Bureau Nieuwe Voedingsmiddelen
Novel Foods Unit

Olie rijk aan docosahexaeenzuur (DHA) en eicosapentaeenzuur (EPA)

Oil rich in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)

Tweede beoordeling van de veiligheid voor de consument, volgens de Europese verordening 258/97 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten

Second opinion regarding consumer safety, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients

aan/to:

de Minister van Volksgezondheid, Welzijn en Sport
the Minister of Health, Welfare and Sport

Nr. 2012-02 BNV, Den Haag, 24 februari 2012
No. 2012-02 BNV, The Hague, February 24, 2012

Bureau Nieuwe Voedingsmiddelen
CBG
Postbus 16229
2500 BE Den Haag
novelfoods@cbg-meb.nl
www.nieuwevoedingsmiddelen.nl

Novel Foods Unit
MEB
P.O. box 16229
2500 BE The Hague
The Netherlands
novelfoods@cbg-meb.nl
www.novel-foods.nl

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Beoordeling

Inleiding

Aan de orde is een tweede beoordeling volgens de Europese Verordening 258/97 (EG97, EG97a), over het gebruik als nieuw voedingsmiddel van een olie afkomstig van de microalg *Schizochytrium sp*. Deze olie bevat tenminste 22,5 % docosahexaeenzuur (DHA) en tenminste 10% eicosapentaeenzuur (EPA). De aanvraag is ingediend door Martek Biosciences Corporation. De aanvrager wil de nieuwe DHA- en EPA-rijke olie, genaamd DHA-O, gaan verwerken in verschillende soorten voedingsmiddelen. Tabel 4 van Bijlage B geeft een overzicht van deze toepassingen[1] die qua productcategorieën en DHA-gehalten grotendeels vergelijkbaar zijn met het gebruik van de al toegelaten DHA-rijke olie, genaamd DHA-S (EG03, EG09). De aanvrager heeft echter enkele typen levensmiddelen toegevoegd aan het voorgestelde productassortiment en heeft van sommige toepassingen de dagelijks te consumeren hoeveelheid van het nieuwe ingrediënt verhoogd. Met dit nieuwe voorstel wil de aanvrager tegemoet komen aan de aanbevolen hoeveelheden DHA en/of EPA zoals recent zijn aangepast door verschillende wetenschappelijke organisaties (zie hoofdstuk IX van Bijlage B).

In het kader van de desbetreffende Europese toelatingsprocedure is deze tweede beoordeling uitgevoerd door het Bureau Nieuwe Voedingsmiddelen van het College ter Beoordeling van Geneesmiddelen. Het bureau heeft hiervoor de Commissie Veiligheidsbeoordeling Nieuwe Voedingsmiddelen geraadpleegd, hierna genoemd 'de commissie VNV'.

Eerste beoordeling

De eerste beoordeling van de aanvraag voor markttoelating is verricht in het Verenigd Koninkrijk door de *Advisory Committee on Novel Foods and Processes* (ACNFP). In het rapport van de eerste beoordeling concludeert deze deskundigencommissie dat de nieuwe DHA- en EPA-rijke olie veilig kan worden geconsumeerd bij de toepassing die de aanvrager voorstelt. Net als bij eerdere beoordelingen van nieuwe algenoliën rijk aan meervoudig onverzadigde vetzuren, benadrukt de ACNFP ook nu dat marktmonitoring nodig is op nationaal en/of Europees niveau omdat zij bezorgd is voor mogelijke gevolgen voor de gezondheid bij langdurig, hoge blootstelling aan deze producten. Deze onzekerheid wordt echter niet alleen bepaald door consumptie van levensmiddelen die zijn verrijkt met de nieuwe DHA- en EPA-rijke olie. Volgens de ACNFP is het daarom belangrijk dat bij onderzoek naar de impact van meervoudig onverzadigde langeketenvetzuren (n-3) alle bronnen van deze vetzuren in de voeding worden meegenomen en ook verschillende leeftijdsgroepen, in het bijzonder kinderen.

