# Application for the authorization of DHA and EPA-rich Algal Oil from *Schizochytrium* sp.

*Submitted pursuant to*
*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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**For the purpose of Regulatory filings Martek considers the marked specific data herein to be proprietary.**

# Application for the authorization of DHA and EPA-rich Algal Oil from *Schizochytrium* sp.

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## EXECUTIVE SUMMARY AND CONCLUSIONS

Martek Biosciences Corporation (Martek) has previously gained approval for docosahexaenoic acid (DHA)-rich oil produced from *Schizochytrium* sp. (hereinafter "DHA-S"), for general use as a nutritional ingredient in foods. Martek has developed an improved strain, from another species of *Schizochytrium* microalgae. This strain produces an oil which contains DHA as in DHA-S along with an eicosapentaenoic acid (EPA) content which is approximately half that of the DHA concentration. This DHA and EPA-rich oil from *Schizochytrium* sp. (hereafter called DHA-O) has a fatty acid profile that more closely represents that of other sources of long chain omega-3 oils. Martek intends to market DHA-O for similar categories to those currently approved for DHA-S, but with minor modifications to use levels to reflect recent developments in recommended daily intakes for DHA and EPA. Approval for uses in biscuits (cookies) and cooking oils is also sought. Because of the higher EPA content in DHA-O and additional uses requested, and following discussions with the Food Standards Agency, Martek is hereby presenting its application for the approval of DHA and EPA-rich algal oil from *Schizochytrium* sp. as a novel food ingredient 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*[1]. Under Article 1, point 2, DHA-O would be classified under group "(d) foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae".

The specification of the DHA-O is well defined with the principle composition being not less than 22.5% DHA and not less than 10% EPA. Oxidative stability is assured by the inclusion of acid value and peroxide value and the non-detectable levels of recovery solvent residues and other contaminants are confirmed by extensive independent analyses. Detailed fatty acid and sterols analyses reveal a profile and ratio of DHA to EPA similar to those of fish/fish oils and with no new components that are not already present in the diet.

The production of DHA-O is tightly controlled using standard fermentation, recovery, and purification techniques. Safe, suitable and approved antioxidants are used and, for commercial reasons, DHA and EPA content may be standardised using food grade vegetable oils, such as high oleic sunflower oil.

The proposed uses of DHA-O are largely the same as currently approved for DHA-S in the EU with a slight increase in levels for three categories to allow for increased EU dietary recommendations for DHA and EPA and to add to biscuits and cooking oils at low levels.

[1] European Parliament and Council of the European Union, 1997 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090807:EN:PDF)

Dietary survey data show that mean estimated daily intakes from all uses would not exceed 0.9 g of DHA+EPA per day (equivalent to 4 maximally fortified portions approximately) and 95$^{th}$ percentile intakes would not exceed 1.5 g (approximately 6 to 7 maximally fortified portions approximately). These estimates are clearly huge over-estimations.

In addition to the extensive safety database already available on *Schizochytrium* sp. algal biomass, on DHA-S and on fish oil, Martek has conducted supporting confirmatory pre-clinical studies on DHA-O, which include a 90-day rat study and a suite of mutagenicity studies. All of these show no significant adverse effects at the maximum dose tested. For the 90 day rat study the NOAEL for DHA-O equivalent to 3149 mg/kg body weight/day and 3343 mg/kg body weight/day for male and female rats respectively equivalent to DHA+EPA doses of 1669 and 1772. For a 60 kg adult this equates to approximately 200 g per person per day of DHA-O/ 100 g DHA +EPA. The absence of significant levels of protein and extensive history of safe consumption of DHA-S indicate there is no significant risk for allergenicity. DHA-O is therefore proposed as a safe and suitable vegetarian and sustainably produced alternative to fish oil for use in foods as a source of the important long-chain (LC) polyunsaturated fatty acids (PUFAs) DHA and EPA.

# INTRODUCTION

In March 2001, an application was submitted under Regulation No 258/97 of 27th January 1997 concerning novel foods and novel food ingredients, for the approval of docosahexaenoic acid (DHA)-rich oil produced from *Schizochytrium* sp. (hereinafter "DHA-S"), for general use as a nutritional ingredient in foods.

The above application and subsequent negotiations resulted in the following approval:

***COMMISSION DECISION of 5 June 2003 authorising the placing on the market of oil rich in DHA (docosahexaenoic acid) from the microalgae Schizochytrium sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (2003/427/EC)* (Commission of the European Communities, 2003)**

The authorized uses for DHA-S under this decision (as detailed in its Annex 2) are reproduced in Table 1.

**Table 1 Authorized Uses of DHA-S Pursuant to Decision 2003/427/EC**

| Food Category Use Group | Maximum Use Level of DHA |
|---|---|
| Dairy products except milk-based drinks | 200 mg/100 g or for cheese products 600 mg/100 g |
| Dairy analogues except drinks | 200 mg/100 g or for analogues to cheese products 600 mg/100 g |
| Spreadable fat and dressings | 600 mg/100 g |
| Breakfast cereals | 500 mg/100 g |
| Food supplements | 200 mg per daily dose as recommended by the manufacturer |
| Dietary foods for special medical purposes | In accordance with the particular nutritional requirements of the persons for whom the products are intended |
| Foods intended for use in energy-restricted diets for weight reduction | 200 mg/meal replacement |

In December 2007 Martek applied for additional use categories for DHA-S, which resulted in the following additional approval:

***2009/778/EC Commission Decision of 22 October 2009 concerning the extension of uses of algal oil from the micro-algae Schizochytrium sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council* (Commission of the European Communities, 2009a)**.

The additional authorised uses for DHA-S under this decision (as detailed in its Annex) are reproduced in Table 2.

| Table 2 Authorized Uses of DHA-rich Algal Oil (DHA-S) Pursuant to Decision 2009/778/EC | |
|---|---|
| **Proposed Food Category Use Groups** | **Maximum Use Level of DHA** |
| Bakery products (Breads and rolls) | 200 mg/100 g |
| Cereal bars | 500 mg/100 g |
| Non-alcoholic beverages (including milk based beverages) | 60 mg/100 mL |

The specification for DHA-S is laid down in Annex 1 of Decision 2003/427/EC, and the fatty acid content reflects a minimum DHA content of 32% (Commission of the European Communities, 2003).

Martek Biosciences Corporation (Martek) has previously gained approval for docosahexaenoic acid (DHA)-rich oil produced from *Schizochytrium* sp. (hereinafter "DHA-S") a microalgae, for general use as a nutritional ingredient in foods. Martek has developed an improved strain from another species of *Schizochytrium* microalgae. This strain produces an oil which contains docosahexaenoic acid (DHA) as in DHA-S along with an eicosapentaenoic acid (EPA) content which is approximately half that of the DHA concentration. This DHA and EPA-rich oil from *Schizochytrium* sp. (hereafter called DHA-O) has a fatty acid profile that more closely represents that of common sources of long chain omega-3 oils. Martek intends to market DHA-O for similar categories to those currently approved for DHA-S, but with minor modifications to use levels to reflect recent developments in recommended daily intakes for DHA and EPA. Approval for uses in biscuits (cookies) and cooking oils are also sought. Because of the higher EPA content in DHA-O and additional uses requested, and following discussions with the Food Standards Agency, Martek is hereby presenting its application for the approval of DHA and EPA-rich algal oil from *Schizochytrium* sp. as a novel food ingredient 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*[2]. Under Article 1, point 2, DHA-O would be classified under group:

"(d) foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae".

This application has been prepared in accordance with the EU recommendation of 29 July 1997, where relevant (Commission of the European Communities, 1997). Under these guidelines DHA-O would fall under class: 2.2 ('complex novel food from a non-GM source', 'the source of the novel food has no history of use in the community'). Consistent with the recommendations, Sections IV to VIII of the EU recommendation are not applicable to DHA-rich algal oil since no GM technology is involved.

[2] European Parliament and Council of the European Union, 1997 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090807:EN:PDF)

# 1. ADMINISTRATIVE DATA

The present petition is submitted by Martek Biosciences Corporation (Martek), manufacturer of DHA and EPA-rich oil from *Schizochytrium* sp. (DHA-O).

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# 2. GENERAL DESCRIPTION

DHA-O is classified as Class 2.2, *i.e.*, “complex Novel Food from non-GM Source”; the source of the NF has no history of use in the Community.

# 3. IDENTIFICATION OF THE ESSENTIAL INFORMATION REQUIREMENTS

In accordance with the EU guidelines, the requirements for the submission of a dossier for this class of Novel Food are as follows:

I. Specification of the Novel Food
II. Effect of the Production Process Applied to the Novel Food
III. History of Source Organism
IX. Anticipated Intake/Extent of Use
XI. Nutritional Information
XII. Microbiological Information
XIII. Toxicological Information

Whilst we do not include “Section X Information from Previous Human Exposure to the Novel Food or Its Source” in this dossier, clear comparisons are made at appropriate points throughout the dossier to both the approved DHA-S and to fish oil(s).

# I SPECIFICATION OF THE NOVEL FOOD

According to the Scientific Committee on Food (SCF) guidelines as published in the EU recommendation of 29 July 1997 (Commission of the European Communities, 1997), the following questions must be asked at this stage:

1. “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?”

2. “Is the information representative of the Novel Food when produced on a commercial scale?”

3. “Is appropriate analytical information available on the potential toxic inherent constituents, external contaminants and nutrients?”

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

## I.1 "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 specification for DHA-O is presented in Table 3 below.

**Table 3 Proposed Specification of DHA and EPA-rich Algal Oil from *Schizochytrium* sp. (DHA-O)**

| Test | Specification |
|---|---|
| Acid value | Not more than 0,5 mg KOH/g |
| Peroxide value (PV) | Not more than 5,0 meq/kg oil |
| Moisture and volatiles | Not more than 0,05% |
| Unsaponifiables | Not more than 4,5% |
| Trans-fatty acids | Not more than 1% |
| DHA content | Not less than 22,5% |
| EPA content | Not less than 10% |

## I.2 "Is the information representative of the Novel Food when produced on a commercial scale?"

### I.2.1 Testing to Specification

Quality control analysis results for 3 batches of DHA-O are provided in Table 4 for those parameters identified in the specification. These clearly show compliance to specification. Certificates of Analysis are presented in Appendix 1.

