)*		

Thraustochytrids are not related to either of the above groups of microalgae (bluegreen or dinoflagellate). The blue-green algae and dinoflagellates are in completely separate Kingdoms. Thrustochytrids are members of the kingdom Chromista which contains the golden algae. Within this kingdom, only two genera of microalgae, *Pseudonitzschia* (phylum: Heterokonta; class: Bacillariophycae) and *Prymnesium* (phylum: Prymnesiophyta) are known to produce toxins. Thraustochytrids are members of the class Thraustochytridae, and no reports of toxins in any member of this class have ever been published.

4. Algal Toxins

(a) Literature Review

Within the microalgae in the Chromista (Stramenopilia), there are two toxins known to be produced, domoic acid and prymnesin. Domoic acid is a potent neurotoxin which causes amnesic shellfish poisoning in humans. It is a naturally occurring amino acid whose production appears to be limited to a few species of microalgae (diatoms) in the genus *Pseudonitzschia* (and possibly by one species of *Chrysochromulina*, a flagellated species of golden algae) (Villac et al., 1993). Species (there are 19) of the genus *Pseudonitzschia* are common members of marine phytoplankton throughout the world. Four of these species of this diatom have been identified as being able to produce domoic acid, and these species can be generally found in the colder coastal waters of the Northern Hemisphere (coastal U.S., Canada and Europe) (Fritz et al., 1992; Garrison et al., 1992; Lundholm et al., 1994).

(b) Domoic Acid Chemical Analysis

In the *phylum* Heterokonta, the thraustochytrids are in a separate subphylum and class from the diatoms, so one would not expect to find domoic acid in *Schizochytrium* sp. As an additional check however, scientists at OmegaTech have analyzed for domoic acid in *Schizochytrium* sp. dried microalgae using the standard HPLC methods (with UV detection) (Lawrence et al., 1991) and did not find any trace of this compound. Additionally, a GCMS method was used (Lawrence et al., 1991) to test for the presence of domoic acid in a derivatized sample of *Schizochytrium* sp. dried microalgae. These analyses showed no evidence of domoic acid. A second laboratory, Monsanto Analytical Science Center, confirmed the absence of domoic acid in *Schizochytrium* sp. dried microalgae samples using an independent HPLC method. Domoic acid was not detected in either the parent *Schizochytrium* sp. ATCC 20888 or the mutant N230D strains, subject to a minimum detection limit of 0.5 ppm (Kuneman and Vinjamoori, 1997a,b).

(c) Prymnesium Toxin Biological Assay

The other toxins found in a member of the Chromista (Stramenopilia) are limited to two species of *Prymnesium* (*P. parvum* and *P. patelliferum*). These toxins (called prymnesins by some) exhibit a broad spectrum of activity including lethal effects on gill breathing animals, cytotoxic effects on erythrocytes, nucleated mammalian cells, protozoa and bacteria. Prymnesin toxins are acidic polar phospho-proteolipids, which because of their chemical nature, form micelles in water. These toxins are not heat stable. *Prymnesium* sp. can be grown both photosynthetically and heterotrophically. Heterotrophic growth of *Prymnesium* is best in a glycerol-rich medium. However, with cultures grown in the dark, there is a marked reduction in the production of prymnesin toxin (Shilo, 1971). Additionally, *Prymnesium* cultures grown in the dark on solid medium (agar plates of glycerol rich medium) exhibit hemolytic activity only after 24 hour exposure to light.

The major economic impact of prymnesin toxins for humans to date has been related to fish kills in aquaculture ponds (mostly occurring in Israel) and in coastal waters associated with intensive aquaculture production (Scandinavia). All gill breathing animals tested to date have proven sensitive to prymnesin toxins. As a result, a sensitive toxicity test for prymnesin toxins has been developed using nauplii of the brine shrimp *Artemia* (Larsen et al., 1993). The LC_{50} values for *Artemia* sp. in 24 hour exposures to toxic strains of *Prymnesium* sp. are only 3,000-5,000 cells/mL (Larsen et al., 1993). Neither OmegaTech nor Monsanto have analyzed directly for the presence of prymnesin toxins in *Schizochytrium* sp. dried microalgae to date due to the unavailability of authentic standards. However, a bioassay for prymnesin has been developed (Vanhaecke et al., 1981) utilizing *Artemia* nauplii as the test organism. Monsanto performed this bioassay for prymnesin on five representative lots of dried microalgae from the N230D strain. The results (Kaneko, 1997) indicate normal growth of *Artemia* culture with all test lots, indicating absence of prymnesin toxin. The absence of prymnesin is indicated because brine shrimp nauplii have been cultured to sexually mature adults in 12-15 days (representative of normal growth) on diets comprising 50-80% *Schizochytrium* sp. dried microalgae (Barclay, unpublished). *Schizochytrium* sp. can be utilized in aquaculture applications, including enrichment of DHA in *Artemia* and rotifers used to feed larval fish and shrimp (Barclay and Zeller, 1996). Monsanto commercialized a product for aquaculture applications (HUFA2000, a spray-dried form of *Schizochytrium* sp. dried microalgae) which has been successfully utilized for over five years with no adverse effects in shrimp larvaculture and finfish (red seabream, Japanese flounder) culture. Use of *Schizochytrium* sp. in these applications promotes larvae survival and growth.

Allergic responses to microorganisms by humans can sometimes be related to microbial toxins. There have been no reports in the literature of allergic responses to any members of the kingdom Chromista, including the thraustochytrids. Reports of respiratory and dermatologic responses (both allergic and chemical irritation) to microalgae have in general been limited to human exposure to toxic bluegreen algae or dinoflagellates, the two groups of algae with the most toxic species (Table III-1). Respiratory responses to members of the Oscillatoraceae (bluegreen algae) have occurred due to contact from swimming in infested waters (Heise 1949, 1951) and from exposure to ocean spray (aerosols) during blooms of *Gymnodinium brevis* (dinoflagellate) (Woodcock, 1948). Dermatologic responses have also been reported from swimming in waters containing both of these types of microalgae (Cohen & Reif, 1953; Grauer, 1959). There has been one report of an allergic response to the green alga *Chlorella* in children (Tiberg et al., 1995).

One worker has reported respiratory difficulties, aches and fever following excessive exposure to an aerosol mist of the fermentation broth that was generated during product recovery of *Schizochytrium* sp. This was determined to be pulmonary hypersensitivity. All symptoms were completely reversible on cessation of the exposure and did not reoccur once additional exposure controls were implemented. There are no reports of symptoms with anyone exposed to the dried microalgae, including the above worker. Pulmonary hypersensitivity and organic dust toxic syndrome are common ailments resulting from overexposure to dust or aerosols containing microorganisms from agricultural materials (grain, hay, silage), wood processing, sewage treatment, garbage composting and industrial bioprocesses (Sorenson & Lewis, 1996). The occurrence of these syndromes is readily prevented by eliminating sources of dusts/aerosols in these work environments or by use of dust masks where these sources cannot be contained (Sorenson & Lewis, 1996).

Based on existing published and unpublished scientific data, it is concluded that: 1) there have never been any published reports on toxic compounds produced by thraustochytrids; 2) most of the toxic compounds produced by microalgae are produced by bluegreen algae or dinoflagellates, and *Schizochytrium* sp. is in a separate kingdom from both of these types of microalgae; 3) the two toxic compounds known to be produced in the Chromista (to which *Schizochytrium* sp. belongs) are largely restricted to two genera (domoic acid in *Pseudonitzschia* and prymnesin in *Prymnesium* spp.) which are in a separate class and phylum, respectively, from the thraustochytrids; 4) chemical tests by scientists at OmegaTech and Monsanto Analytical Labs in St. Louis indicate that domoic acid is not present in *Schizochytrium* sp. microalgae; 5) biological assay for prymnesin toxin is negative; and 6) acute and subchronic dietary toxicity studies in rats and a battery of cytotoxicity/mutagenicity tests have been completed with no effects attributed to algal toxins (see Section XIII.B).

5. Phenotypic characterization

OmegaTech developed an improved strain from the patented wild-type parent strain using a classical mutagenesis/screening program. This program utilized well-accepted techniques commonly used in industrial strain improvement programs. No recombinant DNA technology was employed. N230D was one of more than 1,000 randomly-chosen survivors of chemically mutagenised (NTG; 1-methyl-3-nitro-1-nitrosoguanidine) ATCC 20888 screened for variations in fatty acid content. This particular strain was valued for its improved DHA productivity.

NTG treatment-derived mutants sometimes acquire undesirable traits such as essential growth factor/vitamin requirements leading to growth retardation or altered morphology compared to the parent while attaining characteristics of interest. Therefore, laboratory studies were conducted to phenotypically characterize the mutant N230D and its parent ATCC 20888. These tests included morphological evaluation (light microscopy) throughout their growth cycle under standard growth and fermentation conditions as well as evaluations of differences in growth or substrate utilization patterns in a batch fermentation mode.

Under standard nutrient and environmental conditions for growth and DHA production, N230D performed equivalently to its parent in terms of overall growth and growth rate, carbon consumption and microscopic morphology. Cells can be characterized by a circular shape with varying sizes dependent on growth stage, and can acquire a highly vacuolar appearance later during fermentation and show lipid droplets or lipid bodies. Average cell size in both strains was ~6-8 microns. Glucose uptake, growth profiles and fermentation times were similar in triplicate fermentors for both strains. Moreover, microbial identification panels (Biolog Inc.) based on carbon source utilsation, though not designed for microalgae, showed a high degree of similarity. These results indicate that no adverse traits were produced in N230D due to mutagenesis. Superior performance of this strain could be due to enhanced carbon flow through the lipid production pathway.

IV Effect of the Genetic Modification on the Properties of the Host Organism –
NOT APPLICABLE TO DHA-RICH OIL

V Genetic Stability of the GMO Used as Novel Food Source –
NOT APPLICABLE TO DHA-RICH OIL

VI Speciificity of Expression of Novel Genetic Mate rial –
NOT APPLICABLE TO DHA-RICH OIL

VII Transfer of Genetic Material From GMO –
NOT APPLICABLE TO DHA-RICH OIL

VIII Ability of the GMM to Survive in and Colonise the Human Gut –
NOT APPLICABLE TO DHA-RICH OIL

IX Anticipated intake/extent of use of the Novel Food.

According to the SCF guidelines the following questions must be asked at this stage:

- "Is there information on the anticipated uses of the Novel Food based on its properties?"
- "Is there information to show anticipated intakes for groups predicted to be at risk?"
- "Will introduction of the Novel Food be restricted geographically?"
- "Will the Novel Food replace other foods in the diet?"

The answers to these questions are outlined in the Section below:

Acknowledgement:

We are grateful to Dr D. Tennant of Food Chemical Risk Analysis, Brighton, UK (david-t@dircon.co.uk) for his assistance in the preparation of Sections IX and X of this submission.

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List of Tables

A. Population Intake Recommendations

It has been suggested that overall health may be improved by increasing n-3 PUFA intake (Simopoulos, 1991). Estimates of paleolithic human diets suggest that the amounts of n-3 PUFA and n-6 PUFA were equal during early human evolution. Changes in food production have greatly altered those proportions in the last 100 to 150 years (Simopoulos, 1991). Intakes of n-6 PUFA are now up to 25 times higher than n-3 PUFA in the U.S. (Simopoulos, 1991), and there have been suggestions to decrease this to 5:1 to 10:1 (ISSFAL, 1995).

Canada was the first country to make separate recommendations for n-3 and n-6 fatty acid consumption. Health and Welfare Canada recommends daily intakes of n-3 PUFA in the 1.0 to 1.6 g range for adults older than 19 years with an additional 0.05 to 0.16 g for pregnancy and 0.25 g for lactation (Simopoulos, 1991). Several European Organisations have also made recommendations for total n-3 PUFA intake; a few have made specific recommendations for EPA plus DHA intake (See Table IX-1 below).