Oordeel van de Commissie VNV

De Commissie VNV heeft geen bezwaar tegen de toelating als nieuw voedingsmiddel van de DHA- en EPA-rijke olie en is het eens met de positieve beoordeling door de ACNFP. De commissie VNV heeft haar oordeel gebaseerd op de informatie in het dossier, waarvan de

[1] De categorie bakoliën blijkt per ongeluk te zijn weggevallen in Bijlage A.

samenvatting is opgenomen als bijlage A, en de eerste beoordeling door de ACNFP, toegevoegd als bijlage B.

Gebaseerd op het totaal aan beschikbare gegevens, heeft de commissie VNV geen aanwijzingen dat nadelige gezondheidseffecten kunnen optreden bij voorgesteld gebruik van de nieuwe olie. Net als in haar eerdere adviezen over meervoudig onverzadigde n-3 langeketenvetzuren (CBG08), onderschrijft de commissie de noodzaak van beleidsmaatregelen ten aanzien van de mogelijke cumulatieve inname van deze vetzuren uit alle bronnen in de voeding, bij voorkeur op Europees niveau. De commissie VNV betwijfelt namelijk of de consument zijn dagelijkse inname van deze vetzuren verantwoord kan regelen. Daarom zal bij de verschillende leeftijdsgroepen, in het bijzonder kinderen, moeten worden geverifieerd of de totale consumptie van DHA en EPA uit alle bronnen de veilig geachte bovengrens niet overschrijdt.

Referenties

CBG08 Http://www.cbg-meb.nl/CBG/nl/nieuwe_voedingsmiddelen/beoordelingen/completed%20evaluations/default.htm: Adviezen 'Docosahexaeenzuurrijke olie (2)' van 26 november 2008 en 'Krillolie' van 19 april 2007.

EG97 Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad van 27 januari 1997 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten. Publicatieblad van de Europese Gemeenschappen 1997; L43: 1-6.

EG97a Aanbeveling (EG) nr. 97/618/EG van de Commissie van 29 juli 1997 betreffende de wetenschappelijke aspecten en de presentatie van de informatie die nodig is om aanvragen voor het in de handel brengen van nieuwe voedingsmiddelen en nieuwe voedselingrediënten te ondersteunen alsmede het opstellen van de verslagen van de eerste beoordeling uit hoofde van Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad. Publicatieblad van de Europese Gemeenschappen 1997; L253: 1-36.

EG03 2003/427/EG: Beschikking van de commissie van 5 juni 2003 tot verlening van een vergunning voor het in de handel brengen van DHA-rijke (docosahexaeenzuurrijke) olie van de microalg *Schizochytrium sp*. als nieuw voedselingrediënt krachtens Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad. Publicatieblad van de Europese Unie L 144 (2003): 13-14. Zie http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:144:0013:0014:NL:PDF

EG09 2009/778/EG: Beschikking van de Commissie van 22 oktober 2009 betreffende de uitbreiding van het gebruik van algenolie van de microalg *Schizochytrium sp*. als nieuw voedselingrediënt krachtens Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad. Zie http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:278:0056:0057:NL:PDF

Assessment

Introduction

This report describes a second assessment under European Regulation 258/97 (EC97, EC97a) of the use as a novel food of an oil obtained from the microalgae *Schizochytrium sp*. This algal oil contains not less than 22.5% of docosahexaenoic acid (DHA) and not less than 10% of eicosapentaenoic acid (EPA). The application was submitted by Martek Biosciences Corporation. The applicant will use the DHA and EPA rich oil, referred to as DHA-O, in various types of foods[1] as listed in Table 4 of Annex B. The proposed food use largely resembles the range of food categories as well as the use levels of DHA that are currently approved for the DHA-rich oil of previous applications ('DHA-S') (EC03, EC09). The applicant, however, includes a few additional food categories and proposes an increased daily dose of the novel ingredient for certain uses. The reason for the changes in the current proposal is to reflect the latest advice of several scientific organizations on intakes of DHA and/or EPA (see chapter IX of Annex B).

The second assessment reported here was performed by the Novel Foods Unit of the Medicines Evaluation Board, in accordance with the European authorisation procedure. The Unit consulted the Committee on the Safety Assessment of Novel Foods (referred to below as 'the VNV Committee') regarding its assessment.