**Table 4 Results of Quality Control Testing for DHA-O**

| Tests | DHA-O Batch No | | |
|---|---|---|---|
| | 98-5807 | 98-5814E | 98-5828E |
| **Proposed Specification Parameters Analysis** | | | |
| Acid Value (mg KOH/g) | 0.4 | 0.2 | 0.5 |
| Peroxide Value (meq/kg) | 2.2 | 1.7 | 3.6 |
| Moisture and Volatiles (%) | <0.01 | 0.01 | <0.01 |
| Unsaponifiables (%) | 1.2 | 1.1 | 1.1 |
| Trans-fatty Acids (%) | <0.05 | <0.05 | <0.05 |
| DHA (%) | 35.1 | 33.3 | 32.7 |
| EPA, 20:5n-3 (%) | 15.9 | 14.9 | 17.7 |

### I.2.2 Additional Quality Control Testing

**Table 5 Additional Quality Control Testing for DHA-O**

| Tests | DHA-O Batch No | | |
|---|---|---|---|
| | **98-5807** | **98-5814E** | **98-5828E** |
| Residual solvent - IPA (mg/kg) | <1.0 | <1.0 | <1.0 |
| Protein by Kjeldahl (%N x 6.25) | <0.02 | <0.02 | <0.02 |
| **Additional Analysis** | | | |
| Arsenic (mg/kg) | <0.2 | <0.2 | <0.2 |
| Copper (mg/kg) | <0.02 | <0.02 | <0.02 |
| Iron (mg/kg) | 0.02 | 0.02 | 0.02 |
| Mercury (mg/kg) | <0.04 | <0.04 | <0.04 |
| Lead (mg/kg) [3] | <0.1 | <0.1 | <0.1 |

N/A Not tested
3 Meets limits set for "Fats and oils, including milk fat" of 0.10mg/kg wet weight, set in *Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs*[3]

**Table 6 Test Methods and Laboratory Accreditations**

| Tests | | Test Method | Laboratory Accreditation | Appendix Number |
|---|---|---|---|---|
| Acid Value | | AOCS Cd 3d-63 | ISO 9001:2000 and Good Clinical Practice (GCP) | 1a |
| Peroxide Value | | AOCS Cd 8-53 | ISO 9001:2000 and Good Clinical Practice (GCP) | 1a |
| Moisture and Volatiles | | Ca 2c-25 | American Oil Chemists Society and ISO/IEC 17025:2005 | 1c |
| Unsaponifiables | | Internal Method LAU_G046 | ISO 9001:2000 and Good Clinical Practice (GCP) | 1a |
| Trans-fatty Acids | | Internal Method LAU_G049A | ISO 9001:2000 and Good Clinical Practice (GCP) | 1a |
| DHA | | AOCS Ce 1b-89 (=Mylnefield Method LAU_G049A) | ISO 9001:2000 and Good Clinical Practice (GCP) | 1a |
| EPA, 20:5n-3 | | AOCS Ce 1b-89 (=Mylnefield Method LAU_G049A) | ISO 9001:2000 and Good Clinical Practice (GCP) | 1a |
| **Additional Analysis** | | | | |
| Protein by Kjeldahl | | AOAC 95504 and 97909 | ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories. | 1b |
| IPA (mg/kg) | | AOAC 983.13 | American Oil Chemists Society | 1c |
| Arsenic | | Ca 17-01 | American Oil Chemists Society | 1c |
| Copper | | Ca 17-01 | American Oil Chemists Society | 1c |
| Iron | | Ca 17-01 | American Oil Chemists Society | 1c |
| Mercury | | Ca 17-01 | American Oil Chemists Society | 1c |
| Lead | | Ca 17-01 | American Oil Chemists Society | 1c |

[3] Commission of the European Communities, 2006a (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF)

### I.2.3 Further Compositional Analysis

#### *I.2.3.1 Fatty Acid Profile*

Analysis was conducted at a laboratory that conforms to ISO 9001:2000 and Good Clinical Practice (GCP) requirements. The test method that was used to perform the analysis is based on the following official method:

AOCS Ce 1b-89 – Determination of the fatty acid composition of marine oils and marine oil esters by capillary column gas-liquid chromatography.

All of the fatty acids detected are present already in the diet from a variety of vegetable and animal sources.

**Table 7 Fatty Acid Composition of DHA-O (and Compared to DHA-S)**

| **Fatty Acid** | **Content mg free fatty acids (% w/w oil) Oil** |
|---|---|
| | **MEAN (n=3)** |
| 14:0 Myristic | **1.59** |
| 14:1 Myristoleic | **0.00** |
| 15:0 Pentadecanoic | **0.40** |
| 16:0 Palmitic | **18.56** |
| 17.0 Heptadecanoic | **0.08** |
| 18:0 Stearic | **1.20** |
| 18:1(n-9)* Oleic | **3.90** |
| 18:1(n-7)* cis-vaccenic | **0.03** |
| 18:2 Linoleic | **0.50** |
| 18:4 Octadecatetraenoic | **0.07** |
| 20:0 Eicosanoic | **0.37** |
| 20.1 Eicosenoic acid | **0.01** |
| 20:3(n-6) Eicosatrienoic | **0.04** |
| 20:4(n-6) Arachidonic | **1.37** |
| 20:3(n-3) Eicosatrienoic | **0.12** |
| 20:4(n-3) Eicosatetraenoic | **0.55** |
| 20:5(n-3) Eicosapentaenoic | **16.18** |
| 22:0 Docosanoic | **0.17** |
| 22:1(n-11) Cetoleic | **0.07** |
| 22:4(n-6) Docosatetraenoic | **0.23** |
| 22:5(n-6) Docosapentaenoic | **1.27** |
| 22:5(n-3) Docosapentaenoic | **3.61** |
| 24:0 Tetracosanoic | **0.11** |
| 22:6(n-3) Docosahexaneoic | **33.72** |
| minor components (individual fatty acids <0.005 mg FFA/g) | **0.12** |
| **TOTAL FATTY ACIDS** | **84.27** |

* the source of oleic acid is from the high oleic sunflower oil used as a carrier for the antioxidant system.

### *I.2.3.2 Unsaponifiables*

Testing was also carried out at a laboratory that conforms to ISO 9001:2000 and Good Clinical Practive (GCP) requirements.

The test method that was used to perform the analysis is based on the following official method:

ISO 12228:1999 - Animal and vegetable fats and oils -- Determination of individual and total sterols contents -- Gas chromatographic method.

All of the sterols detected are present already in the diet from a variety of vegetable and animal sources.

**Table 8 Unsaponifiable Composition of DHA-O (and Compared to DHA-S)**

| **Sterol** | **Content (% w/w of total oil)** |
|---|---|
| | **MEAN (n=3)** |
| Cholesterol | **0.182** |
| Cholestanol | **0.000** |
| Brassicasterol | **0.008** |
| 24-Methylene cholesterol | **0.006** |
| Campesterol | **0.005** |
| Campestanol | **0.000** |
| Stigmasterol | **0.505** |
| Δ-7-Campesterol | **0.002** |
| Δ-5,23-stigmastadienol | **0.003** |
| Clerosterol | **0.015** |
| β-sitosterol | **0.033** |
| Sitostanol | **0.001** |
| Δ-5-avenasterol | **0.008** |
| Δ-5,24-stigmastadienol | **0.003** |
| Δ-7-stigmastenol | **0.003** |
| Δ-7-avenasterol | **0.001** |
| **TOTAL STEROLS** | **0.775** |

## I.3 "Is appropriate analytical information available on the potential toxic inherent constituents, external contaminants and nutrients?"

In addition to those parameters routinely tested to specification and the compositional data above, which shows that nothing new has been introduced, Martek has also conducted additional analysis which confirms the absence of significant levels of:

- Dioxins – See Section 1.3.1 below
- Polycylic aromatic hydrocarbons (PAHs) – See Section 1.3.2 below
- Pesticides – See Section 1.3.3 below
- Acrylamide – See Section 1.3.4 below
- Algal Toxins – See Section X below
- Microorganisms – See Section XII below

The analyses for 1 to 4 were performed at a laboratory which is accredited under the the United Kingdom Accreditation Service (UKAS testing laboratory No. 1642 and accreditation is to the ISO 17025 standard for testing laboratories). All specific test methods are detailed on the individual certificates of analysis and comply fully with the appropriate EU recognised test methods.

Maximum limits where applicable are in compliance with the levels laid down in *Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs*[4]

### I.3.1 Dioxins

The results for dioxins and dioxin-like PCBs are presented in Table 9 below.and are below the EU regulatory maximum limit for vegetable oil and fats.

The analytical procedure used is United Kingdom Accreditation Service (UKAS) accredited to the EN45000 and ISO 17025 standards. In order to demonstrate that adequate confidence can be placed in the results obtained, the following requirements are observed.

All analytical data will meet published acceptance criteria for polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) and equivalent criteria for PCBs. The method used has been validated and published after peer review. Each batch of samples analysed incorporates at least one of several reference materials (RMs), for which results are compared with certified or assigned data and laboratory performance (indicative) data. Results for the batch RM must fall within the acceptable range. Each batch of samples analysed includes a full reagent blank extract. The contribution from the batch blank should be negligible. The analytical performance of the laboratory in international inter-comparison studies, using essentially the same method, has been adjudged to be acceptable or better.

[4] Commission of the European Communities, 2006a (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF)

The method used throughout is an in-house developed method, although parts of it have been published in scientific journals [*e.g*., Fernandes *et al.,* 2004]. It has also been circulated amongst other laboratories by the European Committee for Standardization (CEN) and as such is set to be an internationally recognised method.

**Table 9 Dioxin Levels In DHA-O**

| | Maximum limit (vegetable oil and fats) | Batch No | | |
|---|---|---|---|---|
| | | 98-5807 | 98-5814E | 98-5828E |
| Sum of dioxins (WHOPCDD/ F-TEQ) (pg/g) | 0.75 | < 0.75 | < 0.75 | < 0.75 |
| Sum of dioxins and dioxin-like PCBs (WHOPCDD/F-PCB-TEQ) (pg/g) | 1.5 | < 1.5 | < 1.5 | < 1.5 |

### I.3.2 Polycyclic Aromatic Hydrocarbons (PAH)

The results for PAH analysis are below the EU regulatory maximum limit for oils and fats (excluding cocoa butter) intended for direct human consumption or use as an ingredient in foods.and are presented below in Table 10.