Table IX-1. Recommendations for average population intakes of n-3 PUFA

Organization	Recommended Daily Intake			
	Omega-3 PUFA	**LNA (18:3 n-3)**	**EPA (20:5 n-3)**	**DHA (22:6 n-3)**
British Nutrition Foundation[1]	0.5-2.5 % energy	1.0 % energy (2.5 g/d)	• EPA/DHA 0.5% energy (1.2 g/d)	• EPA/DHA 0.5 % energy (1.2 g/d) • 20 mg/kg bw for pre-term infants
COMA[2]	0.1-0.2 g/d 1.5g/week	recommend *« that people eat at least two portions of fish, of which one should be oily, weekly »*		
Health Canada[3]	• 1.0-1.6 g/d min. of 0.5 g/d • pregnancy: + 0.05 g/d 1st + 0.16 g/d 2nd, 3rd trimester • lactation: + 0.25g/d			
ISSFAL[4]			• EPA/DHA 0.3 % energy (0.65 g/d) • EPA minimum 0.22 g/d	• EPA/DHA 0.3 % energy (0.65 g/d) • DHA minimum 0.22 g/d
Nordic Nutrition Council[5]	0.5 % energy (1.2 g/d)			
WHO[6]	*«consumption of adequate amounts of essential fatty acids is also important for normal growth and development.* *Arachidonic acid and docosahexaenoic acid are particularly important for brain development, and breast milk is a good source of these fatty acids »*			
		• formula for preterm babies 50 mg/ kg bw • term infants 50 mg/ kg bw		• formula for preterm babies 40 mg/ kg bw • term infants 20 mg/ kg bw

British Nutrition Foundation[1]. Briefing Paper. N-3 Fatty Acids and Health. July 1999.
Committee on Medical Aspects of Food Policy[2]. Annual Report. London: Department of Health. 1994.
Health and Welfare Canada[3]. Nutrition Recommendations: the report of the Scientific Review Committee. Ottawa: Minister of Supply and Services Canada. 1990.
ISSFAL[4] - Workshop on the Essentiality of and Dietary Reference Intakes (DRIs) for Omega-6 and Omega-3 Fatty Acids. National Institutes of Health. April 7-9, 1999.
Nordic Nutrition Recommendations[5], second edition. Nordic Council of Ministers. 1989.

World Health Organization[6]. Fats and Oils in Human Nutrition: report of a joint expert consultation. FAO Food and Nutrition Paper 57. Rome: FAO and WHO. October 1993.

B. Anticipated intake and extent of use of the Novel Food

1. Background

It is proposed that DHA-rich oil derived from *Schizochytrium* sp. microalgae should be introduced into European countries as a novel food that could allow increased intakes of the long chain polyunsaturated fatty acid (LC PUFA) docosahexaenoic acid (DHA; 22:6n-3) by European consumers. European food and health authorities have recommended that individuals should increase their intakes of omega-3 fatty acids, including DHA, because raised intakes are associated with reduced risks of coronary heart disease. However, it is necessary to show that raising intakes of DHA so that consumers achieve these benefits does not introduce other risks either from DHA itself or from increased intakes of other components of the DHA-rich oil.

The composition of DHA-rich oil is described in Section I. and background intakes of key components, specifically omega-3 fatty acids and sterols, are estimated in Section X. A summary of background intakes of DHA, DPA (n-6), EPA, cholesterol and phytosterols are shown in Table IX-2

Table IX-2. Summary of background intakes of DHA, DPA(n-6), EPA and sterols by UK adults

	Intake mg/day		
Component	**Mean**	**95th %ile**	**97.5th %ile**
DHA	107	330	401
DPA (n-6)	27	66	83
EPA	75	244	303
Cholesterol	191	362	419
Phytosterols	113	198	221

The average DHA intake for UK consumers is 107 mg/day and various estimates have suggested that this should be raised to between 200 and 800 mg per day in order to achieve benefits for coronary health (see Table IX-1). High level consumers at the 97.5th percentile only achieve 401 mg/day DHA but long term high level intake is probably better represented by 330 mg/day at the 95 percentile. For the purposes of this exercise it is assumed that a food manufacturer would wish to raise total daily mean intake target around 550 mg/day DHA per major food group so that intakes of average consumers would be adequate to achieve health benefits without high level consumers being placed at any potential risk.

2. Anticipated levels of usage

The amount of DHA-rich oil that must be added to a given food to achieve a total daily intake of DHA is determined by the quantities of that food consumed on any given day. For example, if the DHA-rich oil were to be added to a dietary staple such as bread, then the concentration required to achieve a mean intake of 550 mg/day DHA would be much lower than, for example, cereal bars that are consumed in smaller amounts. Table IX-3 describes the average daily consumption of a variety of foods to which it is anticipated DHA-rich oil might be included as a nutritional food ingredient. The column headed “Anticipated EU usage level” is the concentration of the oil, in percentage units, that would be required to achieve a mean daily dose of 550 mg DHA per major food group, taking into account the 107 mg per day already present in the diet. In the most extreme case, it is assumed that DHA-oil is used to replace cod-liver oil, which is unpalatable for some consumers. In that case, the mean consumption figure shows the amount that would need to be consumed to achieve a total daily intake of 550 mg.

The food uses provided in Table IX-3 are provided for illustrative purposes so that some realistic exposure scenarios can be developed. In practice usage levels must be determined for the particular product and may require adjustments for different markets within the EU. For example, the category 'Fine bakery wares' includes all kinds of cakes, biscuits and pastries. In reality DHA-rich oil might be added to only one or two such products in which case levels might need to be increased to match potential consumption. Whilst a large number of foodstuffs are included for the purposes of this illustration it is assumed that consumers will rely on only one source to increase their daily DHA intake on any given day. Therefore potential intakes from all uses will not be considered cumulatively.

Table IX-3. Level of use of DHA-rich oil in foods necessary to achieve target dose of 0.55 g in one day.

Food Group	Food Consumption (UK)		Anticipated EU	UK intake
	Consumers %	Mean (g/day)	usage level (%)	Mean (mg/day)*
Fine bakery wares	55.1%	83	**1.53%**	550
Cereal bars	2.3%	39	**3.27%**	550
Bread and rolls	88.3%	125	**1.01%**	550
Breakfast cereals	32.7%	49	**2.57%**	550
Processed and unprocessed cheese	34.3%	50	**2.52%**	550
Ketchup, mayonnaise, etc.	12.2%	27	**4.76%**	550
Dairy products, yoghurt, drinks, etc.	20.7%	94	**1.35%**	550
Puddings and gelatine desserts	2.5%	125	**1.01%**	550
Carbonated drinks, teas, powdered mixes	24.2%	302	**0.42%**	550
Processed fish products	1.5%	114	**1.11%**	550
Fruit-based drinks	4.7%	263	**0.48%**	550
Chewing gum	0.3%	7	**16.96%**	550
Hard confectionery	3.0%	15	**8.25%**	550
Frozen dairy products	9.5%	89	**1.43%**	550
Processed meat products	27.4%	82	**1.55%**	550
Nut and nut-based spreads	1.3%	25	**5.08%**	550
Pasta	5.7%	205	**0.62%**	550
Processed poultry products	0.5%	110	**1.15%**	550
Gravies and sauces	23.9%	91	**1.39%**	550
Potato crisps, etc.	18.0%	75	**1.69%**	550
Chocolate and other confectionery	18.6%	52	**2.44%**	550
Soups and soup mixes	10.1%	258	**0.49%**	550
Soya milks, drinks, creams; whiteners.	4.2%	288	**0.44%**	550
Vegetable oil-based spreads	35.6%	21	**6.16%**	550
Vegetable-based drinks	0.3%	209	**0.61%**	550
Cod-liver oil, etc.	0.0%	**1.16**	**100.00%**	550
Grand Total	100%	419		

* Includes background intake;
Maximum Daily Intake = 1.5g DHA at 350mg DHA per serving;
Average Background Intake = 107 mg DHA;
Desired daily dose = target – background = 443 mg; DHA Content of Oil = 35%

3. Data sources

Data on food consumption by UK adults and for inherent levels of fatty acids and sterols in foods are the same as those described in Section X. Usage of DHA-rich oil is as described in Table IX-3.

4. Intake analyses

Projected average daily intakes after the introduction of DHA-rich oil as a nutritional food ingredient are based on background intakes plus the proposed usage for each food group as described in Table IX-3. However, some consumers will eat more than average amounts of each of the given food groups and so it is necessary to confirm that the intakes of DHA and other components of DHA-rich oils remain within acceptable levels. Intakes will therefore be calculated for DHA, DPA (n-6), EPA, cholesterol and total phytosterols for 95th percentile and 97.5th percentile consumers.

A simple method for calculating high level intakes following addition of DHA-rich oils as a food ingredient might be to sum background intakes at the 97.5th percentile to the intake of a consumer of each food at the 97.5th percentile. However, this would produce erroneous results because a high level consumer of DHA containing foods is not necessarily a high level consumer of foods to which DHA-rich oil has been added. It is therefore necessary to re-calculate the distribution of DHA (and other constituents) intakes for the entire population in order to assess the effect on high level intakes. A better estimate of long-term high level intake is achieved by considering the average weekly consumption of the foods affected (expressed on a g/day basis).

5. High level intakes of EPA, DPA (n-6) and DHA by UK adults.

High level intakes of EPA, DPA (n-6) and DHA are presented in Table IX-4. In most cases high level intakes are higher than background levels but, as expected, they do not equal the sum of 97.5th percentiles of background intakes (mainly from oily fish) plus 97.5th percentiles of intakes from each food. This is because high level consumers are not also high level consumers of DHA-rich oil containing foods. The highest intakes are associated with use of DHA-rich oil in foods such as vegetable fat spreads and bread and rolls probably because these are common constituents of most diets. Note that the fatty acid ratios vary slightly. This is because each intake estimate includes a component from background in the diet and this will vary according to the fatty acid intake profiles of individuals sampled at the 95th or 97.5th percentile.

In some cases (e.g. cereal bars) overall intakes do not appear to have increased significantly. This is because of small numbers of consumers and also because such foods are not eaten every day and so the impact on intakes from such casual consumption is reduced. In reality, consumers might alter their food consumption habits if cereal bars were available that contained DHA-rich oil. In such cases intakes could be estimated by assuming that an individual consumes the typical daily amount of each food presented in Table IX-3 every day plus an average background intake from other sources. This would provide a maximum daily intake 1.5g DHA at 350mg DHA per serving for example. If a high level oily fish consumer were to consume one DHA enriched cereal bar every day then the approximate daily intake of DHA would be 844 mg/day, of DPA (n-6) would be 254 mg/day and of EPA would be 602 mg/day. These projected intakes are very similar to those for other more regularly consumed foods.

Although consumers are intended to use only one food source of DHA-enriched oils the effect of combining the two foods contributing the highest intakes was investigated. For bread and spreadable fats combined, the mean intake was 743 mg/day of DHA.

Table IX-4. Effect on overall EPA, DHA and DPA(n-6) intakes of adding DHA-rich oil to specific foods.

Food description	EPA		DHA		DPA(n-6)		
	95th %ile	97.5th %ile	95th %ile	97.5th %ile	95th %ile	97.5th %ile	
Fine bakery wares	263	329	816	975	280	335	mg/day
	3.7	*4.7*	*12.1*	*14.1*	*4.2*	*5.1*	*mg/kg bw/day*
Cereal bars	245	303	350	435	79	104	mg/day
	3.5	*4.5*	*5.1*	*6.5*	*1.2*	*1.5*	*mg/kg bw/day*
Bread and rolls	277	338	986	1128	341	392	mg/day
	3.8	*4.8*	*13.5*	*15.5*	*4.8*	*5.4*	*mg/kg bw/day*
Breakfast cereals	253	321	707	824	240	292	mg/day
	3.7	*4.6*	*10.5*	*12.8*	*3.6*	*4.3*	*mg/kg bw/day*
Processed and unprocessed cheese	254	316	611	718	203	229	mg/day
	3.7	*4.6*	*8.7*	*10.4*	*2.8*	*3.3*	*mg/kg bw/day*
Ketchup, mayonnaise, etc.	248	307	484	611	133	172	mg/day
	3.5	*4.6*	*6.9*	*8.7*	*1.9*	*2.4*	*mg/kg bw/day*
Dairy products, yoghurt, drinks, etc.	254	315	627	758	200	247	mg/day
	3.7	*4.7*	*9.2*	*11.2*	*3.0*	*3.8*	*mg/kg bw/day*
Puddings and gelatine desserts	245	303	364	454	99	119	mg/day
	3.5	*4.5*	*5.4*	*6.8*	*1.4*	*1.8*	*mg/kg bw/day*
Carbonated drinks, teas, powdered mixes	250	314	613	762	206	263	mg/day
	3.6	*4.5*	*9.2*	*11.1*	*3.1*	*4.0*	*mg/kg bw/day*
Processed fish products	243	303	337	413	73	92	mg/day
	3.5	*4.5*	*4.7*	*6.0*	*1.1*	*1.4*	*mg/kg bw/day*
Fruit-based drinks	246	304	382	496	101	132	mg/day
	3.5	*4.5*	*5.5*	*7.3*	*1.5*	*1.9*	*mg/kg bw/day*
Chewing gum	243	303	330	404	68	86	mg/day
	3.5	*4.5*	*4.6*	*6.0*	*1.0*	*1.3*	*mg/kg bw/day*
Hard confectionery	245	303	350	435	79	104	mg/day
	3.5	*4.5*	*5.1*	*6.5*	*1.2*	*1.5*	*mg/kg bw/day*
Frozen dairy products	246	304	402	495	109	133	mg/day
	3.5	*4.5*	*6.0*	*7.1*	*1.6*	*2.0*	*mg/kg bw/day*
Processed meat products	256	318	745	901	258	316	mg/day
	3.6	*4.7*	*10.7*	*12.5*	*3.8*	*4.6*	*mg/kg bw/day*