Initial assessment

The initial assessment of the application for market authorisation was conducted in the United Kingdom by the Advisory Committee on Novel Foods and Processes (ACNFP). In the report on its initial assessment, this expert Committee concludes that the novel DHA and EPA rich oil can safely be consumed in the manner described in the dossier. Like in previous assessments of novel algal oils rich in polyunsaturated fatty acids, the ACNFP stresses again that intakes of DHA should be monitored at national and/or EU level because concerns have been raised about the impact that long term, high-level consumption of these products may have on health. However, this uncertainty is not solely related to consumption of foods containing the novel DHA and EPA rich oil. According to the ACNFP, it is therefore important that all research that look at the impact of consumption of foods fortified with n-3 long chain polyunsaturated fatty acids should address all dietary sources and different age groups, particularly children.

Findings of the VNV Committee

The VNV Committee has no objection to the authorisation of the DHA and EPA rich oil as a novel food, and concurs with the favourable assessment by the ACNFP. The VNV Committee bases its view on the information in the dossier, which is summarised in Annex A, and the initial assessment by the ACNFP, which is appended as Annex B.

Based on all available information, the VNV Committee has no indications that adverse health effects can occur when the novel oil is used as described in the proposal. Like its previous advices on n-3 long chain polyunsaturated fatty acids (MEB08), the VNV Committee supports the critical view of the ACNFP emphasizing the need of policy measures, preferably

[1] In Annex A, the category "cooking oils" has been left out by mistake.

at European level, with regard to the possible cumulative intake of these fatty acids from all dietary sources. The VNV Committee has expressed its doubts about consumers' ability to reliably regulate their daily consumption of these fatty acids. For that reason, the question of whether the total consumption of DHA and EPA from all sources exceeds the 'safe upper limit' needs to be verified for each of the various age groups, and for children in particular.

References

EC97 Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal of the European Communities 1997; L43: 1-6.

EC97a 97/618/EC. Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament of the Council. Official Journal of the European Communities 1997; L253: 1-36.

EC03 2003/427/EC: Commission Decision of 5 June 2003 authorizing 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. Official Journal L 144 (2003): 13 -14. Available from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:144:0013:0014:EN:PDF

EC09 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. Available from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:278:0056:0057:EN:PDF

MEB08 Http://www.cbg-meb.nl/CBG/en/novel-foods/assessments/finished_assessments/default.htm Advisory reports Docosahexaenoic acid rich oil (November 26, 2008) and Krill oil (April 19, 2007).

De commissie / The Committee

- Prof. dr. G.J. Mulder, *voorzitter / chairman*
  emeritus hoogleraar toxicologie, Universiteit Leiden
  professor emeritus toxicology, Leiden University
- Prof. dr. C.A.F.M. Bruijnzeel-Koomen, *adviseur / advisor*
  hoogleraar dermatologie/allergologie; UMC Utrecht
  professor of dermatology/allergology; University Medical Centre Utrecht
- Dr. ir. M. Dekker
  levensmiddelentechnoloog; Wageningen Universiteit en Researchcentrum
  food technologist; Wageningen University and Research Centre
- Dr. A.F.M. Kardinaal
  voedingskundige; TNO, Zeist
  nutitional expert; TNO, Zeist
- Dr. ir. E.J. Kok
  toxicoloog; RIKILT Instituut voor Voedselveiligheid, Wageningen
  toxicologist; RIKILT, Institute of Food Safety, Wageningen
- Dr. C.F. van Kreijl
  moleculair-bioloog (gepensioneerd); RIVM Bilthoven
  molecular biologist (retired); National Institute of Public Health and the Environment, Bilthoven
- Dr. F.M. Nagengast
  gastro-enteroloog; UMC St Radboud, Nijmegen
  gastro-enterologist; University Medical Centre St Radboud, Nijmegen
- Dr. ir. J.M.A. van Raaij
  voedingsfysioloog; Wageningen Universiteit en Researchcentrum; RIVM Bilthoven
  food physiologist; Wageningen University and Research Centre; National Institute of Public Health and the Environment, Bilthoven
- Dr. G.J.A. Speijers
  toxicoloog (gepensioneerd); RIVM Bilthoven
  toxicologist (retired); National Institute of Public Health and the Environment, Bilthoven
- Dr. ir. E.H.M. Temme
  voedingskundige; RIVM Bilthoven
  nutritional expert; National Institute of Public Health and the Environment, Bilthoven
- Drs. A.I. Viloria Alebesque, *adviseur / advisor*
  Ministerie van VWS, Den Haag
  Ministry of Health, Welfare and Sport, The Hague
- Dr. R.A. Woutersen
  toxicoloog, toxicologisch patholoog; TNO, Zeist
  toxicologist, toxicologic pathologist; TNO, Zeist