The analytical procedure is UKAS accredited and also follows ISO 17025 standards. The method is in-house developed and has been published (Rose, S.White, R.MaCarthur, R.G.Petch, J.Holland, & A.P.Damant (2007). Single-laboratory validation of a GC/MS Method for the determination of 27 PAHs in oils and fats. *Food Additives & Contaminants Vol 24 Number 6 June 2007*, 635-651).

**Table 10 PAH Levels in DHA-O**

| | Maximum limit (Oils and fats (excluding cocoa butter) intended for direct human consumption or use as an ingredient in foods) | Batch No | | |
|---|---|---|---|---|
| | | 98-5807 | 98-5814E | 98-5828E |
| Benzo(a)pyrene (mcg/kg wet weight) | 2.0 | < 2.0 | < 2.0 | < 2.0 |

### I.3.3 Pesticides

Multi-residue pesticide analysis was conducted on 3 batches of DHA-O:

98-5807
98-5814E
98-5828E

The results show the absence of any detectable pesticide residues for all samples tested.

The methods used for the pesticide multi-residue screen are ‘in-house’ methods which have been used for routine analysis of foods for at least 10 years. The methods comply with the

principles of ISO/IEC 17025:2005 and SANCO/10684/2009 ['Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed'].

### I.3.4 Acrylamide

Results for acrylamide analysis are provided in Table 11. No maximum levels have been set and one would not expect there to be acrylamide formation from the process and results are below the detection limit for all batches.

The sample was extracted with hot water. The aqueous extract was brominated, solvent extracted, concentrated then analysed by gas chromatography with mass spectrometry detection (GC-MS). $^{13}$C-acrylamide was used as an internal standard, which gives an implicit correction for recovery. This method is based on a method validated in-house[5] and widely used by international researchers. There are currently no EU regulations governing acrylamide analysis.

**Table 11 Acrylamide Levels in DHA-O**

| | **No designated limits for vegetable oil** | **Batch No** | | |
|---|---|---|---|---|
| | | **98-5807** | **98-5814E** | **98-5828E** |
| Acrylamide level (mcg/kg) | | <30 | <30 | <30 |

[5] Castle L. Determination of acrylamide monomer in mushrooms grown on polyacrylamide gel. *Journal of Agricultural and Food Chemistry* 1993, 41:1261-1263.

# 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:

1. “Does the Novel Food undergo a production process?”
2. “Is there a history of use of the production process for the food?”
3. “Does the process result in a significant change in the composition or structure of the Novel Food compared to its traditional counterpart?”
4. “Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from the use of the process?”
5. “Are the means identified for controlling the process to ensure that the Novel Food complies with its specification?”
6. “Has the process the potential to alter the levels of Substances with an adverse effect on public health, in the Novel Food?”
7. “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.1 "Does the Novel Food undergo a production process?"

Yes the production process is described in Sections 2.1.1 to 2.1.3 and consists of 3 distinct stages:

1. Contained fermentation
2. Oil Recovery
3. Oil purification

### II.1.1 Fermentation

DHA-O oil is produced *via* a self-contained fermentation process using an alga from the genus *Schizochytrium* (see Section III for more detail on the source organism). The algae are grown in a pure culture heterotrophic fed-batch fermentation process and recovered from the fermentation broth. The subsequent oil recovery stages may be applied to either the recovered, dried algae (following reconstitution in water) or the fermentation broth may be used directly in the oil recovery process, in which case a pasteurization step may be employed. Antioxidants may be added to the fermentation broth to aid stability in processing.

### II.1.2 Oil Recovery

Fresh *Schizochytrium* sp. broth or reconstituted dried algae (from *Schizochytrium* sp. fermentation) may be used in the process. The mixture is then heated and centrifuged to separate the oil from the aqueous phase. The oil phase is dried and stored for oil purification.

### II.1.3 Oil Purification

The crude oil is further refined into the finished product using process operations commonly employed in the vegetable oil industry. Approved antioxidants are added to the oil to provide stability. At this stage the DHA and EPA percentage may be standardised by the addition of food grade vegetable oil, for example high oleic sunflower oil.

## II.2 "Is there a history of use of the production process for the food?"

Yes the production process uses unit operations found in traditional vegetable oil processing.

## II.3 "Does the process result in a significant change in the composition or structure of the Novel Food compared to its traditional counterpart?"

The DHA-O oil profile (DHA:EPA ratio) is very similar to that of other fish oils/fish, *e.g.*, tuna oil. Table 12 provides a comparison of the oil profile of DHA-O to other common sources of long chain omega-3 oils.DHA-O delivers on average approximately a ratio of 2 DHA: 1 EPA which is comparable to the ratio of a number of fish species, as shown in Table 13.

**Table 12 Fatty Acid Composition of DHA-O (*Schizochytrium* sp.), DHA-S (*Schizochytrium* sp.), and Other Commercially Available Oils and Fats as Percentage (%) of Total Fatty Acids**

| Fatty Acid | DHA-O (*Schizochytrium* sp.)[1] | DHA-S (*Schizochytrium* sp.)[2] | Tuna Oil[3] | Menhaden oil[3] | Krill Oil[4] | Salmon oil | Cod liver oil | Sunflower oil | Corn oil | Palm oil |
|---|---|---|---|---|---|---|---|---|---|---|
| 12:0 | | | | - | | - | - | - | - | 0.1 |
| 14:0 | 2 | 9.3 | 3 | 9 | - | 3.3 | 3.6 | 0.1 | 0.3 | 1 |
| 16:0 | 22 | 21 | 22 | 19 | - | 9.8 | 10.6 | 5.9 | 10.9 | 43.5 |
| 16:1 | | 0.3 | 3 | 13.3 | - | 4.82 | 8.31 | - | - | - |
| 18:0 | 1.4 | 0.5 | 6 | 3.8 | - | 4.2 | 2.8 | 4.5 | 1.8 | 4.3 |
| 18:1:00 (total n-7+9) | 4.7 | 2 | 21 | 15.5 | 5-11 | 17 | 20.7 | 19.5 | 24.2 | 36.6 |
| 18:2 | 0.6 | 0.4 | 1 | 2 | 1.3-2.4 | 1.5 | 0.9 | 65.7 | 58 | 9.1 |
| 18:3 | 0.2 | 0.8 | 1 | 1 | - | 1.1 | 0.9 | 0.4 | 0.7 | 0.2 |
| 18:4 | 0.1 | 0.3 | 1.9 | 2.4 | - | 2.8 | - | - | - | - |
| 20:3(n-3+6) | 0.1 | 0.5 | | - | - | - | - | - | - | - |
| 20:4 | 0.7 | 1.2 | 2 | 1 | - | 0.7 | 0.9 | - | - | - |
| 20:5 | 19 | 1 | 6 | 12.5 | 15-19 | 13 | 6.9 | - | - | - |
| 22:4 | 0.3 | 0.1 | | - | - | - | - | - | - | - |
| 22:5 (total n-3+6) | 5.8 | 17 | 2 | 1.7 | 0.4-0.7 | 3 | 0.9 | - | - | - |
| 22:6 (n-3) | 40 | 43 | 22 | 7.9 | 7-16 | 18.2 | 11 | - | - | - |

[1] Average of 3 batches of DHA-O corrected to % of total fatty acids (which is why the results are higher than those in table 7, which are expressed as % of total oil.
[2] Average of 3 recent batches of DHA-S
[3] Handbook of Lipid Research
[4] scientific opinion Safety of 'Lipid extract from *Euphausia superba*' as a novel food ingredient 1 Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies (Question No EFSA-Q-2008-027) . http://www.efsa.europa.eu/en/scdocs/doc/938.pdf

**Table 13 Commonly Consumed Oily and White Fish in National Diet and Nutrition Survey of British Adults Aged 19 To 64 Years 2000/01 with Corresponding LC N-3 PUFA Content[1]**

| OILY FISH | % | EPA | DPA | DHA | LC n-3 |
|---|---|---|---|---|---|
| **Type of fish [1]** | **Consumers during the survey week [2]** | **(g/100g)** | **(g/100g)** | **(g/100g)** | **PUFA (g/100g)** |
| Fresh salmon [4] | 9 | 1.2 | 0.2 | 1.3 | 2.7 |
| Canned [3] and smoked salmon | 8 | 0.55 | 0.14 | 0.85 | 1.54 |
| Pickled, smoked and canned sardines and pilchards [3] | 4 | 1.17 | 0.23 | 1.20 | 2.60 |
| Canned sardines [3] | | 0.55 | 0.14 | 0.86 | 1.57 |
| Canned and smoked mackerel | 3 | N/A | N/A | N/A | N/A |
| Fresh trout [3] | 2 | 0.23 | 0.09 | 0.83 | 1.15 |
| Pickled, smoked and canned herring, kipper and bloater | 2 | | | | |
| Herring [3] | | 0.51 | 0.11 | 0.69 | 1.31 |
| Kipper [3] | | 1.15 | 0.10 | 1.34 | 2.49 |
| Fresh tuna [4] | 2 | 0.3 | 0.1 | 1.1 | 1.5 |
| Fresh mackerel [3] | 1 | 0.71 | 0.12 | 1.10 | 1.93 |
| N/A, data not available.<br>Taking into account the relative quantities of fish consumed by an average consumer 100 g of an average oily fish contains approximately 2 g (calculated to 1.99 g); therefore, one portion contains about 2.8 g. | | | | | |
| **WHITE FISH** | **%** | **EPA** | **DPA** | **DHA** | **LC n-3** |
| **Type of fish [1]** | **Consumers during the survey week [2]** | **(g/100g)** | **(g/100g)** | **(g/100g)** | **PUFA (g/100g)** |
| Canned tuna [3] | 27 | 0.06 | 0.04 | 0.27 | 0.37 |
| Fresh cod [3] | 25 | 0.08 | 0.01 | 0.16 | 0.25 |
| Fresh haddock [3] | 9 | 0.05 | 0.01 | 0.10 | 0.16 |
| Fresh plaice [3] and whiting | 2 | 0.16 | 0.04 | 0.10 | 0.30 |
| Smoked and salted haddock | 2 | N/A | N/A | N/A | |
| Fresh sole, including lemon sole [4] and Dover sole | 2 | 0 | 0 | 0.1 | 0.1 |
| Taking into account the relative quantities of fish consumed by an average consumer 100 g of an average white fish contains approximately 0.3 g (calculated to 0.28 g); therefore, one portion contains about 0.4 g. | | | | | |

[1] Includes consumption of fish in dishes.
[2] Percentage who consumed during the seven day dietary recording period.
[3] MAFF fatty acids supplement to McCance & Widdowson's The Composition of Foods, 1998.
[4] MAFF fish and fish products. Third supplement to McCance & Widdowson's The Composition of Foods, 1993.