Food description	EPA 95th %ile	EPA 97.5th %ile	DHA 95th %ile	DHA 97.5th %ile	DPA(n-6) 95th %ile	DPA(n-6) 97.5th %ile	
Nut and nut-based spreads	245	303	330	401	68	85	mg/day
	3.5	*4.5*	*4.6*	*5.9*	*1.0*	*1.3*	*mg/kg bw/day*
Pasta	246	304	380	482	103	119	mg/day
	3.5	*4.6*	*5.7*	*7.0*	*1.5*	*1.8*	*mg/kg bw/day*
Processed poultry products	243	303	330	404	70	89	mg/day
	3.5	*4.5*	*4.7*	*6.0*	*1.0*	*1.3*	*mg/kg bw/day*
Gravies and sauces	246	303	370	467	97	122	mg/day
	3.5	*4.5*	*5.5*	*6.9*	*1.4*	*1.8*	*mg/kg bw/day*
Potato crisps, etc.	247	304	361	436	89	108	mg/day
	3.6	*4.5*	*5.1*	*6.4*	*1.3*	*1.6*	*mg/kg bw/day*
Chocolate and other confectionery	247	307	507	634	152	189	mg/day
	3.5	*4.6*	*7.6*	*9.7*	*2.3*	*3.1*	*mg/kg bw/day*
Soups and soup mixes	243	303	333	403	69	85	mg/day
	3.5	*4.5*	*4.7*	*6.0*	*1.0*	*1.3*	*mg/kg bw/day*
Soya milks, drinks, creams; whiteners.	243	303	330	402	67	84	mg/day
	3.5	*4.5*	*4.6*	*5.9*	*1.0*	*1.2*	*mg/kg bw/day*
Vegetable oil-based spreads	263	325	935	1088	323	394	mg/day
	3.7	*4.7*	*12.9*	*15.0*	*4.7*	*5.5*	*mg/kg bw/day*
Vegetable-based drinks	243	303	330	401	68	86	mg/day
	3.5	*4.5*	*4.6*	*5.8*	*1.0*	*1.3*	*mg/kg bw/day*
Fish oils*	268	333	773	844	237	254	mg/day
	4.5	*10.0*	*12.9*	*14.1*	*4.0*	*4.2*	*mg/kg bw/day*

* Based on a daily dose to deliver 550mg total DHA for a consumer of average weight.

Table IX-5. Effect on overall cholesterol and phytosterol intakes of adding DHA-rich oil to specific foods

Food description	Cholesterol		Phytosterols		
	95th %ile	97.5th %ile	95th %ile	97.5th %ile	
(Background intakes)	(362)	(418)	(248)	(277)	mg/day
Fine bakery wares	370	424	278	304	mg/day
Cereal bars	375	424	249	278	mg/day
Bread and rolls	366	418	287	318	mg/day
Breakfast cereals	367	428	264	293	mg/day
Processed and unprocessed cheese	389	420	264	288	mg/day
Ketchup, mayonnaise, etc.	367	423	254	284	mg/day
Dairy products, yoghurt, drinks, etc.	362	420	256	286	mg/day
Puddings and gelatine desserts	365	421	250	280	mg/day
Carbonated drinks, teas, powdered mixes	362	420	261	285	mg/day
Processed fish products	366	421	248	278	mg/day
Fruit-based drinks	362	418	250	282	mg/day
Chewing gum	362	420	248	278	mg/day
Hard confectionery	368	418	249	278	mg/day
Frozen dairy products	362	418	252	280	mg/day
Processed meat products	368	426	268	295	mg/day
Nut and nut-based spreads	362	418	248	278	mg/day
Pasta	362	418	251	284	mg/day
Processed poultry products	362	418	248	278	mg/day
Gravies and sauces	362	418	252	281	mg/day
Potato crisps, etc.	362	419	250	279	mg/day
Chocolate and other confectionery	365	420	257	286	mg/day
Soups and soup mixes	362	418	249	278	mg/day
Soya milks, drinks, creams; whiteners.	362	418	248	278	mg/day
Vegetable oil-based spreads	367	427	289	324	mg/day
Vegetable-based drinks	362	418	248	278	mg/day
Fish oils	371	427	277	306	mg/day

6. High level intakes of sterols by UK adults

High level intakes of sterols were calculated using the same method as was used for fatty acids. For high level consumers fatty acid intakes were no more that 2% higher than background levels after introduction of DHA-rich oil. Similarly total phytosterol intakes were raised by a maximum of 17%. In both cases the associated uses were bread and spreadable fats again.

7. Conclusions

The use of DHA-rich oils as a food ingredient can raise intakes of DHA to potentially beneficial levels. High level intakes of DHA and EPA can be as much as 1.5g DHA at 350mg DHA per serving but such intakes are unlikely to be sustained in the long term. Intakes of cholesterol were marginally raised for high level consumers but phytosterol intakes were also raised by up to 17% in the same individuals.

C. At-risk Populations

1. Phytosterolemia or sitosterolemia

Phytosterolemia or sitosterolemia (with or without accompanying xanthomatosis) is a rare lipid storage disease inherited in an autosomal recessive pattern (Ling and Jones 1995 and Bhattacharyya et al. 1991). Phytosterolemia is characterized chemically by increased plant sterols and 5á-saturated stanols in plasma and tissue. Phytosterolemia is characterized pathologically by premature atherosclerosis, xanthomas, and some patients have developed hemolytic syndromes. The absorption rate of phytosterol is very high in these patients, and secretion into bile (of at least sitosterol) is less than that of cholesterol, yielding a sluggish turnover and excretion rate of phytosterols. In normal blood serum, the concentration of sitosterol is less than 1 mg/dl, and in untreated patients with phytosterolemia, serum sitosterol values in the range of 10 to 65 mg/dl have been reported. As of 1991, approximately 22 persons with this disease had been identified.

Diagnosis of phytosterolemia can be made in early childhood in patients who develop xanthomas. Treatment is largely palliative, excluding from the diet vegetable oils, shortening, margarine, nuts, seeds, chocolate, olives, avocados and cereal products with the germ remaining. Cholestyramine and neomycin are sometimes prescribed.

2. Bleeding Times

There was early recognition that bleeding times were longer in subjects consuming high amounts of LC, n-3 PUFA. It was also recognized that while Eskimos suffered less from CHD, they experienced a high incidence of cerebral hemorrhage. It appeared that the very factors which may be protective for CHD also may prolong bleeding. However, when bleeding time was investigated clinically with low to moderate doses of fish oil (0.5 to 2.0 g per day of n-3 FA), no significant increases were observed (Connor, 1994). The FDA has concluded that consumption of <3 g/d of EPA plus DHA should not cause increased bleeding times (Fed Reg 62:30751).

3. Glycemic Control

A few studies have reported deterioration of glycemic control in diabetics after fish oil supplementation (Stacpoole et al., 1989; Connor, 1994; Berdanier, 1994). In general no glycemic effects are observed in normal subjects (Berdanier, 1994) or in non-insulin dependent diabetic subjects (Morgan et al., 1995).

X. Information from previous human exposure to the Novel Food or its source.

According to the SCF guidelines the following questions must be asked at this stage:

- "Is there information from previous direct, indirect, intended or unintended human exposure to the Novel Food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?"

- Is there information to demonstrate that exposure to the Novel Food is unlikely to give rise to microbiological, toxicological and/or allergenicity problems?"

The answers to these questions are outlined in the Section below:

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X. Information from Previous Human Exposure

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X. Information from Previous Human Exposure

A. Background

DHA-rich oil consists of approximately 35% docosahexaenoic acid (DHA; 22:6n-3), together with other fatty acids and traces of phytosterols as described in Section I and summarised in Table X-1. The fatty acids and sterols occur naturally in plant and animal products (Southgate et al., 1980, Morton et al., 1995) and it is therefore possible to use established techniques to estimate background levels of intake. Since diets can vary widely it is necessary to consider not only average intake but also the range of intakes from "low-to high-level" consumers. In order to achieve this it is necessary to have access to data about individual's food consumption patterns so that nutrient intakes can be modeled for each individual in the study population. Statistics can then be applied to these data to describe the population as a whole. One of the few sources of individual food consumption data are the UK National Diet and Nutrition Surveys (NDNS). However, since these data reflect only consumption in the UK it will be necessary to use supplementary data to ensure that the UK NDNS data are sufficiently representative of other European consumers. Only adult food consumption data have been used in this analysis because DHA-rich oil containing foods are aimed specifically at this age-group.

Table X-1. Composition of DHA-rich Oils derived from Schizochytrium sp. microalgae.

Class	Name	Mean mg /g oil
	Eicosapentaenoic (EPA) (20:5n-3)	26.3
	Docosapentaenoic n-6 (DPA (n-6) (22:5n-6))	135.0
	Docosahexaenoic (DHA) (22:6n-3)	350.0
Sterols	Cholesta-5-en-3-ol (Cholesterol)	7.8
	Phytosterols	17.1

B. Data sources

1. Levels of fatty acids and sterols in food

The first supplement to McCance and Widdowson's 'The Composition of Foods' provides comprehensive information about the levels of fatty acids including EPA, DPA (n-6) and DHA present in a wide range of food-stuffs (some additional data about DPA (n-6) levels are drawn from Table XIII-5). Information about levels of cholesterol and phytosterols are provided in a paper by Morton et al., 1995. The data in the Morton paper are grouped according to UK Total Diet Survey (TDS) food groups and so, for convenience, all of the sterol and fatty acid levels have been assembled in these categories in Table X-2. The Morton data have been updated with new data for some foods published in the seventh supplement to McCance and Widdowson. For the fatty acids, the TDS category for fish has been sub-divided into white fish and oily fish because of the very different levels of EPA and DHA in these types of fish.

2. Food consumption

The source of food consumption data for the UK used in this study was the National Dietary and Nutrition Surveys (NDNS) of adults aged 16 to 65 (1986/87) which was commissioned jointly by the UK Ministry of Agriculture Foods and Fisheries (MAFF) and Department of Health (DH). These data have been made available for use in scientific research projects and enable the estimated intakes of Sterols and Fatty Acids and naturally occurring fatty acids to be projected. The data comprise 7 day records of all foods consumed by approximately 2000

individuals. Each food that was consumed was individually weighed and recorded and over 2000 food descriptions are included in the databases.

Since data are available for each individual who participated in the surveys it is possible to derive statistics. In addition to the mean number, the 95th and 97.5th percentiles of the distribution are therefore provided to represent 'high-level' consumption. Data can also be provided on a bodyweight basis by dividing food consumption by each individual's body weight.

Table X-3 provides data on the amounts of foods consumed, as used in the intake calculations for fatty acids and sterols. Consumption figures are provided only for those who reported consumption of each food (consumers only).

The Food and Agriculture Organization of the United Nations compiles information and data on various aspects of food and agriculture from a large number of countries. FAO Food Balance Sheets are based on reported domestic production plus imports, less exports, divided by the population count. They include some additional adjustments for stockpiling, spoilage and wastage. Food balance sheet data for European countries are given in Table X-4. Note that food balance sheets are compiled as raw agricultural commodities and therefore do not correspond directly to groups used in the National Food Survey or the National Dietary and Nutrition Survey which are based on foods as consumed.

C. Intake Analyses

Intakes were calculated initially using distributional modeling based on raw data from the food consumption survey. The UK food consumption databases are comprised of lists of the amounts of each food consumed by each individual on each eating occasion during the survey. A fatty acid or sterol concentration is assigned to each relevant food and then multiplied by the amount of food consumed on each eating occasion to generate an intake figure. The total intake is summed for each individual and then divided by the number of days in the survey to give the average daily intake for that individual. This provides a distribution of intake levels from which means and percentiles for the population can be estimated. To calculate intakes on a bodyweight basis, individual intake figures are divided by each individual's bodyweight as recorded in the surveys.

Food Balance Sheets (FBS) provide data on population *per* capita food consumption and so only mean intake figures can be generated. European intakes based on FBS data will tend to differ from those generated from food consumption surveys because they are based on different population groups and factors such as wastage and processing in the home are not taken into account. Comparisons are also made difficult because foods are classified in different ways. Nevertheless, intake based on FBS data can provide useful international comparisons.

1. Intakes of EPA, DPA (n-6) and DHA by UK adults

Intakes of EPA, DHA and DPA(n-6) by UK adults are reported in Table X-5. Mean intakes of EPA, DHA and DPA(n-6) are 75 mg/day, 107 mg/day and 27 mg/day respectively (1.08 mg/kg bw/day, 1.54 mg/kg bw/day and 0.39 mg/kg bw/day) and range up to 303 mg/day, 401 mg/day and 83 mg/day (4.46 mg/kg bw/day, 5.85 mg/kg bw/day and 1.22 mg/kg bw/day) at the 97.5th percentile. The principal sources of EPA and DHA in the diet are fatty fish and for DPA(n-6) the main source is offal. Fatty fish are the principal source of EPA and DHA in the diet. However, only 35% of adults regularly consume fatty fish. In the absence of fatty fish in the diet average intakes of EPA and DHA would be 33 mg/day and 54 mg/day respectively, for the same group of consumers.