Bureau Nieuwe Voedingsmiddelen, CBG/ Novel Foods Unit, MEB

- Dr. C.M.A. van Rossum, *beoordelaar voedselveiligheid / scientific assessor food safety*
- Dr. ir. M. Rutgers, *beoordelaar voedselveiligheid / scientific assessor food safety*
- Drs. E. van Galen, *hoofd BNV / head NFU*

A Samenvatting van het dossier / Summary of the dossier

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

SUMMARY

NON-CONFIDENTIAL

Prepared by: Martek Biosciences Corporation
6480 Dobbin Road
Columbia, MD 21045
USA

December 15, 2010

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

For all correspondence regarding this dossier, please refer to:

Martek Biosciences Corporation
6480 Dobbin Road
Columbia
Maryland 21045
USA

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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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 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*[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".

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.

I SPECIFICATION OF THE NOVEL FOOD

The specification for DHA-O is presented in Table 3 below.

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

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%

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. 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 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.

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

The production of DHA-O is tightly controlled using standard fermentation, recovery - and purification techniques used in the food industry. 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 DHA-O oil profile (DHA:EPA ratio) is very similar to that of other fish oils/fish, *e.g.*, tuna oil. DHA-O delivers on average approximately a ratio of 2 DHA: 1 EPA which is comparable to the ratio in a number of fish species.

All processes are set up using a Hazard Analysis Critical Control Point (HACCP) approach.

III HISTORY OF SOURCE ORGANISM

DHA-O is obtained from *Schizochytrium* sp. microalgae using a classic screening program that utilized well-accepted techniques commonly employed in industrial strain improvement programs. **No recombinant DNA technology was employed.**

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

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.

IX ANTICIPATED INTAKE/EXTENT OF USE

DHA-O is clearly a close alternative to other currently available DHA and EPA sources and is from sustainable and vegetarian sources. It's 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.

In this application we wish to apply for the uses laid down in Table 4 below. These are largely very similar to the uses currently approved for DHA-S.

Table 4 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

Table 4	**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)**
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 in levels from those approved for DHA-S 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*[2]

*"*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

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

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 5). 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 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).

Table 5 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 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.

Table 5	World-wide DHA Intake Recommendations for Pregnant and Lactating Women	
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*[3]

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.

We will consider each in turn in the following sections:

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

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

advised intake of long chain omega-3 fatty acids of 250 mg per day, or in the case of pregnant and lactating women 450 mg per day (including at least 200 mg of DHA). 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*[4].

For meal replacements 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[5] 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.

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). 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.

On an all-user basis, the highest mean and 95th percentile intakes of DHA+EPA from DHA-O by the UK population from all proposed food-uses 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 95th 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 95th 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.

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.

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[4] 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)
[5] Commission of the European Communities, 1996 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1996L0008:20070620:EN:PDF)

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)

There are no proposed geographical restrictions. DHA-O is an environmentally sustainable vegetarian alternative to fish oil. 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.

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

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 and 450 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.

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[6] 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*

[6] European Commission, 2007 (http://ec.europa.eu/food/food/biotechnology/novelfood/notif_list_en.pdf)

European Parliament and of the Council[7]. *Ulkenia sp.* has essentially the same approval specification and uses as DHA-S.

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*[8] has approved uses of DHA and EPA. These are essentially equivalent in terms of use groups and maximum levels of specified LC PUFAs as those in Decision 2003/427/EC.

XII MICROBIOLOGICAL INFORMATION

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.

Microbiological testing clearly shows 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* [9].

XIII ADDITIONAL TOXICOLOGICAL AND HUMAN SAFETY INFORMATION

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.

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.

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.

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[7] Commission of the European Communities, 2009b (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:278:0054:0055:EN:PDF)
[8] Commission of the European Communities, 2009c (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:268:0033:0034:EN:PDF)
[9] Corrigendum to Regulation (EC) No 852/2004 (European Parliament and the Council of the European Union, 2004a,b - http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:226:0003:0021:EN:PDF)

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

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[10,11] 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.