(Reproduced from Table 4.2 of the Scientific Advisory Committee on Nutrition Report on Advice on Fish Consumption; Benefits and Risks[6])

[6] SACN, 2004 (http://www.sacn.gov.uk/pdfs/fics_sacn_advice_fish.pdf)

## II.4 "Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from the use of the process?"

Details of the analysis of contaminants are provided in Section I above. The absence of algal toxins is specifically discussed in Section II. The absence of microbiological contamination is confirmed in Section XII.

## II.5 "Are the means identified for controlling the process to ensure that the Novel Food complies with its specification?"

Details for batch results to specification are provided in Section I above. All processes are set up using a Hazard Analysis Critical Control Point (HACCP) approach. They are documented according to current Good Manufacturing Practices regulation (cGMP) for foods and the identified critical control points (CCPs) are monitored. Quality Control (QC) personnel record the results of laboratory tests as well as sterility checks. Production personnel record the continuous batch monitoring results within the batch records, according to cGMP. Quality Assurance personnel monitor the production records to ensure that batch process changes have been properly authorized, documented, and recorded in the records for each batch.

## II.6 "Has the process the potential to alter the levels of substances with an adverse effect on public health, in the Novel Food?"

We have clearly, in our view demonstrated that this is not the case within Sections I, II and XII. To the contrary this novel food has the potential to have a positive effect on public health.

## II.7 "After processing is the Novel Food likely to contain micro-organisms of adverse public health significance?"

Details of micro-organisms analyses are provided in Section XII below. These results confirm the absence of pathogens.

# III HISTORY OF SOURCE ORGANISM

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to the history of the source organism (Commission of the European Communities, 1997):

1. “Is the novel food obtained from a biological source, *i.e*., a plant, animal or microorganism?”

2. “Has the organism used as the source of the novel food been derived using GM?”

3. “Is the source organism characterized?”

4. “Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?”

We will address each point in turn in this section.

### III.1 "Is the novel food obtained from a biological source, i.e., a plant, animal or microorganism?"

DHA-O is obtained from *Schizochytrium* sp. microalgae. More detail on the source organism is provided in Section III.3 below.

### III.2 "Has the organism used as the source of the novel food been derived using GM?"

As explained in more detail in Section 2.3.2 below the *Schizochytrium* sp. microalgae is obtained using a classic screening program that utilized well-accepted techniques commonly employed in industrial strain improvement programs. No recombinant DNA technology was employed.

### III.3 "Is the source organism characterized?"

#### *III.3.1 Taxonomy*

The taxonomy for the source microalgae for DHA-O like that of DHA-S is as follows:

- Kingdom – Chromista (Stramenopilia)
- Phylum – Heterokonta
- Class – Thaustochytridae
- Order – Thaustochytriales
- Family – Thaustochytridriaceae
- Genus – Schizochytrium

#### *III.3.2 The Production Strain for DHA-O*

The microalgaewas isolated from the intertidal coastline in 2007. Preliminary examination of the organism the microalgae is a thraustochytrid. Subsequent detailed examination of the microalgae indicated that it possessed the definitive characteristics of the genus *Schizochytrium* and was a previously unpublished member of that genus.

Martek developed an improved strain from the wild-type parent strain using a classic screening program that utilized well-accepted techniques commonly employed in industrial strain improvement programs. No recombinant DNA technology was employed. Following multiple serial dilutions, the improved strain was chosen for its improved production of DHA and EPA compared to the current *Schizochytrium* sp. production strain.

Laboratory studies were conducted to phenotypically characterize the sub-isolate and its parent. These tests included morphological evaluation (light microscopy) throughout their growth cycle under standard growth and fermentation conditions as well as evaluations of multicellular aggregates and differences in growth or substrate utilization patterns in a batch fermentation mode.

After establishing the strain was monophenotypic at laboratory scale, the productivity of the organism was improved through optimization of fermentation nutrients and manufacturing conditions.

## III.4 "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

### *III.4.1 Algal Toxins*

It has long been known that some species of microalgae produce toxic substances. 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 by 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 (blue-green algae) can produce neurotoxic, hepatotoxic, and dermatoxic 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 species of *Lyngbya*, *Oscillatoria* and *Schizothrix*.

Thraustochytrids, including *Schizochytrium* sp., are not related to either of the above groups of microalgae (bluegreen or dinoflagellates). The blue-green algae and dinoflagellates are in completely separate Kingdoms. Thraustochytrids are members of the kingdom Chromista which contains the golden algae. Within this kingdom, only 2 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.

Within the microalgae in the Chromista kingdom (Stramenopilia), 2 toxins are known to be produced, domoic acid and prymnesin.

### *III.4.2 Domoic acid*

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 phytoplankton throughout the world. Four of these diatom species 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).

Confirmatory testing of *Schizochytrium* sp. dried microalgae, derived from the proposed production strain, for domoic acid was performed using standard HPLC - ultraviolet (modified version of AOAC Official Method 991.26) and ELISA (Biosense) methods. Both methods have the capacity to quantitatively detect domoic acid and these analyses showed no evidence of domoic acid present in *Schizochytrium* sp. dried microalgae from the proposed production strain.

The analysis report from Martek is presented in Appendix 3a.

### *III.4.3 Prymnesins*

The other toxins found in a member of the Chromista (Stramenopilia) are limited to 2 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 haemolytic 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 to 5,000 cells/mL (Larsen *et al*., 1993).

Human consumption of Thraustochytrids, especially the genus *Schizochytrium*, is by consumption of mussels and clams and through the food chain (fish and shell fish).

Due to the unavailability of authentic prymnesin standards, Martek is unable to analyze *Schizochytrium* sp. directly for the presence of prymnesin toxins. However we have conducted our own artemia assay on this new strain. The test protocol used and more precisely the cells/ml were based on 2 previously published papers:

1) Levels Toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta)

A. Houdan*, A. Bonnard, J. Fresnel, S. Fouchard, C. Billard And I. Probert. Journal Of Plankton Research 26: 875–883 (2004)

2) Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions (Granéli, 2003).

### *III.4.4 Conclusions on Algal Toxins*

Based on existing published and unpublished scientific data, it is concluded that: 1) there have never been any published reports of toxic compounds, or association with 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 indicate that domoic acid is not present in *Schizochytrium* sp. microalgae; and 5) a biological assay for prymnesin toxin is negative.

## SECTIONS IV TO VIII

Sections IV to VIII of the EU recommendation are not applicable to DHA and EPA-rich algal oil since no GM technology is involved.

# IX ANTICIPATED INTAKE/EXTENT OF USE

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to the intake/extent of use of the novel food (Commission of the European Communities, 1997):

1. “Is there information on the anticipated uses of the novel food based on its properties?”
2. “Is there information to show anticipated intakes for groups predicted to be at risk?”
3. “Will introduction of the novel food be restricted geographically?”
4. “Will the novel food replace other foods in the diet?”

We will address each point in turn in this section.

## IX.1 Is there information on the anticipated uses of the novel food based on its properties?

DHA-O is clearly a close alternative to other currently available DHA and EPA sources and is from sustainable and vegetarian sources. Its DHA and EPA ratio mimics that of fish oil which is freely and without restriction used in many fortified food products. It is also important to note at this point that there are clear limits to which such oils can be added to foods due to sensory and economic issues. Even without restrictions there would be no realistic possibility that significant bolus doses could arise that would have any impact on safety.

### IX.1.1 Proposed Uses

In this application we wish to apply for the same uses currently approved for DHA-S with 5 small modifications:

1. To adjust the maximum permitted level in food supplements for the normal population to “250 mg per daily dose as recommended by the manufacturer” in keeping with the 2010 EFSA recommendation

2. Food supplements for pregnant and lactating women – specifically for this population group and in line with EFSA’s scientific advice we propose to add a maximum level of “450 mg per daily dose as recommended by the manufacturer”, to enable the full “Adequate daily intake” to be delivered in supplement form (see below for further explanation).

3. To modify the level permitted in Foods intended for use in energy-restricted diets for weight reduction to “250 mg per meal replacement”.

4. To adjust the maximum permitted level for Non-alcoholic beverages (including milk based beverages) to “80 mg/100 mL” to meet the minimum requirement for a nutrition claim for high in long chain omega-3s = 80 mg/100 kcal and 100 g.

5. To include the use of DHA+EPA from DHA-O in biscuits at a maximum of 200 mg/100 g.

6. To include the use of DHA+EPA from DHA-O in cooking oils to meet the minimum requirement for a nutrition claim for high in long chain omega-3s = 80 mg/100 kcal and 100 g.