2. Intakes of sterols by UK adults

Intakes of individual plant sterols are reported in Table X-6 and of total phytosterols (excluding cholesterol) in Table X-7. Average cholesterol intakes for UK adults are 303 mg/person/day, which is consistent with the intakes of UK adults reported by Morton *et al.* Intakes from different food groups differ slightly from the Morton *et al.* paper because food groups have been categorised in different ways. Estimates based on UK adults are higher than those reported for 'Total Diet' studies reported by Morton *et al.* Morton *et al.* explain this as being due to the Total Diet survey including low consumers such as children and the elderly.

Intakes of phytosterols range from about 1 mg/day to almost 125 mg/day. The highest contributor to total phytosterol intakes is β-sitosterol (mean = 64 mg/day; 97.5th percentile = 123 mg/day), followed by campesterol (mean = 27 mg/day; 97.5th percentile = 55 mg/day). The principal sources of intake are from oils and fats, followed by bread and other cereals.

Average daily intakes are slightly lower that those reported by Morton *et al.* for Total Diet samples in 1991 (although similar to those reported for 1987). This discrepancy is mainly related to intakes from oils and fats with intakes from other sources being broadly consistent. The difference is probably due to difficulties in recording oil and fat consumption in the adults survey since they tend to be 'hidden' in other foods.

Intakes of total phytosterols for UK adults range from 113 mg/day (1.64 mg/kg bw/day) at the mean to 221 mg/day (3.31 mg/kg bw/day) at the 97.5th percentile. These intakes are comparable to national intakes reported in an opinion on the safety assessment of phytosterols by the EU Scientific Committee on Foods (Opinion 6th April 2000).

3. Potential intakes of omega fatty acids and sterols in other EU Member States

Tables X-8, X-9 and X-10 summarise the food balance sheet data for European countries described in Table X-4 grouped into Total Diet Study categories. Note that correspondence can only be approximate because of the different ways in which the FBS and TDS data were collected and presented. Consumption of foods that are critical to intakes omega-3 of fatty acids and sterols (fatty fish, oils and fats, bread and other cereals) appear to vary between countries and this could have an impact on national intakes.

Combined EPA and DHA intakes appear to vary from country to country from 261 mg/day in Ireland to 566 mg/day in Greece. The differences are due largely to differences in fish consumption that may, in part, reflect national diets but might which also reflect the accuracy of data in FBS tables. Although there are some national differences, UK consumers appear to provide a reasonable representation of typical European consumers.

Average intakes of cholesterol are higher when calculated using FBS data, as compared to UK consumers using food consumption data. Intakes of total phytosterols are also higher, with average intakes ranging from 190 mg/day in Finland to 410 mg/day in Greece. Phytosterol intakes are driven by estimates of fat and oils consumption and the resulting β-sitosterol intakes and are thus subject to uncertainties about the amounts of oil used in cooking but not consumed. Nevertheless, UK consumers again appear to provide a reasonable representation of typical European consumers.

4. Conclusions

Levels of key omega fatty acids and sterols can be combined with the consumption of foods by UK adults to provide estimates of intake that concur with previous estimates published in the scientific literature. Average intakes predicted using FAO Food Balance Sheets tend to over-estimate intakes and this is believed to be associated with uncertainties in making

reliable estimates of fat and oil consumption. However, on the basis of these data it can be observed that UK consumers provide a reasonable model for typical consumers in European countries.

Allergenicity and toxicology will be addressed in Chapter XIII.

***Table X-2. Levels of EPA, DPA(n-6), DHA and sterols in foods* (mg/kg).**

GROUP	EPA	DPA	DHA	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	b - Sitosterol	Fucostanol	d5-Avenasterol	d7-Stigmasterol	d7-Avenasterol
Bread	0.00	0.000	0.00	0.0	0.2	5.0	0.5	15.1	1.8	0.9	0.4	0.3
Other cereals	0.00	0.000	0.00	0.0	0.3	37.0	1.4	19.0	2.6	1.3	0.5	0.4
Milk	0.00	0.000	0.00	7.7	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0
Dairy products	0.00	0.000	0.00	9.1	0.0	0.8	0.0	0.8	0.0	0.0	0.0	0.0
Eggs	0.00	0.040	0.11	380.5	1.3	3.2	0.0	0.7	0.0	0.0	0.0	0.0
Oils & fats	0.01	0.000	0.00	76.0	14.3	90.0	7.7	143.0	0.0	8.2	7.0	2.1
Carcase meats	0.02	0.002	0.01	0.0	0.0	0.5	0.0	0.2	0.0	0.0	0.0	0.0
Offals	0.03	0.160	0.04	270.0	0.0	2.0	0.0	0.3	0.0	0.0	0.0	0.0
Meat products	0.00	0.000	0.00	37.8	1.5	6.3	1.0	11.9	0.4	0.0	0.0	0.0
Poultry	0.02	0.007	0.03	108.0	0.4	0.9	0.0	0.7	0.0	0.1	0.0	0.0
White fish	0.10	0.036	0.12	65.0	0.8	3.5	0.4	5.0	0.0	0.1	0.0	0.0
Oily fish	0.62	0.030	0.78	65.0	0.8	3.5	0.4	5.0	0.0	0.1	0.0	0.0
Potatoes	0.00	0.000	0.00	0.0	0.0	0.2	0.2	1.0	0.0	0.4	0.0	0.0
Other vegetables	0.00	0.000	0.00	1.0	0.1	2.9	1.5	7.7	0.0	0.4	1.1	0.1
Canned vegetables	0.00	0.000	0.00	0.0	0.0	0.4	2.0	3.7	0.0	0.6	0.0	0.0
Nuts	0.00	0.000	0.00	0.0	0.2	11.4	6.9	68.0	0.9	9.4	0.5	0.7
Sugars & preserves	0.00	0.000	0.00	6.0	0.1	2.2	4.5	11.1	0.0	0.5	0.2	0.0
Beverages	0.00	0.000	0.00	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	g/100g	g/100g	g/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g

Table X-3. Weekly consumption by UK adults of foods containing fatty acids and sterols.

TDS Code	Food description	Consumers %	Food Consumption, g/day* Mean	95th%ile	97.5th%ile
1	Bread	99%	109	220	258
2	Other cereals	98%	120	278	324
3	Carcass meats	93%	54	128	149
4	Offals	29%	16	43	53
5	Meat products	91%	73	189	217
6	Poultry	70%	34	84	105
7	Fish	73%	33	81	97
7A	White fish	60%	27	63	75
7B	Oily fish	35%	19	49	65
8	Oils & fats	81%	11	29	34
9	Eggs	80%	26	61	73
10	Sugars & preserves	91%	38	101	118
11	Potatoes	98%	114	241	291
12	Other vegetables	99%	114	252	305
13	Canned vegetables	66%	36	94	114
14	Beverages	87%	449	1534	2062
15	Milk	97%	237	533	616
16	Dairy products	95%	57	144	168
17	Nuts	20%	10	29	36
			1394	2679	3063

* All food consumption figures represent only individuals who reported consumption during the period of the survey (i.e. non-consumers of each food are excluded).

Table X-4. Consumption of agricultural commodities by European consumers.

Product	Austria	Belgium-Luxembourg	Denmark	Finland	France	Germany	Greece	Ireland	Italy	Netherlands	Norway	Portugal	Spain	Sweden	United Kingdom
Wheat	190.19	251.23	217.42	196.36	257.32	199.70	389.26	272.74	405.04	174.19	256.77	261.73	252.63	209.62	235.67
Barley	0.77	1.78	0.05	9.97	0.55	0.71	0.00	8.74	2.14	3.40	27.12	2.63	0.36	4.63	1.37
Maize	11.29	4.00	26.82	0.00	34.79	20.22	4.49	47.75	9.56	7.67	1.86	21.67	4.14	5.53	9.37
Rye	28.93	4.08	45.42	42.99	1.45	34.08	0.60	1.81	0.25	4.96	19.97	11.12	4.38	23.84	0.93
Oats	2.27	0.71	11.07	14.52	0.79	6.08	0.19	8.38	0.47	4.19	15.59	2.49	0.71	12.11	9.59
Millet	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorghum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cereals, Other	0.00	3.51	0.00	0.00	0.77	1.78	3.04	4.88	0.05	0.30	0.05	5.92	0.00	0.63	0.00
Potatoes	161.04	298.96	195.84	188.93	182.90	200.63	186.74	346.88	104.00	231.12	212.38	345.18	245.12	178.00	311.37
Cassava	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sweet Potatoes	0.00	0.00	0.00	0.03	0.00	0.00	0.66	0.00	0.55	0.08	0.03	1.23	1.40	0.00	0.00
Roots, Other	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.00
Sugar Cane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sugar Beet	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00
Sugar (Raw Equivalent)	123.34	121.45	113.48	97.89	97.29	100.99	82.00	108.27	78.08	137.89	123.73	95.56	81.95	125.62	107.51
Sweeteners, Other	3.78	4.52	21.56	6.00	6.38	14.05	2.00	7.70	2.77	1.45	1.59	4.44	0.99	3.67	3.56
Beans	0.77	2.03	0.14	0.05	2.71	0.58	8.52	1.15	4.47	1.75	0.27	7.37	4.58	0.82	2.93
Peas	0.77	3.64	2.08	4.11	1.75	2.55	0.03	4.27	3.32	4.66	2.41	1.40	4.08	1.97	10.99
Pulses, Other	0.30	0.99	0.36	0.03	1.23	1.12	5.29	0.08	6.47	1.62	0.11	4.47	12.74	0.08	0.60
Soyabeans	0.82	0.05	0.03	0.11	0.05	2.08	0.00	0.03	0.00	0.16	0.08	0.00	0.03	0.16	0.11
Groundnuts (Shelled Eq)	1.07	0.90	0.22	1.42	1.95	2.74	0.52	1.40	0.68	2.33	1.15	0.38	2.30	1.70	5.67
Sunflowerseed	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	2.77	0.00	0.00
Rape and Mustardseed	0.33	1.67	0.52	0.33	0.38	0.85	0.33	0.33	0.08	1.92	0.52	0.11	0.05	0.19	0.30

Product	Austria	Belgium-Luxembourg	Denmark	Finland	France	Germany	Greece	Ireland	Italy	Netherlands	Norway	Portugal	Spain	Sweden	United Kingdom
Cottonseed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coconuts - Incl Copra	1.81	10.68	2.03	0.60	1.40	1.92	1.04	2.66	0.77	7.78	2.16	2.82	1.53	1.84	4.52
Sesameseed	0.41	0.00	0.00	0.11	0.00	0.00	1.84	0.14	0.00	0.00	0.36	0.00	0.03	0.25	0.27
Palmkernels	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Olives	0.55	1.04	1.21	0.82	2.52	0.79	34.19	0.03	6.68	0.88	0.55	1.34	7.67	1.26	0.25
Oilcrops, Other	0.66	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.03
Soyabean Oil	0.00	12.99	7.15	1.34	3.56	11.18	1.12	4.44	10.11	27.53	23.59	12.99	9.92	9.26	10.16
Groundnut Oil	0.44	1.70	0.00	0.03	3.37	0.68	1.12	0.44	2.22	6.14	1.67	0.63	0.03	0.03	0.11
Sunflowerseed Oil	13.04	5.23	1.89	1.81	19.12	10.25	14.71	8.55	9.48	2.88	1.64	15.95	27.62	2.33	7.32
Rape and Mustard Oil	20.03	12.27	0.00	21.92	7.97	15.07	0.00	19.12	4.05	0.00	8.14	0.00	1.95	25.73	22.96
Cottonseed Oil	0.00	4.68	0.00	0.00	0.05	0.00	1.32	0.00	0.00	0.00	0.00	0.00	1.37	0.22	0.00
Palmkernel Oil	0.00	1.34	5.23	0.00	0.25	0.00	0.00	0.05	0.25	0.00	0.00	0.47	0.05	0.00	0.00
Palm Oil	1.26	5.23	0.00	0.00	1.40	3.37	0.00	0.00	1.92	1.75	0.00	6.47	1.04	4.66	3.89
Coconut Oil	2.77	5.53	0.00	0.00	0.00	4.49	0.00	6.11	2.30	3.48	0.00	0.00	0.16	1.97	0.71
Sesameseed Oil	0.03	0.03	0.55	0.00	0.11	0.16	0.96	0.03	0.08	0.00	0.03	0.00	0.00	0.03	0.03
Olive Oil	0.82	1.81	1.23	0.27	2.38	0.60	53.51	1.10	33.81	0.63	1.45	10.52	31.40	0.71	1.21
Maize Germ Oil	1.84	3.18	0.99	0.00	1.45	0.63	7.89	1.04	4.96	0.96	0.33	0.71	1.10	0.03	0.47
Oilcrops Oil, Other	8.99	6.52	2.00	3.56	6.66	1.67	0.00	0.05	0.05	0.00	1.23	0.22	0.08	4.05	1.21
Tomatoes	32.33	77.18	41.78	38.47	64.60	36.74	334.93	24.85	178.00	40.27	34.38	115.45	93.18	48.66	47.15
Onions	16.00	20.33	26.88	9.15	14.00	12.93	39.78	16.99	16.88	28.36	0.96	21.01	47.01	12.82	22.22
Vegetables, Other	210.63	262.30	191.73	142.05	245.29	171.92	256.74	152.99	268.63	186.38	134.82	265.18	193.95	126.11	153.18
Oranges, Mandarines	51.37	78.36	88.03	58.22	91.40	32.99	126.27	44.08	109.56	111.53	138.14	58.14	110.33	86.99	50.27
Lemons, Limes	6.03	5.73	4.71	2.30	5.51	4.00	25.73	2.96	26.30	3.29	1.92	3.18	9.21	3.75	2.63