Confirmatory Pre-clinical Safety Studies have been conducted with DHA-O:

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.

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

[10] DHA Gold
February 2001: Application from OmegaTech for approval of DHA Gold, a DHA-rich oil. Authorised June 2003.
[11] 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.

(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). 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**

An *in-vivo* mouse micronucleus test was conducted to GLP and in accordance with OECD Guideline No 474 "Mammalian Erythrocyte Micronucleus Test" (OECD, 1997c).

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 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 95th 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.

REFERENCES

AFSSA, Opinion Regarding the Update of the Recommended Dietary Intake for Fatty Acids. AFSSA-Hearing n2006-SA-0359. 2010.

ANZ National Health and Medical Research Council. Nutrient reference values for Australia and New Zealand including recommended dietary intakes. 2006. Available from: www.nhmrc.gov.au/publications/synopses/_files/n35.pdf.

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

Cohen SG, Reif CB. Cutaneous sensitization to blue-green algae. *The Journal of Allergy* 1953, 24:452-457.

Commission of the European Communities. Commission Directive 96/8/EC of 26 February 1996 on foods intended for use in energy-restricted diets for weight reduction [L55]. *Official Journal of the European Communities* 1996, 39:22-26. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1996L0008:20070620:EN:PDF [Consolidated version: 2007-06-20].

Commission of the European Communities. Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council [L253]. *Official Journal of the European Communities* 1997, 40:1-36. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31997H0618:EN:HTML.

Commission of the European Communities. Commission Decision of 5 June 2003 authorizing 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) [L144]. *Official Journal of the European Union*, 2003, 40:13-14. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:144:0013:0014:EN:PDF.

Commission of the European Communities. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs [L364]. *Official Journal of the European Union*, 2006a. 49:5–24. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF.

Commission of the European Communities. Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs [L70]. *Official Journal of the European Union* 2006b. 49:12–34. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R0401:20100313:EN:PDF [Consolidated version: 2010-03-13].

Commission of the European Communities. 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 [L278]. *Official Journal of the European Union* 2009a, 52:56-57. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:278:0056:0057:EN:PDF.

Commission of the European Communities. 2009/777/EC: 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 [L278]. *Official Journal of the European Union* 2009b, 52:54–55. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:278:0054:0055:EN:PDF.

Commission of the European Communities. 2009/752/EC: 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 [L268]. *Official Journal of the European Union* 2009c, 52:33–34. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:268:0033:0034:EN:PDF.

Council of the European Communities. Council Directive 88/344/EEC of 13 June 1988 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients [L157]. *Official Journal of the European Communities* 1988, 31:28–33. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1988L0344:19971223:EN:PDF [Consolidated version: 1997-12-23].

EFSA. Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids 1 [Question No EFSA-Q-2009-00548 - Adopted on 30 June 2009 by the European Food Safety Authority (EFSA)]. *The EFSA Journal* 2009, 1176:1-11. Available from: http://www.efsa.europa.eu/en/scdocs/scdoc/1176.htm.

EFSA. Response to comments with regard to two EFSA opinion regarding the scientific substantiation of health claims related to docosahexaenoic acid (DHA) and support of the visual development of the unborn child and breasfed infant (EFSA-Q-2008-675) and on DHA and support of the cognitive development of the unborn child and breastfed infant (EFSA-Q-2008-772) pursuant to Article 14 Regulation (EC) No 1924/2006. 2009. Available from: http://ec.europa.eu/food/efsa/comments/Q-2008-675-reply.pdf

EFSA. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol (EFSA Panel on Dietetic Products, Nutrition, and Allergies/NDA). [Question number: EFSA-Q-2008-466 - Adopted: 4 December 2009 by the European Food Safety Authority (EFSA). The EFSA Journal 2010 8:1461 [5 pp.]. Available from: http://www.efsa.europa.eu/en/scdocs/scdoc/1461.htm.European Commission. *Notifications Pursuant to Article 5 of Regulation (Ec) N°258/97 of the European Parliament and of the Council.* European Commission. Available from: http://ec.europa.eu/food/food/biotechnology/novelfood/notif_list_en.pdf [April 2007].