**Table 14 Summary of the Individual Proposed Food Uses and Use-Levels for DHA+EPA from DHA-O in the EU**

| Food Category | Food-Use | Maximum Use-Level (mg DHA+EPA/100 g unless otherwise stated) |
|---|---|---|
| Food Supplements | Food Supplements for the normal population | 250 mg per daily dose as recommended by the manufacturer |
| | Food Supplements for pregnant and lactating women | 450 mg per daily dose as recommended by the manufacturer |
| Dietary foods for special medical purposes | Dietary foods for special medical purposes | In accordance with the particular nutritional requirements of the persons for whom the products are intended |
| Foods intended for use in energy-restricted diets for weight reduction | Foods intended for use in energy-restricted diets for weight reduction | 250 mg per meal replacement |
| Bakery Products, Breads and Rolls | Sweet Biscuits | 200 |
| | White Bread and rolls | 200 |
| | Wholemeal Bread and rolls | 200 |
| Breakfast Cereals | Breakfast Cereals (not wholegrain) | 500 |
| | Wholegrain and High Fibre Breakfast Cereals | 500 |
| Cooking Fats | Cooking oils | 360 |
| Dairy Analogues (except drinks) | Cheese Analogues | 600 |
| | Soy and Imitation Milk Products (Excluding Drinks) | 200 |
| Dairy Products (except milk-based drinks) | Cheese | 600 |
| | Milk Products (Including Milk, Fromage Frais, and Yogurt Products; Excluding Drinks) | 200 |
| Non-alcoholic Beverages (including dairy analogue and milk-based drinks) | Carbonated Beverages | 80 |
| | Dairy Analogue Drinks (Soy-based Beverages) | 80 |
| | Fruit Juice and Nectar | 80 |
| | Fruit Juice-based Drinks (Excluding Nectars and Fruit Juices) | 80 |
| | Milk and Milk-based Drinks | 80 |
| | Non-Alcoholic, Non-Carbonated, Water-based Flavoured Drinks (Including Energy Drinks, Sports Drinks) | 80 |
| Nutrition Bars | Cereal Bars and Nutrition Bars | 500 |
| Spreadable Fats and Dressings | Spreadable Fats and Dressings | 600 |

The reason for the small changes from those approved for DHA-S in levels is to reflect the latest scientific advice on intakes of DHA and EPA from the European Food Safety Authority (EFSA) in 2010, specifically:

1. *Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol*[7] which stated:

"An intake of 250 mg per day of eicosapentaenoic acid plus docosahexaenoic acid appears to be sufficient for primary prevention in healthy subjects. Therefore, and taking into account that available data are insufficient to derive an Average Requirement, the Panel proposes to set an Adequate Intake of 250 mg for eicosapentaenoic acid plus docosahexaenoic acid for adults based on cardiovascular considerations."

To support brain and eye development during pregnancy and early post-natal life numerous government authorities and expert groups have recommended that pregnant and nursing women consume up to 450 mg EPA+DHA, including at least 200 mg DHA, per day (Table 15). Adequate daily DHA consumption by pregnant and nursing women is needed to compensate for increased metabolic demands associated with pregnancy and lactation, and accumulation of DHA by the foetus/infant while meeting minimum adult requirements for cardiovascular health (EFSA, 2010). It has been calculated that in order to maintain human milk levels of DHA concentrations at levels necessary to achieve functional benefits for the infant, a woman must consume 170 mg DHA/d throughout her lifetime (Van Goor *et al.*, 2008). However, if a mother's habitual intake of DHA has been low throughout her lifetime but increases during pregnancy much higher intake levels of 200 to 300 mg DHA/day, in addition to cardiovascular health requirements, are needed if she is expected to achieve and maintain meaningful levels of DHA in breast milk (Van Goor *et al.*, 2008). Since breast milk provides the best nutrition for infants, raising awareness among mothers of the importance of consuming increased DHA during pregnancy and nursing is vital. DHA dietary supplements are important to bridge the gap between the low DHA intake provided by the habitual diet of most women and the recommendations for increased DHA intake. Achieving DHA maternal intake requirements, up to 450 mg/day during pregnancy and nursing, helps promote brain and eye development of the growing foetus and infant (EFSA, 2009).

[7] EFSA, 2010 (http://www.efsa.europa.eu/en/scdocs/doc/s1461.pdf)

**Table 15 World-wide DHA Intake Recommendations for Pregnant and Lactating Women**

| Organization | EPA and/or DHA Recommendation | Reference |
|---|---|---|
| European Food Safety Authority (EFSA) | 250 mg DHA+EPA/d for all women plus an additional 100-200 mg DHA/d for pregnant and nursing women | Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal 2010; 8; 1461. http://www.efsa.europa.eu/en/scdocs/doc/1461.pdf |
| Agence Français de Sécurité Sanitaire des Aliments | 250 mg DHA/d for pregnant women<br>250 mg DHA/day for breastfeeding women | AFSSA Opinion Regarding the Update of the Recommended Dietary Intake for Fatty Acids, AFSSA-Hearing n2006-SA-0359.2010. |
| International Society for the Study of Fats and Lipids | At least 200 mg DHA/d during pregnancy and nursing | ISSFAL Policy Statement 4: Recommendations for intake of polyunsaturated fatty acids by pregnant and lactating women. 2009. |
| March of Dimes | At least 200 mg DHA/d during pregnancy and nursing | http://www.marchofdimes.com/pnhec/15955030.asp. 2009. |
| FAO/WHO Expert Consultation | At least 200 mg DHA/d toward total 300 mg n-3 EPA+DHA for pregnant and nursing women | From the Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition, November 10-14. 2008. WHO HQ, Geneva. |
| Perinatal Lipid Intake Working Group | At least 200 mg/day DHA | Koletzko B, Cetin I, and Brenna TJ (2007) Perinatal Lipid Intake Working Group Consensus Statement: *Dietary fat intakes for pregnant and lactating women*. Brit J Nutr 98:873-7. |
| BE Superior Health Council | Consume approximately 250 mg (200 to 300 mg) DHA on a daily basis | Superior Health Council, Advisory Report, Recommendations and claims made on omega-3 fatty Acids (SHC 7945). https://portal.health.fgov.be/pls/portal/docs/PAGE/INTERNET_PG/HOMEPAGE_MENU/ABOUTUS1_MENU/INSTITUTIONSAPPARENTEES1_MENU/HOGEGEZONDHEIDSRAAD1_MENU/ADVIEZENENAANBEVELINGEN1_MENU/ADVIEZENENAANBEVELINGEN1_DOCS/OMEGA-3%20ENGLISH.PDF |
| ANZ NHMRC | Adequate Intakes - Pregnancy – 110- 115 mg/day DHA+EPA+DPAn-3; Lactation – 140-145 mg/day DHA+EPA+DPAn-3 | National Health and Medical Research Council. Nutrient reference values for Australia and New Zealand including recommended dietary intakes. www.nhmrc.gov.au/publications/synopses/_files/n35.pdf |

2. *Scientific Opinion on Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids*[8], which stated:

"The Panel proposes 250mg/d as the labelling reference intake value for the long-chain n-3 PUFAs EPA plus DHA, which is in agreement with most recent evidence on the relationship between the intake of these fatty acids and cardiovascular health in healthy populations."

The Commission has, on the basis of these opinions amended the Nutrition Claims Annex to the Nutrition and Health Claims Regulation in the following Regulation:

[8] EFSA, 2009 (http://www.efsa.europa.eu/en/scdocs/doc/1176.pdf)

*Commission Regulation (EU) No 116/2010 of 9 February 2010 amending Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims*[9]

In this regulation the following requirements are formally laid down into EU legislation:

"SOURCE OF OMEGA-3 FATTY ACIDS

A claim that a food is a source of omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0.3 g *alpha*-linolenic acid per 100 g and per 100 kcal, or at least 40 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal.

HIGH OMEGA-3 FATTY ACIDS

A claim that a food is high in omega-3 fatty acids, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 0.6 g *alpha*-linolenic acid per 100 g and per 100 kcal, or at least 80 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal."

These updated opinions and the resulting amended legislation provide companies, wishing to formulate food and food supplement products containing DHA-rich oil, upon which they may wish to make nutrition and/or health claims, with practical issues about delivering the recommended daily intake of long-chain PUFA. Principally we would like to address these issues by amending the permitted maximum use levels for the following categories as follows:

1. Food supplements for the normal population – we would like to adjust the maximum level to "250 mg per daily dose as recommended by the manufacturer", to enable the full "Adequate daily intake" to be delivered in supplement form.

2. Food supplements for pregnant and lactating women – specifically for this population group and in line with EFSA's scientific advice we propose to add a maximum level of "450 mg per daily dose as recommended by the manufacturer", to enable the full "Adequate daily intake" to be delivered in supplement form.

3. Non-alcoholic beverages (including milk based beverages) – we would like to adjust the maximum level to "80 mg/100 mL", to enable the claim "high in omega-3 fatty acids" to be made.

[9] European Commission, 2010 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:037:0016:0018:EN:PDF)

### *IX.1.1.2 Food Supplements*

Since in most cases, food supplements are consumed as an alternative to fortified food products, we do not believe that increasing the maximum level of permitted DHA and EPA per daily recommended serving would represent a significant increase in intake, from a safety perspective. Indeed the sole purpose of this proposed intake is to enable the daily advised intake of long chain omega-3 fatty acids of 250 mg per day. This is indeed no more than the fish oil supplements that it would replace (*e.g.*, for vegetarians) on the market currently provide. Furthermore the conditions laid down for labelling and presentation under food supplements legislation would prevent involuntary excessive dosing. Specifically these conditions are laid down in Article 6, point 3 of *Directive 2002/46/EC on food supplements*[10], as follows:

3. Without prejudice to Directive 2000/13/EC, the labelling shall bear the following particulars (European Parliament and Council of the European Union, 2000):

(a) the names of the categories of nutrients or substances that characterise the product or an indication of the nature of those nutrients or substances;

(b) the portion of the product recommended for daily consumption;

(c) a warning not to exceed the stated recommended daily dose;

(d) a statement to the effect that food supplements should not be used as a substitute for a varied diet;

(e) a statement to the effect that the products should be stored out of the reach of young children.

### *IX.1.1.3 Foods intended for use in energy-restricted diets for weight reduction*

The proposed maximum inclusion level of 250 mg reflects the reference daily advisory level for Long Chain Omega-3 fatty acids. These products are controlled under the requirements of Commission Directive 96/8/EC of 26 February 1996 on foods intended for use in energy-restricted diets for weight reduction[11] specifically with regard to the labelling and delivery of daily servings. So this category is excluded from the intake calculations presented below for fortified individual foods.

[10] European Parliament and the Council of the European Union, 2002 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:183:0051:0057:EN:PDF)
[11] Commission of the European Communities, 1996 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1996L0008:20070620:EN:PDF)

## IX.2 Consumption Estimates Based on UK National Diet and Nutrition Survey Data

The full report of "Estimated Daily Intake of DHA and EPA-rich Algal Oil from *Schizochytrium* sp. by the UK Population from Proposed Food-Uses in the EU", containing detailed additional individual food use group intake data is provided in Appendix 4.