Product	Austria	Belgium-Luxembourg	Denmark	Finland	France	Germany	Greece	Ireland	Italy	Netherlands	Norway	Portugal	Spain	Sweden	United Kingdom
Grapefruit	2.55	12.44	2.14	2.79	15.34	3.92	2.63	3.45	3.59	11.70	1.29	3.26	0.58	3.64	4.82
Citrus, Other	0.96	0.79	0.00	0.03	0.05	1.45	0.99	1.70	0.30	0.00	0.79	0.03	0.33	0.96	0.82
Bananas	26.99	55.12	26.33	30.33	3.21	34.38	14.88	26.49	19.84	3.51	34.66	36.79	24.00	41.26	27.97
Plantains	0.00	0.63	0.03	0.00	0.16	0.05	0.03	0.00	0.05	0.00	0.00	1.84	0.03	0.03	0.27
Apples	125.48	76.19	86.79	40.05	26.66	137.42	67.92	36.77	49.26	84.05	53.40	70.25	51.18	63.04	39.34
Pineapples	3.12	9.97	4.19	6.30	3.86	4.30	1.10	2.27	4.05	7.89	4.03	9.10	4.03	3.32	3.81
Dates	0.22	0.55	0.74	0.14	0.38	0.14	0.08	0.08	0.25	0.14	0.16	0.03	0.82	0.16	14.93
Grapes	20.14	13.26	22.77	12.33	7.01	22.74	64.44	20.88	35.18	30.52	20.49	8.03	7.56	15.15	27.18
Fruits, Other	112.38	62.33	54.88	43.73	88.68	78.99	210.93	49.51	116.66	58.68	75.73	120.11	139.29	66.93	50.58
Coffee	21.29	11.56	24.85	31.32	15.89	19.12	10.71	6.08	13.75	25.64	25.59	9.53	11.51	27.32	7.04
Cocoa Beans	4.79	3.97	14.03	0.77	8.68	7.48	4.36	6.79	4.00	3.15	7.59	4.19	5.81	5.07	6.38
Tea	0.38	0.47	1.07	0.60	0.79	0.66	0.14	8.63	0.22	2.96	0.82	0.11	0.08	0.88	7.01
Pepper	0.60	0.52	0.60	0.27	0.33	0.52	0.25	0.22	0.16	0.74	0.30	0.08	0.14	0.41	0.30
Pimento	0.77	0.19	0.33	0.22	0.14	0.38	0.19	0.05	0.03	0.66	0.16	0.22	0.49	0.41	0.19
Cloves	0.03	0.00	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.08	0.00	0.00	0.00	0.03	0.03
Spices, Other	0.74	0.90	1.07	0.30	0.36	0.58	0.63	0.71	0.11	2.14	0.82	0.22	0.22	0.52	1.01
Wine	85.37	60.38	85.92	16.55	166.38	50.71	44.58	21.70	156.90	29.62	26.55	158.22	103.78	33.48	39.01
Beer	309.23	332.30	330.99	229.42	82.33	344.16	105.75	364.16	61.51	289.29	145.75	188.14	181.10	182.55	287.29
Beverages, Fermented	6.30	0.22	0.33	6.60	31.15	0.00	0.16	4.77	0.03	0.00	1.73	0.41	1.89	0.19	0.49
Beverages, Alcoholic	13.64	7.51	5.73	9.12	6.85	17.78	7.75	23.97	2.38	7.26	4.74	29.15	6.71	12.44	7.45
Alcohol, Non-Food	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bovine Meat	54.63	58.25	56.55	52.88	75.45	41.04	53.95	46.36	66.71	56.33	57.10	40.52	35.12	52.99	46.30
Mutton & Goat Meat	3.18	4.90	2.30	1.04	11.89	2.71	35.59	25.92	4.41	3.04	15.67	9.29	16.52	1.97	17.73

Product	Austria	Belgium-Luxembourg	Denmark	Finland	France	Germany	Greece	Ireland	Italy	Netherlands	Norway	Portugal	Spain	Sweden	United Kingdom
Pigmeat	175.12	93.67	157.37	90.03	97.59	142.22	82.99	96.79	96.08	130.58	63.29	109.23	154.11	99.86	67.34
Poultry Meat	42.85	51.53	50.55	28.11	66.66	34.68	45.37	65.73	50.60	48.90	20.88	64.36	65.51	23.95	70.27
Meat, Other	3.07	10.77	2.41	6.68	15.51	5.34	3.67	0.03	15.10	5.01	4.00	4.38	9.97	6.58	0.38
Fats, Animals, Raw	26.82	37.15	33.64	4.08	13.56	18.33	1.29	13.78	13.92	15.64	14.63	24.00	6.47	13.59	10.96
Butter, Ghee	12.71	17.53	5.07	11.95	24.90	19.34	3.01	9.73	5.86	6.22	6.36	4.30	0.93	11.62	7.95
Cream	12.77	18.52	21.89	17.95	14.36	19.32	5.81	23.23	7.92	0.00	21.59	2.52	3.15	26.96	0.05
Honey	2.79	1.75	0.90	1.37	1.75	2.85	4.03	1.12	0.99	0.93	1.04	1.15	1.75	1.18	1.10
Freshwater Fish	7.37	7.97	11.26	23.23	8.25	5.78	6.41	8.77	3.95	6.08	12.99	0.82	4.33	7.34	5.29
Demersal Fish	7.89	20.05	7.62	13.70	22.22	14.47	26.38	24.79	20.49	11.81	61.18	104.05	36.36	20.52	27.78
Pelagic Fish	9.53	11.15	15.01	48.96	11.51	14.52	18.47	10.79	11.26	9.62	21.73	30.05	18.58	23.10	9.34
Marine Fish, Other	2.38	5.78	0.00	5.42	5.29	1.67	5.15	0.00	4.93	0.00	1.23	7.70	5.37	11.51	0.30
Crustaceans	1.40	6.88	10.90	5.37	9.70	1.40	2.16	5.04	3.89	3.21	29.78	4.11	11.59	17.53	9.10
Cephalopods	0.11	0.49	0.08	0.03	1.75	0.30	6.16	0.00	7.95	2.74	0.16	7.97	8.05	0.05	0.38
Molluscs, Other	2.60	3.78	20.82	1.67	17.64	4.44	4.79	5.29	10.58	5.86	9.64	5.45	16.85	3.97	2.55
Aquatic Animals, Others	0.00	0.33	0.00	0.00	0.19	0.05	0.05	0.00	0.00	0.00	0.00	0.41	0.22	0.00	0.03
Fish, Body Oil	0.00	1.18	14.11	0.00	1.23	1.56	0.00	0.00	0.00	2.08	0.00	0.14	0.00	0.00	1.18
Fish, Liver Oil	8.47	0.00	0.00	0.00	0.08	0.00	0.00	0.03	0.08	0.00	0.00	0.03	0.00	0.00	0.00
Rice (Milled Equivalent)	0.00	17.97	8.68	12.19	12.41	8.27	19.18	8.58	15.64	11.01	10.25	44.08	21.23	10.66	5.15
Miscellaneous	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Milk - Excluding Butter	768.77	649.37	652.16	935.78	688.30	654.33	680.03	698.93	689.26	1016.47	739.73	526.25	449.48	952.38	639.21
Eggs	38.25	39.12	43.64	26.52	43.48	34.60	27.15	16.41	34.52	44.49	27.67	24.71	35.62	31.73	27.86
TOTAL	2810.68	2923.15	2788.19	2561.26	2663.45	2650.19	3154.00	2739.64	2826.38	2937.62	2538.60	2936.52	2596.68	2674.52	2504.74

Table X-5. Background intakes of EPA, DHA and DPA (n-6) from levels inherent in foods.

Food description	EPA intake, mg/day			DHA intake, mg/day			DPA (n-6) intake, mg/day		
	Mean	95th%ile	97.5th%ile	Mean	95th%ile	97.5th%ile	Mean	95th%ile	97.5th%ile
Carcase meats	11	26	30	5	13	15	1	3	3
Offals	5	13	16	1	2	2	26	69	84
Poultry	7	17	21	10	25	32	2	6	7
White fish	27	63	75	32	76	90	10	23	27
Fatty fish	119	306	400	150	385	503	6	15	19
Oils & fats	1	3	3	0	0	0	0	0	0
Eggs	0	0	0	28	68	81	10	25	29
Grand Total	75	244	303	107	330	401	27	66	83

Food description	EPA intake, mg/kg bw/day			DHA intake, mg/kg bw/day			DPA(n-6) intake, mg/kg bw/day		
	Mean	95th%ile	97.5th%ile	Mean	95th%ile	97.5th%ile	Mean	95th%ile	97.5th%ile
Carcase meats	0.16	0.37	0.44	0.08	0.18	0.22	0.02	0.04	0.04
Offals	0.07	0.18	0.22	0.01	0.02	0.03	0.38	0.98	1.18
Poultry	0.10	0.24	0.31	0.15	0.37	0.46	0.03	0.09	0.11
White fish	0.39	0.89	1.06	0.47	1.07	1.27	0.14	0.32	0.38
Fatty fish	1.70	4.27	6.01	2.14	5.37	7.56	0.08	0.21	0.29
Oils & fats	0.02	0.04	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Eggs	0.00	0.00	0.00	0.41	0.98	1.16	0.15	0.36	0.42
Grand Total	1.08	3.49	4.46	1.54	4.55	5.85	0.39	0.96	1.22

Food description	EPA intake, % total energy			DHA intake, % total energy			DPA(n-6) intake, % total energy		
	Mean	95th%ile	97.5th%ile	Mean	95th%ile	97.5th%ile	Mean	95th%ile	97.5th%ile
Carcase meats	0.005	0.013	0.015	0.003	0.006	0.007	0.001	0.001	0.001
Offals	0.001	0.004	0.006	0.000	0.001	0.001	0.004	0.022	0.030
Poultry	0.002	0.008	0.010	0.004	0.012	0.015	0.001	0.003	0.003
White fish	0.008	0.028	0.035	0.010	0.034	0.042	0.003	0.010	0.013
Fatty fish	0.021	0.104	0.146	0.027	0.131	0.184	0.001	0.005	0.007
Oils & fats	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Eggs	0.000	0.000	0.000	0.012	0.033	0.040	0.004	0.012	0.015
Grand Total	0.038	0.125	0.166	0.055	0.168	0.225	0.014	0.035	0.043

Table X-6. Background intakes of sterols from levels inherent in foods.