European Commission. 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 [L37]. *Official Journal of the European Union* 2010, 53:16–18. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:037:0016:0018:EN:PDF.

European Parliament and Council of the European Union. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients [L43]. *Official Journal of the European Communities* 1997, 40:1-6. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090807:EN:PDF [Consolidated to: 2009-08-07].

European Parliament and Council of the European Union. Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs [L109]. *Official Journal of the European Communities* 2000, 43:29–42. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2000L0013:20090807:EN:PDF [Consolidated version: 2009-08-07].

European Parliament and the Council of the European Union. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements [L183]. *Official Journal of the European Communities* 2002, 45:51–57. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0046:20091221:EN:PDF [Consolidated version: 2009-12-21].

European Parliament and the Council of the European Union. Corrigendum to Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs [L226]. *Official Journal of the European Union*, 2004a, 41:3-21. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:226:0003:0021:EN:PDF.

European Parliament and the Council of the European Union. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs [L139]. *Official Journal of the European Union*, 2004b, 41:1-54. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2004R0852:20090420:EN:PDF [Consolidated version: 2009-04-20].

FAO/WHO Expert Consultation. From the Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition. 2008.

Fernandes A, White S, D'Silva K, Rose M. Simultaneous determination of PCDDs, PCDFs, PCBs and PBDEs in food. Talanta 2004, 63:1147-1155.

Fritz L, Quilliam MA, Wright JLC, Beale AM, Work T. An outbreak of domoic acid poisoning attributed to the pinnate diatom *Pseudonitzschia-australis*. *Journal of Phycology* 1992, 28:439-442.

Garrison DL, Conrad SM, Eilers PP, Waldron EH. Confirmation of domoic acid productions by *Pseudonitzschia australis* (Bacillariophyceae) cultures. *Journal of Phycology* 1992, 28:604-607.

Granéli E, Johansson N. Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. Harmful Algae 2003, 2:135-145.

Grauer FH. Dermatitis escharotica caused by a marine alga. *Hawaii Medical Journal* 1959 19:32-34.

Gregory JR, Collin DL, Davies PSW, Hughes JM, Clarke PC. Appendix J: Number and pattern of recording days and the effect of weighting. In: *National Diet and Nutrition Survey: Children Aged 1+1/2 to 4+1/2 Years. Vol. 1: Report of the Diet and Nutrition Survey.* Her Majesty's Stationary Office (HMSO), London, Engl.; 1995, 345-347.

Harris WS. Expert opinion: omega-3 fatty acids and bleeding-cause for concern? *The American Journal of Cardiology* 2007, 99:44C-46C.

Heise HA. Symptoms of hay fever caused by algae. The Journal of Allergy 1949, 20:383-385.

Heise HA. Symptoms of hay fever caused by algae. II. Microcystis, another form of algae producing allergenic reactions. Annals of Allergy 1951, 9:100-101.

Houdan A, Bonnard A, Fresnel J, Fouchard S, Billard C, Probert I. Toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta). Journal of Plankton Research 2004, 26:875-883.

International Society for the Study of Fats and Lipids (ISSFAL). 2009. ISSFAL Policy Statement 4: Recommendations for intake of polyunsaturated fatty acids by pregnant and lactating women. Available from: http://www.issfal.org/index.php/lipid-matters-mainmenu-8/issfal-policy-statements-mainmenu-9/121-issfal policy statement-4.

Koletzko, B., Cetin, I., and Brenna T.J. 2007. Perinatal Lipid Intake Working Group Consensus Statement: *Dietary fat intakes for pregnant and lactating women* 2009, Brit J Nutr 98:873-7.

Larsen A, Eikrem W, Paasche E. Growth and toxicity in Prymnesium patelliferum (Prymnesiophyceae) isolated from Norwegian waters. Canadian Journal of Botany 1993, 71:1357-1362.

Lien EL. Toxicology and safety of DHA. Prostaglandins, *Leukotrienes and Essential Fatty Acids* 2009, 81:125-132.

LSRO. *Third Report on Nutrition Monitoring in the United States.* Prepared by Bethesda (MD): Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) for the Interagency Board for Nutrition Monitoring and Related Research. Washington (DC): U.S. Government Printing Office, 1995, vol 1, pp. 19-31 & III-1 to III-10 and vol 2, pp. VB-1 to VB-2.