Estimates for the intake of DHA+EPA from DHA-O in the EU were based on the proposed use-levels and food consumption data collected as part of the United Kingdom (UK) Food Standards Agency's, Dietary Survey Programme (DSP). Calculations for the mean and high-level (95$^{th}$ percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for DHA-O. Similar calculations were used to determine the estimated total intake of DHA+EPA from DHA-O from all proposed food-uses combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

children, ages 1½ to 4½ ;
young people, ages 4 to 10;
female teenagers, ages 11 to 18;
male teenagers, ages 11 to 18;
female adults, ages 16 to 64;
male adults, ages 16 to 64.

### IX.2.1 Survey Description

The Ministry of Agriculture, Fisheries, and Food (MAFF) and the Department of Health were responsible for the joint commission of the National Diet and Nutrition Survey (NDNS) program in 1992. The responsibility for the program was subsequently transferred from MAFF to the FSA upon its inception in April 2000. The NDNS program itself consists of 4 different surveys targeting specific age groups, which were conducted every 3 years in succession. Separate survey data are available from the UK Data Archive (UKDA) for the NDNS: Adults Aged 16 to 64 years collected in 2000-2001 (NDNS 2000-2001) (Office for National Statistics, 2005), the National Diet, Nutrition and Dental Survey of Children Aged 1½ to 4½ Years, 1992-1993 (NDNS 1992-1993) (UKDA, 1995), the National Diet and Nutrition Survey: Young People aged 4 to 18 Years (NDNS 1997) (UKDA, 2001), and the National Diet and Nutrition Survey: People Aged 65 Years and Over, 1994-1995. Although all four surveys are available, only the former three were utilized in the generation of estimates in the current intake analysis. When combined, the survey results provide the most current data for use in the evaluation of food-use, food-consumption patterns, and nutritional status for individuals residing within the UK. Weighted 4- or 7-day food records for individuals were selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors (UKDA, 1995, 2001; Office for National Statistics, 2005) as the primary sampling unit.

NDNS data were collected from individuals as well as households *via* 4- (children, aged 1½ to 4½) or 7-day (young people, aged 4 to 18 and adults, aged 16 to 64) weighed dietary intake records throughout all 4 seasons of the year (4 fieldwork waves of 3 months duration), in order to address variability in eating behaviours due to seasonality. Dietary data were recorded by survey respondents or by parents or guardians in the case of the children's survey for the duration of the survey period. NDNS 2000-2001 contains 7-day weighted dietary records for more than 1,724 individuals aged 16 to 64, while, NDNS 1992-1993 contributes 4-day data from an additional 1,592 children 1½ to 4½ years of age. NDNS 1997 adds 7-day records for approximately 1,700 youth aged 4 to 18 (UKDA, 1995, 2001; Office for National Statistics, 2005). Initial postal questionnaires and interviews were employed to identify eligible children, youth, or adults, respectively, for the surveys. Overall, response rates of 93, 92, and 73% were achieved; the maximum response rate (individuals agreeing to the initial dietary interview) from the eligible sample selected for participation in the survey were, 88, 80, and 61%, respectively, while only 81, 64, and 47% of surveyed individuals completed a full dietary record (Gregory *et al*., 1995; UKDA, 2001; Office for National Statistics, 2005).

The NDNS program collects physiological, anthropometric and demographic information from individual survey participants, such as sex, age, measured height and weight (by the interviewer), blood analytes, and other variables useful in characterizing consumption in addition to collecting information on the types and quantities of foods being consumed. Further assessment of food intake based on consumption by specific population groups of interest within the total surveyed samples was made possible by the inclusion of this information. In order to compensate for the potential under-representation of intakes from specific population groups resulting from sample variability due to differential sampling probabilities and differential non-response rates [particularly the lower response rate among males aged 15 to 18 years (UKDA, 2001)], sample weights were developed and incorporated with the youth survey (NDNS 1997) [UKDA, 2001].

Weighting the children's survey data to 7 days facilitated the comparison of adult and youth 7-day dietary survey data to dietary data obtained in the 4-day children's survey. This change was based on the assumption that intake patterns on non-recording weekdays were similar to the intakes on recorded weekdays. The 2 weekend days were not re-weighted. All food and drinks consumed on the 2-recorded weekdays were averaged to obtain a daily intake value, which was then multiplied by 5 to approximate intakes for all weekdays. This data was combined with consumption data from weekend dietary records. The full details of the weighting method employed are provided in Appendix J of the report on the children's diet and nutrition study (Gregory *et al*., 1995).

### IX.2.2 Statistical Methods

Estimates for the intake of DHA+EPA from DHA-O by the UK population were generated and collated by computer, using consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days. Estimates for the daily intake of DHA+EPA from DHA-O represent projected 7-day averages for each

individual from Days 1 to 7 of NDNS data. The distribution from which mean and percentile intake estimates were produced was comprised of these average amounts. Mean and percentile estimates were generated using ratio estimation and nonparametric techniques, incorporating survey weights where appropriate (*i.e.,* when using youth data to estimate intakes, as described in Section 2.1) in order to provide representative intakes for specific UK population groups. All-person intake refers to the estimated intake of DHA+EPA from DHA-Oil averaged over all individuals surveyed regardless of whether they consumed food products in which DHA-O is currently proposed for use, and therefore includes "zero" consumers (those who reported no intake of food products for which DHA-O is proposed for use during the 7 survey days). All-user intake refers to the estimated intake of DHA+EPA from DHA-O by those individuals consuming food products in which the use of DHA-O is currently under consideration, hence the 'all-user' designation. Individuals were considered users if they consumed 1 or more food products in which DHA-O is proposed for use on 1 of the 7 survey days.

Mean and 95$^{th}$ percentile intake estimates based on sample sizes of less than 30 and 160, respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). As such, the reliability of estimates for the intake of DHA+EPA from DHA-O based on the consumption of these foods may be questionable for certain individual population groups.

#### *IX.1.2.3 Food Usage Data*

The individual proposed use-levels for DHA+EPA from DHA-O employed in the current intake analysis are summarized in Table 14. Food codes representative of each proposed food-use were chosen from the MAFF food code list associated with each food consumption survey and grouped in food-use categories according to the food type, main and subsidiary food group classifications detailed within the NDNS reports (UKDA, 1995, 2001; Office for National Statistics, 2005). A given food code may not be associated with all 3 surveys; as with each new survey the food code list has been updated to reflect the availability of new foods and the discontinuation of certain obsolete codes.

#### *IX.1.2.4 Food Survey Results*

Estimates for the total daily intakes of DHA+EPA from all proposed food-uses of DHA-O are provided in Tables 15 and 16. Estimates for the daily intake of DHA+EPA from individual proposed food-uses of DHA-O in the EU are summarized in Tables A-1 to A-6 and B-1 to B-6 of Appendix A and B, respectively. Tables A-1 to A-6 provide estimates for the daily intake of DHA+EPA from DHA-O in the UK per person (mg/day), whereas Tables B-1 to B-6 provide estimates on a per kilogram body weight basis (mg/kg body weight/day).

**Estimated Daily Intake of DHA-O from All Proposed Food-Uses in the EU**

Table 16 summarizes the estimated total intake of DHA+EPA (g/person/day) from all proposed food-uses of DHA-O in the EU by UK population group. As would be expected for

a 7-day survey, the percentage of users was high among all age groups evaluated in the current intake assessment; greater than 94.3% of the population groups consisted of users of those food products in which DHA-O is currently proposed for use (Table 15). Young people had the greatest percentage of users at 99.6%. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be discussed in detail.

Of the individual population groups, male teenagers were determined to have the greatest mean and 95$^{th}$ percentile all-user intakes of DHA+EPA from DHA-O on an absolute basis, at 0.88 and 1.50 g/person/day, respectively, while children had the lowest mean and 95$^{th}$ percentile all-user intakes of 0.42 and 0.77 g/person/day, respectively (Table 16).

**Table 16 Summary of the Estimated Daily Intake of DHA+EPA from All Proposed Food Categories of DHA-O in the U.K. by Population Group (NDNS Data)**

| Population Group | Age Group (Years) | % User | Actual # of Total Users | All-Person Consumption | | | | All-Users Consumption | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean (g) | Percentile (g) | | | Mean (g) | Percentile (g) | | |
| | | | | | 90 | 95 | 97.5 | | 90 | 95 | 97.5 |
| Children | 1½ - 4½ | 98.8 | 1,628 | 0.42 | 0.67 | 0.77 | 0.89 | 0.42 | 0.66 | 0.77 | 0.89 |
| Young People | 4-10 | 99.6 | 834 | 0.65 | 0.99 | 1.13 | 1.23 | 0.65 | 0.99 | 1.13 | 1.23 |
| Female Teenager | 11-18 | 97.8 | 436 | 0.67 | 1.05 | 1.20 | 1.31 | 0.67 | 1.05 | 1.17 | 1.30 |
| Male Teenager | 11-18 | 99.5 | 414 | 0.88 | 1.33 | 1.51 | 1.68 | 0.88 | 1.33 | 1.50 | 1.72 |
| Female Adults | 16-64 | 94.3 | 903 | 0.60 | 0.95 | 1.10 | 1.21 | 0.60 | 0.96 | 1.12 | 1.23 |
| Male Adults | 16-64 | 95.0 | 728 | 0.76 | 1.23 | 1.45 | 1.66 | 0.77 | 1.23 | 1.45 | 1.65 |

On a body weight basis, children were identified as having the highest mean and 95$^{th}$ percentile all-user intakes of any population group, of 29.5 and 53.6 mg/kg body weight/day. Female adults had the lowest mean and 95$^{th}$ percentile all-user intakes of 8.9 and 16.4 mg/kg body weight/day, respectively (Table 17).