TDS Code	Food description	Cholesterol (mg/day)			Brassicasterol (mg/day)			Campesterol (mg/day)		
		Mean	95th %ile	97.5th%ile	Mean	95th %ile	97.5th %ile	Mean	95th %ile	97.5th%ile
1	Bread	0.0	0.0	0.0	0.2	0.4	0.5	5.9	11.8	14.2
2	Other cereals	4.2	10.3	13.0	0.3	0.6	0.8	5.9	14.4	18.2
3	Carcase meats	51.9	123.5	142.9	0.0	0.0	0.0	0.3	0.7	0.8
4	Offals	45.6	118.7	145.6	0.0	0.0	0.0	0.3	0.9	1.1
5	Meat products	46.5	119.0	140.2	0.3	0.7	0.9	0.3	0.9	1.0
6	Poultry	36.6	89.3	113.8	0.0	0.0	0.0	0.4	1.1	1.4
7	Fish	20.2	48.2	57.8	0.2	0.6	0.7	1.1	2.6	3.1
8	Oils & fats	8.3	21.6	25.6	1.6	4.1	4.8	9.8	25.6	30.3
9	Eggs	103.4	246.0	295.8	0.3	0.8	1.0	0.8	2.0	2.4
10	Sugars & preserves	2.3	6.1	7.3	0.0	0.1	0.1	0.8	2.2	2.7
11	Potatoes	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.5	0.5
12	Other vegetables	1.1	2.5	3.0	0.1	0.3	0.3	3.3	7.3	8.7
13	Canned vegetables	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.5
14	Beverages	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	Milk	23.8	53.3	61.6	0.2	0.4	0.5	0.0	0.0	0.0
16	Dairy products	40.1	100.0	117.9	0.0	0.0	0.0	0.4	1.1	1.3
17	Nuts	0.0	0.0	0.0	0.0	0.1	0.1	1.1	3.2	3.8
	Total	303.5	535.0	585.6	2.8	5.7	6.5	27.1	49.9	55.3

TDS Code	Food description	Stigmasterol (mg/day)			b - Sitosterol (mg/day)			Fucostanol (mg/day)		
		Mean	95th %ile	97.5th%ile	Mean	95th%ile	97.5th %ile	Mean	95th %ile	97.5th%ile
1	Bread	0.6	1.1	1.3	16.7	33.6	40.4	2.0	4.0	4.8
2	Other cereals	1.2	2.9	3.6	16.1	39.0	49.4	2.2	5.3	6.8
3	Carcase meats	0.0	0.0	0.0	0.1	0.3	0.3	0.0	0.0	0.0
4	Offals	0.0	0.0	0.0	0.1	0.1	0.2	0.0	0.0	0.0
5	Meat products	0.1	0.1	0.2	0.7	1.7	2.0	0.0	0.1	0.1
6	Poultry	0.0	0.0	0.0	0.1	0.3	0.4	0.0	0.0	0.0
7	Fish	0.1	0.3	0.4	1.6	3.7	4.4	0.0	0.0	0.0
8	Oils & fats	0.8	2.2	2.6	15.6	40.6	48.2	0.0	0.0	0.0
9	Eggs	0.0	0.0	0.0	0.2	0.4	0.5	0.0	0.0	0.0
10	Sugars & preserves	1.7	4.6	5.4	4.3	11.4	13.4	0.0	0.0	0.0
11	Potatoes	0.2	0.5	0.5	1.1	2.4	2.7	0.0	0.0	0.0
12	Other vegetables	1.7	3.8	4.5	8.7	19.3	23.2	0.0	0.0	0.0
13	Canned vegetables	0.7	1.9	2.3	1.3	3.5	4.2	0.0	0.0	0.0
14	Beverages	0.0	0.0	0.0	0.5	1.6	2.1	0.0	0.0	0.0
15	Milk	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	Dairy products	0.0	0.0	0.0	0.4	1.1	1.3	0.0	0.0	0.0
17	Nuts	0.6	2.0	2.3	6.4	19.4	22.9	0.1	0.3	0.3
	Total	6.6	11.4	12.5	63.8	111.5	122.9	4.2	7.9	9.2

TDS Code	Food description	d5-Avenasterol (mg/day)			d7-Stigmasterol (mg/day)			d7-Avenasterol (mg/day)		
		Mean	95th %ile	97.5th %ile	Mean	95th %ile	97.5th %ile	Mean	95th %ile	97.5th%ile
1	Bread	1.0	2.0	2.4	0.4	0.9	1.1	0.3	0.3	0.7
2	Other cereals	1.1	2.7	3.4	0.4	1.0	1.3	0.3	0.3	0.8
3	Carcase meats	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	Offals	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	Meat products	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	Poultry	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	Fish	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
8	Oils & fats	0.9	2.3	2.8	0.8	2.0	2.4	0.2	0.2	0.6
9	Eggs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	Sugars & preserves	0.2	0.5	0.6	0.1	0.2	0.2	0.0	0.0	0.0
11	Potatoes	0.5	0.9	1.1	0.0	0.0	0.0	0.0	0.0	0.0
12	Other vegetables	0.5	1.0	1.2	1.2	2.8	3.3	0.1	0.1	0.3
13	Canned vegetables	0.2	0.6	0.7	0.0	0.0	0.0	0.0	0.0	0.0
14	Beverages	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	Milk	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	Dairy products	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	Nuts	0.9	2.7	3.2	0.0	0.1	0.2	0.1	0.1	0.2
	Total	4.2	7.4	8.1	2.8	5.1	5.7	1.0	1.0	1.8

Table X-7. Background intakes of total phytosterols from levels inherent in foods.

TDS Code	Food description	Total phytosterols* (mg/day)			Total phytosterols* (mg/kg bw/day)		
		Mean	95th%ile	97.5th%ile	Mean	95th%ile	97.5th%ile
1	Bread	27.2	54.6	65.5	0.39	0.78	0.92
2	Other cereals	27.5	66.7	84.4	0.41	1.00	1.27
3	Carcase meats	0.4	0.9	1.1	0.01	0.01	0.02
4	Offals	0.4	1.0	1.2	0.01	0.01	0.02
5	Meat products	1.4	3.5	4.2	0.02	0.05	0.06
6	Poultry	0.6	1.4	1.8	0.01	0.02	0.03
7	Fish	3.0	7.3	8.7	0.04	0.10	0.12
8	Oils & fats	29.8	77.3	91.8	0.43	1.11	1.26
9	Eggs	1.3	3.2	3.9	0.02	0.05	0.06
10	Sugars & preserves	7.2	19.0	22.5	0.11	0.28	0.36
11	Potatoes	2.1	4.2	4.9	0.03	0.06	0.07
12	Other vegetables	15.6	34.6	41.5	0.23	0.51	0.60
13	Canned vegetables	2.4	6.4	7.6	0.04	0.09	0.12
14	Beverages	0.5	1.6	2.1	0.01	0.02	0.03
15	Milk	0.2	0.4	0.5	0.00	0.01	0.01
16	Dairy products	0.9	2.1	2.5	0.01	0.03	0.04
17	Nuts	9.2	27.9	33.0	0.14	0.39	0.53
	Total	112.5	198.1	221.3	1.64	2.90	3.31

* Excluding cholesterol.

Table X-8. Average per capita consumption of foods grouped into TDS categories.

TDS		Austria	Belgium-Luxembourg	Denmark	Finland	France	Germany	Greece	Ireland	Italy	Netherlands	Norway	Portugal	Spain	Sweden	UK	EU
1	Bread	190	251	217	196	257	200	389	273	405	174	257	262	253	210	236	251
2	Other cereals	43	32	92	80	51	71	28	80	28	32	75	88	31	57	26	54
3	Carcass meats	233	157	216	144	185	186	173	169	167	190	136	159	206	155	131	174
4	Offals	27	37	34	4	14	18	1	14	14	16	15	24	6	14	11	17
5	Meat products	3	11	2	7	16	5	4	0	15	5	4	4	10	7	0	6
6	Poultry	43	52	51	28	67	35	45	66	51	49	21	64	66	24	70	49
7	Fish	58	81	61	30	75	66	108	38	100	88	56	72	108	51	52	70
7a	White	20	37	23	68	39	31	50	36	37	21	84	142	60	55	37	49
7b	Fatty	7	8	11	23	8	6	6	9	4	6	13	1	4	7	5	8
8	Oils & fats	20	42	17	3	32	31	81	22	65	43	29	48	73	19	24	37
9	Eggs	38	39	44	27	43	35	27	16	35	44	28	25	36	32	28	33
10	Sugars & preserves	123	121	113	98	97	101	82	108	78	138	124	96	82	126	108	106
11	Potatoes	161	299	196	189	183	201	187	347	104	231	212	345	245	178	311	226
12	Other vegetables	262	367	263	194	330	228	645	200	478	263	173	415	356	191	237	307
13	Canned vegetables	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	Beverages	436	412	449	294	303	432	169	429	235	355	205	386	305	257	348	334
15	Milk	769	649	652	936	688	654	680	699	689	1016	740	526	449	952	639	716
16	Dairy products	25	36	27	30	39	39	9	33	14	6	28	7	4	39	8	23
17	Nuts	3	12	2	2	3	5	2	4	1	10	3	3	4	4	10	5

Table X - 9. Average intakes of DHA, DPA(n-6) and EPA by EU consumers (plus Norway).

	Austria	Belgium-Luxembourg	Denmark	Finland	France	Germany	Greece	Ireland
EPA	141	160	249	145	119	137	149	111
DHA	196	213	317	203	156	171	171	150
DPA(n-6)	98	91	53	64	62	39	52	57

	Italy	Netherlands	Norway	Portugal	Spain	Sweden	United Kingdom	EU average*
EPA	118	205	202	148	148	116	151	155
DHA	162	258	249	188	186	155	196	174
DPA(n-6)	60	73	107	56	61	51	67	137

* EU average is unweighted for population size.

Table X-10. Average intakes of omega-3 fatty acids by EU consumers (plus Norway).

	Austria	Belgium-Luxembourg	Denmark	Finland	France	Germany	Greece	Ireland
Cholesterol	427	462	269	379	350	284	360	346
Brassicasterol	6	9	5	4	7	8	15	7
Campesterol	65	101	68	54	81	84	132	73
Stigmasterol	16	16	11	13	13	19	18	15
ß-Sitosterol	132	153	103	100	129	176	213	152
Fucostanol	6	6	6	6	6	8	7	8
ä5-Avenasterol	9	9	6	6	8	10	13	9
ä7-Stigmasterol	7	7	5	5	6	11	10	9
ä7-Avenasterol	2	2	1	1	2	3	3	2
Total phytosterol	242	304	206	190	253	318	410	274

	Italy	Netherlands	Norway	Portugal	Spain	Sweden	United Kingdom	EU Average*
Cholesterol	477	317	338	361	395	316	347	362
Brassicasterol	13	9	7	10	14	5	6	8
Campesterol	100	94	92	87	114	55	72	85
Stigmasterol	18	15	16	16	17	13	14	15
ß-Sitosterol	179	152	151	161	188	113	129	149
Fucostanol	4	7	7	5	5	5	6	6
ä5-Avenasterol	11	9	10	10	11	8	8	9
ä7-Stigmasterol	9	7	8	9	9	5	7	7
ä7-Avenasterol	2	2	2	2	3	2	2	2
Total phytosterol	336	295	293	300	360	205	244	282

* EU average is unweighted for population size.

XI. Nutritional Information on the Novel food

According to the SCF guidelines the following questions must be asked at this stage:

- "Is there information to show that the Novel Food is nutritionally equivalent to existing foods that it might replace in the diet?"

The answers to these questions are outlined in the Section below:

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XI. Nutritional Information

A. Nutritional Equivalence to Existing Foods

The sources in the diet of LC PUFA's and other components identified DHA-rich oil have been discussed extensively in Sections IX, X and XIII. It has been shown that all the key components are present in varying amounts in the human food chain (this is discussed in detail in Section XIII). However, it can be said that DHA-rich oil will in many cases be used to provide DHA that would typically be provided by fish or fish oil. Whilst fish stocks are in decline and concern is raised about the levels of contaminants present (PCB, pesticides etc.), DHA-rich oil from algae represents a valuable alternative. Indeed algal oil derived from the species *Cryptocodinium cohnii* is already available on the European Market for use in infant foods and nutritional supplements, having been reviewed by the Netherlands and UK Voluntary Novel Foods Committees in 1996 (ACNFP Annual Report 1996).

It has been shown that the individual ratios of PUFA vary according to the food source and for DHA-rich oil the key points to note are the much lower levels of eicosapentaenoic acid (EPA 20:5n-3) and the increased content of docosapentaenoic acid (DPA 22:5n-6). DPA(n-6) is present in a wide variety of foods (see Sections IX, XI and XIII), but to specifically review the compositional ratio of DPA(n-6):DHA(n-3) in human breast milk, it is reported to range normally from 1:1 to 1:6 (Clandinin et al., 1981; Putnam et al., 1982; Carlson et al., 1986; Sanders et al., 1978; Sanders et al., 1979; Koletzko et al., 1992). The ratio of DPA(n-6) to DHA(n-3) in *Schizochytrium* and it's DHA-rich oil is 1:3, within the range reported for breast milk.

B. Nutritional Benefits of DHA-rich oil

Intervention trials support the strength of the association between heart disease and omega-3 fatty acids (EPA and DHA) and the consistency of the observed endpoint. The GISSI trial (Valgussa et al. 1999) in particular, supports the specificity of the EPA and DHA (1:2) components as responsible for the improvement in cardiovascular endpoints since it utilized dietary supplementation with known amounts of EPA and DHA and controlled for appropriate factors.

DHA is a fatty acid which is not effectively synthesized in the body and therefore must be obtained in the diet. DHA is a precursor of cellular membrane lipids, eicosanoids that are important in normal physiological and inflammatory processes. In these processes, EPA and DHA compete with another class of lipids, the omega-6 fatty acids that are the more prevalent in the Western Diet. DHA has many effects that could contribute to prevention of heart disease. These include:

- Prevention of arrhythmia (ventricular tachycardia and fibrillation)(Albert et al. 1998, Nair et al. 1997);
- Antithrombotic activity (Cerbone et al., 1999);
- Hypolipidemic properties on triglycerols and VLDLs (Grimsgaard 1997, Agren et al. 1996);
- Precursors of prostaglandins and leukotrienes (Scheulen et al., 1993, Mann et al., 1997);
- Stimulation of endothelial-derived nitric oxide (Johansen et al., 1999);
- Anti-inflammatory properties (Johansen et al., 1999); and
- Inhibition of atherosclerosis (Von Schacky et al., 1999).

The effects listed above have been demonstrated in *in vitro* and *in vivo* studies and clinical trials continue to examine the role of omega-3 fatty acids (EPA and DHA) (EPA and DHA) in modulating these effects. However, the data to support the link between triglycerol lowering,

antithrombotic activity, and anti arrhythmic activity and omega-3 fatty acids (EPA and DHA) has reached a significant level. The trials currently available demonstrate that these substances have clinical benefit when each of the three endpoints are examined.

In the US, during the rulemaking on health claims for omega-3 fatty acids (EPA and DHA) and heart disease, FDA concluded that "fish oils reduce plasma triglycerides." Studies published since that time using both dietary supplement and food sources of omega-3 fatty acids (EPA and DHA) in both normal and specialized (hyperlipidemic and heart disease and diabetes) populations continue to support the effectiveness and safety of the substances for this action.

DHA has many potential physiologic effects. Of these effects, increasing heart rate variability over a 24 hour period which has been shown to reduce cardiac arrythmia appears to have support as a mechanism by which these substances prevent death from heart disease. DHA is incorporated into phospholopid membranes of many types of cells and it is here where they are postulated to exert their effects. It has been shown in animals that omega-3 fatty acids (EPA and DHA) are antiarrythmic. Omega-3 fatty acids (EPA and DHA) stabilize the electrical activity of isolated cardiac myocytes. These essential fatty acids also inhibit voltage-dependent sodium currents and L-type calcium currents appear to be the major antiarrhythmic mechanisms of Omega-3 fatty acids (EPA and DHA) (EPA and DHA) (Leaf *et al.* 1999a.) Clinical trials including the large GISSI trial, 1999 support these actions of EPA and DHA.

As a precursor in eicosanoid production, DHA has many potential physiological effects including their effects on platelets and clotting many trials support the beneficial effects of heart disease. Thrombosis is a complication of atherosclerosis that can lead to myocardial infarction. (Heemskerk 1996) and Engler 1994) have reviewed the mechanisms of omega-3 fatty acids (EPA and DHA) on thrombosis. Omega-3 fatty acids (EPA and DHA) are incorporated into phospholopid membranes of many types of cells and it is here where they are postulated to exert their effects. In platelets, omega-3 fatty acids (EPA and DHA) inhibit the synthesis of thromboxane A_2 by replacing arachodonic acid in the membrane phospholipids. Thromboxane A_2 causes platelet aggregation, activation and vasoconstriction. As a result, a less potent eicosanoid, prostacyclin is produced and effects on platelet aggregation, activation and vasoconstriction are reduced.

XII Microbiological Information on the Novel Food

According to the SCF guidelines the following questions must be asked at this stage:

- "Is the presence of any microorganisms or their metabolites due to Novelty of the Process?"

The answers to these questions are detailed in this Section below:

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XII Microbiological Information on the Novel Food

DHA-rich oil is manufactured under the general guidelines of food chemical Good Manufacturing Practices (Food Chemical Codex pp xxvii, 4th edition). The incorporation of typical food borne microbes is inhibited by a combination of heat treatment applied to the cultured algal cells, the environmental conditions of the oil extraction and processing, and the extremely low water activity of the finished oil product.

XIII. Toxicological Information on the Novel Food.

According to the SCF guidelines the following questions must be asked at this stage:

- "Is there a traditional counterpart to the Novel Food that can be used as a baseline to facilitate the toxicological assessment?"

- "Compared to the traditional counterpart, does the Novel Food contain any new toxicants or changed levels of existing toxicants?"

OR

- "Is there information from a range of toxicological studies appropriate to the Novel Food to show that the Novel Food is safe under anticipated conditions of preparation and use?"

- "Is there information which suggests that the Novel Food might pose an allergenic risk to humans?"

The answers to these questions are outlined in the Section below:

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XIII. Toxicological Information

The safety of DHA-rich oil intended for consumption as a nutritional food ingredient is established by the history of safe consumption of the components of the oil. The safety is further supported by published safety studies on a similar single-cell oil and by the historical safe use of fish oils of similar composition. The *Schizochytrium* sp. marine microalgae is the source of the oil. *Schizochytrium* sp. is known to be consumed by marine animals which, in turn, are consumed by humans. Neither of two toxins found in other genera of the same kingdom of microorganisms are present in the source species. Finally, the safety of the oil is confirmed by safety studies utilising the intact, dried microalgae or the DHA-rich oil.

A. Summary of Confirmatory Safety Studies with Dried *Schizochytrium* sp. Microalgae and its Constituent DHA-rich Oil

Schizochytrium sp. microalgae contains oil rich in polyunsaturated fatty acids (PUFAs). DHA is the most abundant PUFA component of the oil (approx. 35% w/w). DHA-rich oil extracted from *Schizochytrium* sp. microalgae is currently used in the U.S. as a nutritional supplement, 1 gram of oil delivering approximately 350 mg DHA, and has been Generally Regarded as Safe as a nutritional food ingredient up to 1.5 g DHA per day. Assuming an average human body weight of 60 kg, ingestion of 1 gram of oil would result in a dosage of 16.6 mg oil/kg bodyweight. Fatty acid and sterol components of oil from *Schizochytrium* sp. microalgae have been characterized and identified as normal constituents of common human foods. Therefore, the components of *Schizochytrium* sp. microalgal oil have a history of safe consumption. Exposure to components of *Schizochytrium* sp. microalgal oil from its use as a nutritional food ingredient are within the normal range of exposures from consumption of foods with these components.

Several safety studies have been conducted with dried DHA-rich microalgae from *Schizochytrium* sp. These studies were performed according to 1982 FDA Redbook Guidelines (US FDA, 1982) and in compliance with FDA Good Laboratory Practice (GLP) regulations. For more detailed information on the safety studies, refer to Overview of Confirmatory Safety Studies with *Schizochytrium* sp. Dried Microalgae, Appendix D. Draft manuscripts, accepted and pending publication in peer-reviewed journals, are available in Appendix E of this submission. All of the safety studies provide confirmatory safety information on the source microalgae and the oil it contains since the fatty acid and sterol components already have a history of safe consumption in food. These confirmatory safety studies have been conducted on *Schizochytrium* sp. dried microalgae produced in three separate production campaigns (fermentation processes PB26, AS4, HD1). During these campaigns, process improvements were implemented to increase the production of oil and DHA content and add supplemental vitamin E acetate to the oil. Vitamin E acetate provides a supplemental nutritional source of antioxidant given the high PUFA content of oil. Compositional analysis found the same fatty acids and sterols present in oil produced during the three production campaigns. Process improvements did not result in the introduction of new fatty acids or sterols into the oil.

A battery of mutagenicity studies was carried out with: 1) intact *Schizochytrium* sp. microalgal cells (Ames, In Vitro Human Lymphocytes, Mouse lymphoma assays); 2) lysed *Schizochytrium* sp. microalgal cells (Ames, AS52/XPRT Gene Locus, Mouse Micronucleus assays); and 3) an Ames test with DHA-rich oil, extracted and refined as described in Section II, from *Schizochytrium* sp. microalgae.

A one-generation rat reproduction study was carried out with dried DHA-rich microalgae from *Schizochytrium* sp. administered at average dosages up to 17,800 mg/kg per day (males) and 20,700 mg/kg per day (females) when animals were fed up to 30% dried microalgae in the diet. Dietary teratology studies with the dried microalgae were conducted in the rat and

the rabbit. Rats were fed up to 30% dried microalgae in the diet (up to 22,000 mg/kg per day); the rabbit dietary teratology study could not be completed due to technical problems. A rabbit gavage teratology study was conducted at dosages up to 1,800 mg/kg per day dried microalgae.

An acute gavage study in mice was conducted with DHA-rich oil, extracted and refined as described in Section II, from *Schizochytrium* sp. microalgae, administered at a single high dosage of 2,000 mg/kg.

Thirteen-week rat feeding studies were carried out with dried DHA-rich microalgae from *Schizochytrium* sp. in one study at dietary levels up to 30% (approximately 18,000 mg/kg per day) and in a second study at dosages up to 4,000 mg/kg per day.

Target animal safety trials were conducted with dried DHA-rich microalgae from *Schizochytrium* sp. , specifically in laying hens and broiler chickens. For the laying hens, dose levels of 165, 495 and 825 mg DHA/hen per day were administered in the form of dried microalgae. For the broiler chickens, treatments of 82, 240 and 408 mg DHA/broiler per day were administered in the form of dried microalgae.

Results of confirmatory safety studies establish that dried DHA-rich microalgae from *Schizochytrium* sp. and its component oils are not mutagenic in bacterial and mammalian test systems and are not teratogenic in a rat dietary teratology study and a rabbit gavage teratology study. There is no evidence that DHA-rich microalgae interfered with reproductive performance or progeny development in a rat one-generation dietary reproduction study. The algae was fed to rats for 13 weeks in two separate feeding studies. In the first study, the dietary levels of microalgae were too high and caused nutritional imbalances in test animals. In the second 13-week feeding study, lower dietary levels of the DHA-rich microalgae were fed. In this study, there was no evidence of toxicity, and the only findings were anticipated changes in clinical chemistry parameters and microscopic changes commonly observed in rats following consumption of diets high in PUFA's. In two target animal safety studies, namely, laying hens and broiler chickens, no evidence of any adverse effect on any parameters evaluated in either study were noted. DHA-rich oil, extracted and refined from *Schizochytrium* sp. microalgae, produced no effects when administered by gavage as a single high dose to mice. There were no adverse effects observed in confirmatory safety studies that could be attributed to algal toxins which supports the absence of detectable algal toxins from the microalgae (see Section III).

1. Sub-Chronic Feeding Studies

Dried DHA-rich microalgae from *Schizochytrium* sp. was administered in the diet to rats for at least thirteen weeks. The algae was administered in the diet to groups of twenty male and twenty female Sprague-Dawley derived rats to provide dosages of 0, 400, 1,500 and 4,000 mg/kg/day for at least 13 weeks. The algae contained high levels of fat (approximately 41% w/w) of which long chain polyunsaturated fatty acids (PUFA's) were a major component. Vitamin E acetate was added to the dried microalgae at manufacture to provide supplementary dietary antioxidant given the highly unsaturated fat content of the microalgae. Untreated controls received basal diet only. An additional group of twenty males and twenty females received rodent diet mixed with fish oil (Arista) to provide a target dosage of 1,628 mg/kg/day, an amount of fat comparable to that received by rats administered the highest dose of the DHA-rich microalgae. Vitamin E acetate was also added to the fish oil to provide a comparable level of dietary antioxidant provided to high dose DHA-rich microalgae rats.

There were no treatment-related effects in clinical observations, body weights or weight gains, food consumption, haematologic or urinalysis values, gross necropsy findings or organ weights. The only treatment-related changes in clinical chemistry parameters were decreases

in high-density lipoproteins (HDL) and cholesterol in the microalgae and fish oil groups when compared to the untreated controls. These changes were expected based on the PUFA content of dried microalgae and fish oil. There were no microscopic findings suggestive of toxicity. Periportal hepatocellular fat vacuolation (accumulation of fat) was observed only in the livers of female rats in both the algae (all dosages) and fish oil groups. This finding was expected given the higher fat content of both the algae and fish oil diets compared to the basal diet fed to the untreated controls. A slight increase in the incidence, but not severity, of cardiomyopathy was observed only in the 4,000 mg/kg/day algae males. This finding was not considered adverse because cardiomyopathy occurs spontaneously in rats, and especially male rats of the Sprague-Dawley strain when fed high levels of fat. Since cardiomyopathy does not develop in other species including primates fed high fat diets, its occurrence in rats is considered to have little relevance to human health.

This study demonstrates that administration of dried microalgae did not produce any treatment-related adverse effects in Sprague-Dawley rats at dosages up to 4,000 mg/kg/day for 13 weeks (Hammond et al., 2001a) (see also Appendix D and E).