**Table 17 Summary of the Estimated Daily Per Kilogram Body Weight Intake of DHA+EPA from All Proposed Food Categories of DHA-O in the U.K. by Population Group (NDNS Data)**

| Population Group | Age Group (Years) | % User | Actual # of Total Users | All-Person Consumption | | | | All-Users Consumption | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean (mg/kg) | Percentile (mg/kg) | | | Mean (mg/kg) | Percentile (mg/kg) | | |
| | | | | | 90 | 95 | 97.5 | | 90 | 95 | 97.5 |
| Children | 1½ - 4½ | 98.8 | 1,628 | 29 | 47 | 54 | 62 | 30 | 48 | 54 | 62 |
| Young People | 4-10 | 99.6 | 834 | 25 | 39 | 44 | 49 | 25 | 39 | 44 | 49 |
| Female Teenager | 11-18 | 97.8 | 436 | 13 | 21 | 24 | 26 | 13 | 21 | 24 | 26 |
| Male Teenager | 11-18 | 99.5 | 414 | 16 | 26 | 28 | 32 | 16 | 26 | 28 | 32 |
| Female Adult | 16-64 | 94.3 | 903 | 8 | 14 | 16 | 19 | 9 | 14 | 16 | 19 |
| Male Adult | 16-64 | 95.0 | 728 | 9 | 15 | 17 | 20 | 9 | 16 | 18 | 20 |

**Estimated Daily Intake of DHA+EPA from Individual Proposed Food-Uses of DHA-O in the EU**

*All-Person Intakes*

Estimates for the mean and 95$^{th}$ percentile daily intakes of DHA+EPA from DHA-O from each individual food category are summarized in Tables A-1 to A-6 and B-1 to B-6 of Appendix 4 on a mg/day and mg/kg body weight/day basis, respectively. The total UK population was identified as being significant consumers of white bread (88.6 to 97.1% users), carbonated beverages (51.8 to 90.9% users), cooking oils (62.0 to 86.1% users), sweet biscuits (51.8 to 86.4% users) and cheese (66.0 to 82.4% users). The UK population did not significantly consume soy and imitation milk products, and dairy analogue drinks with less than 5% users in all population groups.

Male teenagers consuming carbonated beverages experienced the highest mean and 95$^{th}$ percentile all-user intakes of DHA+EPA from DHA-O of 246 and 609 mg/person/day, respectively. The lowest reliable mean all-user intake of DHA+EPA from DHA-O were estimated to occur in young people consuming cereal and nutrition bars, at 2 mg/person/day, while the lowest reliable 95$^{th}$ percentile all-user intake of DHA+EPA from DHA-O was estimated to occur in children consuming cooking oils, at 16 mg/person/day, respectively.

On a per kilogram body weight basis, children and young people consuming white bread were identified as having the highest all-person mean intake of DHA+EPA from DHA-O of 4.5 mg/kg body weight/day. The highest all-person 95$^{th}$ percentile intakes of DHA+EPA from DHA-O were observed in children consuming carbonated beverages, with a value of 15.7 mg/kg body weight/day.

*All-User Intakes*

Tables A-1 to A-6 and B-1 to B-6 also summarize the estimates for the mean all-user intakes of DHA+EPA by the individual surveyed populations from each of the individual food-uses of DHA-O on a mg/person/day and mg/kg body weight/day basis, respectively. Consumption of carbonated beverages made the greatest contribution to the mean all-user intake of DHA+EPA from DHA-O among female and male teenagers, while white bread made the greatest contribution to the mean all-user intake of DHA+EPA from DHA-O among children, young people, female adults, and male adults. Carbonated beverages made the greatest contribution to the 95$^{th}$ percentile all-user intakes among children, young people, female and male teenagers, and female adults, while the greatest contribution to the 95$^{th}$ percentile all-user intake of DHA+EPA from DHA-O among male adults was made by white bread.

The greatest contribution to the mean and 95$^{th}$ percentile all-user intake of DHA+EPA from DHA-O by the UK population were made by male teenagers consuming carbonated beverages, with values of 246 and 609 mg/person/day (4.5 and 11.8 mg/kg body weight/day, respectively), respectively. On a body weight basis, the highest mean and 95$^{th}$ percentile all-

user intakes of DHA+EPA from DHA-O were identified in children consuming fruit juice-based drinks, at 9.4 and 33.5 mg/kg body weight/day, respectively.

### IX.2.3 Conclusions

Consumption data and information pertaining to the individual proposed food-uses for DHA-O were used to estimate the all-person and all-user DHA+EPA from DHA-O intakes of specific demographic groups in the UK population. This type of intake methodology is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the 4-day children's survey, may overestimate consumption of food products that are consumed relatively infrequently, particularly when weighted to 7 days (Gregory *et al*., 1995).

In summary, on an all-user basis, the highest mean and 95$^{th}$ percentile intakes of DHA+EPA by the UK population from all proposed food-uses of DHA-O in the EU, observed in male teenagers were estimated to be 0.88 and 1.50 g/person/day, respectively. Children consumed the greatest amount of DHA+EPA from DHA-O on a per body weight basis with the highest mean and 95$^{th}$ percentile all-user intake of 29.5 and 53.6 mg/kg body weight/day, respectively. Furthermore, male teenagers consuming carbonated beverages were estimated to make the greatest contribution to the mean and 95$^{th}$ percentile all-user intake of DHA+EPA from DHA-O, with values of 246 and 609 mg/person/day (4.5 and 11.8 mg/kg body weight/day, respectively), respectively.

## IX.3 Is there information to show anticipated intakes for groups predicted to be at risk?

DHA-O has been developed to provide an alternative choice of DHA and EPA to other omega-3 sources in food and food supplement products. Whilst very high doses of fish oils have been suggested as increasing bleeding time, patients taking anticoagulation therapy are advised by their healthcare professionals not to consume large doses of fish oil. However this should be of no concern in relation to the consumption of products fortified with DHA-O. In 1997 and again in 2005, the U.S. Food and Drug Administration (U.S. FDA) has stated that there is not a significant bleeding risk at intake levels of DHA+EPA at levels up to 3 g/day.

Results from both short and intermediate-length clinical trials indicate that:

- "..the experience has been virtually unanimous: omega-3 fatty acid supplements do not increase the risk for clinically significant bleeding, even in patients also being treated with anti-platelet or antithrombotic medications." (Harris, 2007)

## IX.4 Will introduction of the novel food be restricted geographically?

There are no proposed geographical restrictions. DHA-O is an environmentally sustainable vegetarian alternative to fish. This should be a highly desirable prospect for all Member States at a time of stretched fishing stocks and the increasing amount of evidence supporting the importance of DHA and EPA to their citizens' health.

## IX.5 Will the novel food replace other foods in the diet

As stated earlier, DHA-O is a simple replacement for fish and other algal oils in the European diet. It is proposed at "like-for like" uses and levels of addition, with the same nutritional value.

# XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

1. “Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?”

We will address this point within this section.

## X.1 Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?

As stated in detail in the introduction to this dossier the proposed maximum use level reflects the nutrition recommendations of EFSA and the regulatory requirements of the Annex to Regulation 1924/2006. Specifically the advisory intake/daily reference labelling value of 250 mg DHA plus EPA per day (for food supplements and meal replacements) and the requirements for "High in Omega-3 Fatty Acids" (for non-alcoholic beverages).

We are simply matching the latest developments in "generally accepted scientific evidence" to ensure adequate levels of DHA and EPA are provided to European consumers and making small modifications in line with uses that are already approved for DHA and EPA-rich oils.

In addition to fish oils a number of specific DHA and EPA-rich oils have received novel food approvals.

### X.1.1 Approved DHA-rich Microalgal Oils

As discussed earlier DHA-rich oil from the microalgae *Schizochytrium* sp. (DHA-S) is already approved under Commission Decisions 2003/427/EC and 2009/778/EC (Commission of the European Communities, 2003, 2009a). The approval uses are laid down in Tables 1 and 2 above. Lonza (formerly Nutrinova) have also notified for substantial equivalence on 24 Dec 2003 to Decision 2003/427/EC[12] and have also obtained full approval under *Commission Decision of 21 October 2009 concerning the extension of uses of algal oil from the micro-algae Ulkenia sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council*[13]. *Ulkenia sp.* has essentially the same approval specification and uses as DHA-S.

### X.1.2 Approved DHA and EPA-rich Krill Oil

Additionally *Commission Decision of 12 October 2009 authorising the placing on the market of a lipid extract from Antarctic Krill Euphausia superba as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council*[14] has approved the uses of DHA and EPA in Table 18 below. These are essentially equivalent in terms of use groups and maximum levels of specified LC PUFAs as those in Decision 2003/427/EC.

[12] European Commission, 2007 (http://ec.europa.eu/food/food/biotechnology/novelfood/notif_list_en.pdf)
[13] Commission of the European Communities, 2009b (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:278:0054:0055:EN:PDF)
[14] Commission of the European Communities, 2009c (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:268:0033:0034:EN:PDF)

| Table 18 Authorized Uses of Krill Oil Pursuant to Decision 2003/427/EC | |
|---|---|
| **Food Category Use Group** | **Maximum Use Level of combined DHA and EPA** |
| Dairy products except milk-based drinks | 200 mg/100 g or for cheese products 600 mg/100 g |
| Dairy analogues except drinks | 200 mg/100 g or for analogues to cheese products 600 mg/100 g |
| Spreadable fat and dressings | 600 mg/100 g |
| Breakfast cereals | 500 mg/100 g |
| Food supplements | 200 mg per daily dose as recommended by the manufacturer |
| Dietary foods for special medical purposes | In accordance with the particular nutritional requirements of the persons for whom the products are intended |
| Foods intended for use in energy-restricted diets for weight reduction | 200 mg/meal replacement |

# XII MICROBIOLOGICAL INFORMATION

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to microbiological information available for the novel food (Commission of the European Communities, 1997):

1. “Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?”

We will address this point in the following section.

## XII.1 Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?

In Section I we have presented details of aflatoxin analysis and below in Table 19 we present the results of microbiological analysis of DHA-O Pasteurisation is employed in the manufacture of DHA-O, which is itself 100% lipid with very low water activity. Thus neither the source organism nor microbial contaminants are able to survive.