2. Developmental Toxicity Evaluation in Rats and Rabbits

The developmental toxicity of dried DHA-rich microalgae from *Schizochytrium* sp. was assessed in Sprague-Dawley-derived rats (25 per group provided dried microalgae in the diet at 0.6, 6 and 30% on gestation days [GD] 6-15 and in New Zealand White Rabbits, (22 per group, dosed with dried microalgae at levels of 180, 600 and 1,800 mg/kg/day by oral gavage on GD 6-19). Fish oil was used as a negative control at dose levels to provide an equivalent amount of fat to that received by the high dose dried microalgae rabbits. Maternal food consumption, body weights and clinical signs were recorded at regular intervals throughout these studies. Animals were sacrificed on GD 20 (rats) and GD 29 (rabbits) and examined for implant status, fetal weight, sex and morphologic development. No clinical signs of toxicity were observed. Maternal exposure to dried microalgae during organogenesis did not adversely affect the frequency of postimplantation loss, mean fetal body weight per litter, or external, visceral or skeletal malformations in either the rat or the rabbit.

In the rats, neither maternal nor developmental toxicity was observed at any dietary concentration of DHA-rich microalgae. Thus 22 g/kg/day of the DHA-rich microalgae administered in the feed to pregnant rats during organogenesis was the NOEL (no observed effect level) for both maternal and developmental toxicity.

3. Single Generation Rat Reproduction Toxicity

The reproductive toxicity of dried DHA-rich microalgae from *Schizochytrium* sp. was examined in Sprague-Dawley-derived rats Crl:CD®(SD)BR (30 per sex per group) provided dried microalgae in the diet at concentrations of 0, 0.6, 6.0 and 30%. These dietary levels corresponded to overall average dosages of approximately 400, 3,900 and 17,800 mg/kg/day for F_0 males (pre-mating) and 480, 4,600 and 20,700 mg/kg/day for F_0 females, respectively. Prior to mating, males and females of the F_0 generation were treated for 10 weeks and 2 weeks, respectively. Treatment of males continued throughout mating and until termination (approximately 3 weeks after mating). Treatment of the females was continued throughout gestation and through lactation day 21. The females were killed after raising their young to weaning at 21 days of age. Food consumption was measured weekly throughout the study (except during mating) and body weights were recorded at least weekly during premating, gestation and lactation. Reproductive parameters including oestrus cycle duration, mating performance, fertility, gestation length, parturition and gestation index were evaluated. Litter size, and offspring body weights were recorded, offspring viability indices were calculated, and physical development (vaginal opening and preputial separation) was assessed for the F_1 generation. All adult F_0 and F_1 animals were subjected to a detailed necropsy.

The DHA-rich microalgae treatment had no effects on oestrus cycles or reproductive performance including: mating performance, fertility, gestation length, parturition or gestation index. Litter size, sex ratio and offspring viability indices were similarly unaffected and there were no effects of dried microalgae treatment to the physical development of F_1 animals. (Hammond et al., 2001c) (see also Appendix D and E).

4. Mutagenicity Studies

A battery of mutagenicity studies was carried out with: 1) intact *Schizochytrium* sp. microalgal cells (Ames, In Vitro Human Lymphocytes, Mouse lymphoma assays); 2) lysed *Schizochytrium* sp. microalgal cells (Ames, AS52/XPRT Gene Locus, Mouse Micronucleus assays); and 3) an Ames test with DHA-rich oil, extracted and refined as described in Section II, from *Schizochytrium* sp. microalgae. Results of these studies establish that dried DHA-rich microalgae from *Schizochytrium* sp., its component oils, and extracted and refined DHA-rich oil are not mutagenic in bacterial and mammalian test systems. (Hammond et al., 2001d) (see also Appendix D and E).

5. Acute Study for DHA-rich Oil

An acute gavage study in mice was conducted with DHA-rich oil, extracted and refined from *Schizochytrium* sp. microalgae, administered at a single high dosage of 2,000 mg/kg. No effects attributed to DHA-rich oil were observed when administered by gavage as a single high dose to mice (Bechtel, 24 October 1997) (see also Appendix D and E).

6. Laying Hen Study

A target animal safety trial with laying hens was conducted using dried DHA-rich microalgae from *Schizochytrium* sp. at dose levels of 165, 495, and 825 mg DHA/hen/day. Each treatment consisted of 64 laying hens divided into eight replicates (cages) per group for a total of 320 animals on study. As required by FDA laying hen target animal safety protocols, all of the hens were preconditioned for one month prior to the start of dosing period by feeding a basal commercial type layer feed. Body weights, food conversion, egg production, egg weight, shell thickness, and interior quality were measured at the end of each of the four months during the dosing period. Eggs were also collected and analysed at the end of months 2 and 4 for their weight, shell thickness, interior egg quality, and fatty acid profile. At the end of the 4 month dosing period, terminal sacrifices were conducted and two randomly selected hens from each dose level and replicate were evaluated for haematological and histopathological changes. Haematological analyses included the following: red blood cell count, haematocrit, differential leukocyte count and hemoglobin. As dietary omega-3 fatty acids are known to decrease platelet reactivity, blood clotting time was also determined. Gross necropsy was completed on all layers found dead during the trial or killed for scheduled evaluation. Weights were determined for the following organs: liver, kidney, heart, bursa of Fabricus, brain, spleen, thymus, bone marrow, and ovaries. Tissues were collected for histopathology, preserved, and evaluated. Breast tissue samples were evaluated for fatty acid profile by gas chromatography. Consequence of experimental diets was determined via statistical analysis of feed consumption/efficiency, egg production, egg weight, egg quality, body weight, organ weight, and histopathology.

There were no significant differences in any of the organ weights measured and there were no significant differences in the feathering score between any of the treatments. The results of the histopathological examination also indicated that no alterations could be observed in the

tissues examined that would differentiate between treatment groups. There were also no significant differences between treatments for any of the haematological analyses

It was concluded, based on results from this study, that dried DHA-rich microalgae from *Schizochytrium* sp., is safe as a feed ingredient for laying hens at 3,040 mg/kg/day dried microalgae delivering approximately 532 mg DHA/kg/day and 177 mg DPA(n-6)/kg/day (Abril et al., 2000).

7. Broiler Chicken Study

A target animal safety trial with broiler chickens was conducted with two thousand two hundred and forty birds, sexed at day of hatch, wingbanded, and randomly assigned to one of four dietary treatments. In addition to a control broiler ration, dietary treatments of dried DHA-rich microalgae from *Schizochytrium* sp. delivered 82, 240, and 408 mg DHA/bird per day. Each dietary treatment contained 560 broilers divided among eight replicates (n=70; 35 males; 35 females). All rations were pelletized for feeding to the birds. Group body weights for each pen were determined on days 0, 21, and 49 of the feeding trial. Feed consumption was evaluated for each pen on days 21, 42, and 49 of the trial and used to determine feed efficiency for feeding periods 0-21 days and 0-49 days. On days 4 and 49, birds (n=2 per replicate) were bled for haematological analyses and sacrificed for histopathologic evaluation. Haematological analyses included the following: red blood cell count, haematocrit, differential leukocyte count, and haemoglobin. As dietary n-3 FA are known to decrease platelet reactivity, blood clotting time was also determined. Gross necropsy was completed on all broilers found dead during the trial or killed for scheduled evaluation. Weights were determined for the following organs: liver, kidney, heart, bursa of Fabricus, brain, spleen, thymus, bone marrow, and ovaries. Tissues were collected for histopathology, preserved, and evaluated. Breast samples were evaluated for fatty acid profile by gas chromatography. Consequence of experimental diets was determined by statistical analysis of feed consumption/efficiency, body weight, organ weight, and histopathology.

The results of this study indicate that there was no effect of treatment level on any of the evaluated broiler growth performance measures. There was no significant difference between treatment level regarding weight gain, feed intake, or feed conversion. There was no significant difference between treatments on organ weight for the liver, kidney, heart, bursa of Fabricus, brain, spleen, thymus, bone marrow, or ovaries. The histopathological examination also indicated that no alterations could be observed in the tissues examined that would differentiate between treatment groups. There was no significant difference between treatment for any of the haematological analyses conducted.

It is concluded, based on results from this study, that dried DHA-rich microalgae from *Schizochytrium* sp., is safe as a feed ingredient for broiler chickens at 2,331 mg/bird/day dried microalgae delivering approximately 408 mg DHA/bird/day and 136 mg DPA(n-6)/bird/day.

8. Summary of Toxicology Studies

A summary of the no observed effect level (NOEL) of dried DHA-rich microalgae from *Schizochytrium* sp. and DHA-rich oil used in safety studies are presented in Table XIII-7. DHA and DPA(n-6) content in the *Schizochytrium* sp. microalgae and the oil extracted from this microorganism are calculated and used to estimate NOEL of both DHA and DPA(n-6). The NOEL for DHA in these animal studies ranged from 153-1,868 mg/kg/day. The NOEL for DPA(n-6) (in the presence of dietary DHA) in these animal studies ranged from 53-645 mg/kg/day. The results of these studies along with the studies presented here and the available scientific literature, indicate that dietary DPA(n-6) in the presence of dietary DHA is safe.

DHA-rich oil derived from *Schizochytrium* sp. microalgae has recently been determined by a panel of U.S. food safety experts to be GRAS (Generally Recognized as Safe) in food applications at a level providing 1.5 g/day of DHA and 0.5 g/day of DPA(n-6). This represents a daily DPA(n-6) intake (on a mg/kg basis) similar to that of breast feeding infants. While the panel recognised that the safety data indicated the oil was safe at higher intake levels than this, it is a principle of the GRAS process that ingredients should only be used in accordance with good manufacturing practices (cGMP's), a basic principle of cGMP is that ingredients added to the food can only be used at levels adequate to achieve their intended effect. DHA intake levels of 1.5 g/day are recognised by many experts as the level necessary to provide key cardiovascular and immune system health benefits.

Table XIII-7. Summary of the "No Observed Effect Level" (NOEL) for DPA(n-6) and DHA from the animal safety trials with dried* Schizochytrium *sp. microalgae and oil containing DPA(n-6) and DHA

Trial	Test article	NOEL(mg/kg/day) Test Article	DPA(n-6)	DHA
Acute Feeding				
Mouse - acute oral limit study	oil	2,000	284	693
Sub-chronic Feeding				
Rat - 90 day[1]	algae	4,000	117	340
Laying Hen -112 day[2]	algae	3,040	177	532
Broiler chicken – whole life	algae	2,331	136	408
Developmental & Reproductive Toxicity				
Rat – developmental toxicity[3]	algae	22,000	645	1,868
Rat – single generation reproduction[4]	algae			
males		17,800	522	1,512
females		20,700	607	1,757
Rabbit – developmental toxicity[3]	algae	1,800	53	153

[1] *Hammond et al., 2001a.*
[2] *Abril et al., 2000.*
[3] *Hammond et al., 2001b*
[4] *Hammond et al., 2001c.*

C. Conclusions

The safety of fatty acids present in DHA-rich oil is based on four factors: 1) extensive knowledge of FA metabolism; 2) experience of use as a result of their abundant natural presence in food, and the small quantities expected to be consumed; 3) published literature on the safety of the fatty acid components and of comparable oils; and 4) confirmatory safety studies with the dried microalgal source of DHA-rich oil and DHA-rich oil.

As described in this Section and in Section IX, the FA's are expected to be consumed in relatively small quantities, at levels similar to current consumption from natural sources. Fatty acids are easily converted to one or another form as needed or simply metabolized as a source of energy. Pathological accumulation of a specific FA type producing the sequelae found in animal studies would only occur in humans in cases of extreme abuse.

The human experience with these FA has been extensive and has demonstrated that, when part of a balanced diet, supplemental FA are beneficial. The few pathological findings in animals following supplementary FA administration are likely one or a combination of an inappropriate animal model or an excessive dose far beyond that anticipated for humans.

The safety of phytosterols found in DHA-rich oil has basis in five factors: 1) experience of use as a result of their abundant natural presence in food and the small quantities expected to be consumed; 2) extensive knowledge of the absorption, distribution, metabolism and excretion of phytosterols in mammalian species; 3) extensive safety information as the result of testing these and similar phytosterols; 4) easy identification of at-risk populations (i.e., sitosterolemia); and 5) results of confirmatory safety studies. In summary, these factors allow a conclusion that intake of phytosterols present in DHA-rich oil, taken as a nutritional food ingredient, is safe.

DHA-rich oil and the source of DHA-rich oil, *Schizochytrium* sp., a thraustochytrid and member of the kingdom Chromista (Stramenopilia), is safe based on published and unpublished scientific data and further corroborated by confirmatory safety studies. *Schizochytrium* sp. occurs widely in the marine environment and is an indirect component of the human food chain through indirect consumption of fish and other marine animals which feed on the microalgae. There have never been any reports of toxic compounds being produced by members of the thraustochytrids. Analytical tests indicate that the two toxins known to be produced by two other genera on the Chromista (in completely separate classes from the thraustochytrids) are not present in *Schizochytrium*.

Assessment of DHA-Rich Oil Derived from *Schizochytrium* sp. Dried Microalgae

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