**Table 19 Microbiological Analysis Results for DHA-O**

| Test Method | Specification | 98-5807 | 5814 | 5828 |
|---|---|---|---|---|
| Standard Plate Count<br>AOAC 966.23 | <10 CFU/g | <10 | <10 | <10 |
| Yeast<br>FDA BAM Ch 18 | <10 CFU/g | <10 | <10 | <10 |
| Mold<br>FDA BAM Ch 18 | <10 CFU/g | <10 | <10 | <10 |
| Total Coliforms (Petrifilm)<br>AOAC 988.19 Mod. | <10 CFU/g | <10 | <10 | <10 |
| *Escherichia coli* by Petrifilm<br>AOAC 988.19 Mod. | <10 CFU/g | <10 | <10 | <10 |
| *Staphylococci Coagulase*+<br>AOAC 1003.07 | <10 CFU/g | <10 | <10 | <10 |
| *Salmonella*<br>AOAC 2003.09 | Negative /25g | Negative | Negative | Negative |

The results clearly show the absence of both source organism and opportunistic contamination. DHA-O is manufactured using full Good Manufacturing Procedures and Martek continues to comply with the requirements of *Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs*[15].

[15] European Commission, 2004 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:006:0030:0034:EN:PDF)

# XIII ADDITIONAL TOXICOLOGICAL AND HUMAN SAFETY INFORMATION

Based on Commission Recommendation 97/618/EC decision trees the following questions must be addressed pertaining to toxicological information available on the novel food (Commission of the European Communities, 1997):

1. “Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?”

2. “Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?”

OR

3. “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?”

4. “Is there information which suggests that the novel food might pose an allergenic risk to humans?”

We will address each point in turn in this section.

## XIII.1 Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?

### XIII.1.1 Background and Comparison to Fish Oil

The traditional counterpart to DHA-O is fish oil which is widely used for food supplements and food fortification throughout the EU and without restriction. DHA-O is a simple replacement. Table 12 above provides a comparison of DHA-O to a range of commercially available oils. The ratios of DHA and EPA are very similar, given the seasonal and geographical variation.

### XIII.1.2 Background and Comparison to DHA-S

Morphological, biochemical, and DNA sequence characteristics indicate that the current production organism and DHA-O are both *Schizochytrium* species and phenotypically very similar. This, plus the compositional similarities between the DHA (S) Algal Oil and DHA-O Algal Oil allow use of the safety data generated with DHA-S Algal Oil to support the safety of the intended uses of DHA-O Algal Oil. . In addition confirmatory pre-clinical studies and genotoxicity studies have been completed on DHA-O.

## XIII.2 Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?

We have clearly demonstrated in the Sections above that there are no added toxicants. Indeed the nature of manufacturer of DHA-O in closed vessels with tight production and environmental controls mean that the risk of contamination from environmental sources is much lower than that for fish and fish oil.

Manufacturing controls for DHA-O are the same as for DHA-S.

## XIII.3 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?

The pre-clinical studies conducted with *Schizochytrium* sp. biomass and subsequent pre-clinical and extensive clinical studies conducted in DHA-S have been reviewed previously by the ACNFP in two previous opinions[16,17] and we do not intend to discuss them further in this petition. In the following sections we provide details of the confirmatory pre-clinical studies that have been completed on DHA-O in accordance with the SCF guidance.

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[16] DHA Gold
February 2001: Application from OmegaTech for approval of DHA Gold, a DHA-rich oil. Authorised June 2003.
[17] DHA Rich Microalgal Oil
January 2008: Application from Martek Biosciences Corporation, for the extension of use of a DHA-rich algal oil from the microalgae *Schizochytrium* sp under the novel food Regulation (EC) 258/97.

### XII.3.1 Confirmatory Pre-clinical Safety Studies

*Sub-chronic Studies*

14 day Dose-ranging Study in the rat - (Martek, 2010 – submitted for publication) **Proprietary Data**

In order to set the correct doses for the following 90-day study, and in agreement with OECD Guidelines for Testing of Chemicals Section 4 (Part 407): Health Effects (OECD, 1995), a 14-day dose-ranging study was conducted with DHA-O.

One hundred healthy rats (50 male and 50 females per dietary level). Dietary levels of 60,000 mg/kg (Group 2) Fish Oil as well as, 10,000 mg/kg (Group 3), 30,000 mg/kg (Group 4), and 60,000 mg/kg (Group 5) of the test substance, as well as Basal Diet control (Group 1), were selected for the test.

The results of the study demonstrated that the animals were expected to tolerate at least 60,000 mg/kg DHA-O in a study of longer duration.

The full test report is considered to be confidential (*i.e.*, Martek have exclusive right to the data and it is considered proprietary).

90-day toxicity study in the rat – (Martek, 2010 – submitted for publication) **Proprietary Data**

A 90 day repeated dose dietary toxicity study in rats was conducted on DHA-O. The study was conducted to good laboratory practices (GLP) following the OECD Guidelines for the Testing of Chemicals and Food Ingredients, Section 4 (Part 408): Health Effects, *Repeated Dose 90-Day Oral Toxicity Study in Rodents.* (OECD, 1981) (as specified by the SCF guidance for novel foods) and U.S. FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 4. a. *Subchronic Toxicity Studies with Rodents* (U.S. FDA, 2003).

One hundred healthy rats (50 males and 50 females) were selected for the test and equally distributed into 5 groups (10 males and 10 females per dietary level). Dietary levels of 50,000 mg/kg (Group 2) Fish Oil as well as, 50,000 mg/kg (Group 3), 15,000 mg/kg (Group 4), and 50,000 mg/kg (Group 5) of the test substance, as well as Basal Diet control (Group 1), were selected for the test.

Under the conditions of this study, there was no toxicity related to administration of DHA-O in male or female Sprague-Dawley rats. Under the conditions of this study and based on the toxicological endpoints evaluated, the NOAEL for DHA-O in the diet was judged to be 50,000 mg/kg for male and female rats, equivalent to 3149 mg/kg body weight/day and 3343 mg/kg body weight/day, for male and female rats respectively.

*Genotoxicity Studies*

Reverse Mutation (Ames) Assay **Proprietary Data**

A reverse mutation assay in *Salmonella typhimurium* and *Escherichia coli* was conducted in accordance with OECD Good Laboratory Practice "Bacterial Reverse Mutation Test": OECD Guideline for the Testing of Chemicals, Test Guideline 471 (OECD, 1997a).

No biologically relevant increases in revertant colony numbers of any of the 5 tester strains were observed following treatment with DHA-O or at any concentration level, neither in the presence or absence of metabolic activation. The study authors conclude that DHA-O did not induce gene mutations by base-pair changes or frameshifts in the genomes of the tester strains used and therefore was non mutagenic.

*In-vitro* Mammalian Chromosome Aberration Test – (Martek, 2010 – submitted for publication) **Proprietary Data**

An *in-vitro* mammalian chromosome aberration test in human lymphocytes was conducted to GLP and OECD Guideline No 473 "*In-vitro* Mammalian Chromosomal Aberration Test" (OECD, 1997b), also at BSL Bioservice GmbH. The genotoxicity was assessed in the presence and absence of metabolic activation by S-9 homogenate. There was no induction of clastogenicity (chromosomal aberrations) in any of the does tested.

*In-vivo* Mouse Micronucleus Test – (Martek, 2010 – submitted for publication) **Proprietary Data**

A *in-vivo* mouse micronucleus test was conducted to GLP and in accordance with OECD Guideline No 474 "Mammalian Erythrocyte Micronucleus Test" (OECD, 1997c), also at Bioservice GmbH. DHA-O did not induce structural or numerical chromosomal damage in the immature erythrocytes of the mouse

## XIII.4 Is there information which suggests that the novel food might pose an allergenic risk to humans?

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 blue-green algae or dinoflagellates, the 2 groups of algae with the most toxic species. 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 and Reif, 1953; Grauer, 1959). There has been one report of an allergic response to the green alga *Chlorella* in children (Tiberg *et al*., 1995).

There is no indication to suggest that DHA-O should elicit allergenic responses. It is also worth noting that to date there have been no reported serious adverse events related to allergenicity from the consumption of DHA-S oil.

## CONCLUSIONS

Martek Biosciences Corporation (Martek) has previously gained approval for docosahexaenoic acid (DHA)-rich oil produced from *Schizochytrium* sp. (hereinafter "DHA-S") a microalgae, for general use as a nutritional ingredient in foods. Martek has developed an improved strain, from another species of *Schizochytrium* microalgae. This strain produces an oil which contains a similar amount of docosahexaenoic acid DHA as in DHA-S along with an eicosapentaenoic acid (EPA) content which is approximately half that of the DHA concentration. This in effect makes it closer still to other approved sources, which it is intended to replace in foods and food supplements. Indeed the fatty acid and sterol profiles of DHA-O contain no new fatty acids that are not already consumed in either fish or vegetable oils. Extensive analysis shows the absence of significant levels of impurities or contaminants.

The proposed uses of DHA-O are largely the same as currently approved for DHA-S in the EU with a slight increase in levels for 2 categories to allow for increased dietary recommendations for DHA and EPA and to add biscuits and cooking oils at low levels. Dietary survey data shows that mean estimated daily intakes from all uses would not exceed 0.9 g of DHA+EPA per day (equivalent to 4 maximally fortified portions approximately) and 95$^{th}$ percentile intakes would not exceed 1.5 g (approximately 6 to 7 maximally fortified portions approximately). These estimates are clearly huge over-estimations.

In addition to the extensive safety database already available on *Schizochytrium* sp. algal biomass, on DHA-S and on fish oil itself, Martek has conducted supporting confirmatory pre-clinical studies on DHA-O, which include a 90-day rat study and a suite of mutagenicity studies. All of these show no significant adverse effects at the maximum dose tested. For the 90 day rat study the NOAEL for DHA-O equivalent to 3149 mg/kg body weight/day and 3343 mg/kg body weight/day for male and female rats respectively equivalent to DHA+EPA doses of 1669 and 1772. For a 60 kg adult this equates to approximately 200 g per person per day of DHA-O/ 100 g DHA +EPA. The absence of significant levels of protein and extensive history of safe consumption of DHA-S indicate there is no significant risk for allergenicity. DHA-O is therefore proposed as a safe and suitable vegetarian and sustainably produced alternative to fish oil for use in foods as a source of the important LC PUFAs DHA and EPA.

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