Lundholm N, Skov J, Pocklington R, Moestrup O. Domoic acid, the toxic amino acid responsible for amnesic shellfish poisoning, now in Pseudonitzschia seriata (Bacillariophyceae) in Europe. Phycologia 1994, 33:475-478.

MacDonald SJ, Anderson S, Brereton P, Wood R, Damant A. Determination of zearalenone in barley, maize and wheat flour, polenta, and maize-based baby food by immunoaffinity column cleanup with liquid chromatography: interlaboratory study. *Journal of AOAC International* 2005, 88:1733-1740.

March of Dimes. 2009. Omega-3 fatty acids during pregnancy. Available from: http://www.march of dines.com/professionals/nutrition_omega3more.html.

OECD. Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-Operation and Development (OECD), Paris, France; 1981. OECD Guideline No. 408. Available from: http://puck.sourceoecd.org/vl=15180684/cl=21/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s8/p1.

OECD. Repeated dose 28-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-Operation and Development (OECD), Paris, France; 1995. OECD Guideline No. 407. Available from: http://www.oecd.org/dataoecd/62/14/45123613.pdf.

OECD. Bacterial reverse mutation test. In: *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-Operation and Development (OECD), Paris, France; 1997a. OECD Guideline No. 471. Available from: http://titania.sourceoecd.org/vl=720763/cl=18/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s37/p1.

OECD. In vitro mammalian chromosome aberration test. In: *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-Operation and Development (OECD), Paris, France; 1997b. OECD Guideline No. 473. Available from: http://titania.sourceoecd.org/vl=720763/cl=18/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s38/p1.

OECD. Mammalian erythrocyte micronucleus test. In: *OECD Guidelines for the Testing of Chemicals*. Organisation for Economic Co-Operation and Development (OECD), Paris, France; 1997b. OECD Guideline No. 474. Available from: http://titania.sourceoecd.org/vl=1010303/cl=19/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s39/p1.

Office for National Statistics. *The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years: 2000-2001* [Computer File]. Office for National Statistics, Social and Vital Statistics Division & Food Standards Agency (FSA), Colchester, Engl., UK Data Archive [distributor]; 2005. SN: 5140. Available from: http://www.food.gov.uk/multimedia/pdfs/ndns5full.pdf.

Rose M, Lewis J, Langford N, Baxter M, Origgi S, Barber M, MacBain H and Thomas K. Arsenic in seaweed-forms, concentration and dietary exposure. *Food and Chemical Toxicology* 2007, 45:1263-1267.

SACN. Advice on Fish Consumption: Benefits and Risks. Committee on Toxicity (COT), Scientific Advisory Committee on Nutrition (SACN), London, Engl.; 2004. Available from: http://www.sacn.gov.uk/pdfs/fics_sacn_advice_fish.pdf.

Schothorst RC, Jekel AA, Van Egmond HP, De Mul A, Boon PE, Van Klaveren JD. Determination of trichothecenes in duplicate diets of young children by capillary gas chromatography with mass spectrometric detection. *Food Additives and Contaminants* 2005, 22:48-55.

Shilo M. Toxins of chrysophyceae. In: Kadis S, Ciegler A, Ajl AJ, eds. *Microbial Toxins. Vol. III: Algal and Fungal Toxins*. Academic Press, New York, 1971, pp. 67-103.

Superior Health Council (2004) Advisory Report: Recommendations and claims made on omega-3 fatty Acids *(SHC 7945).* Available from: 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, accessed December 2, 2008.

Tiberg E, Dreborg S, and Bjorksten B. Allergy to green algae (*Chlorella*) among children. *Journal of Allergy and Clinical Immunology* 1995, 96:257-259.

UKDA. *National Diet, Nutrition and Dental Survey of Children Aged 1 ½ to 4 ½ Years, 1992-1993* [computer file]. Office of Population Censuses and Surveys, Social Survey Division, Medical Research Council Centre for Human Nutrition Research, Ministry of Agriculture, Fisheries and Food (MAFF), and U.K. Department of Health. Colchester, Engl., Data Archive (UKDA) [distributor]; 13 December 1995. SN: 3481.

UKDA. *National Diet and Nutrition Survey: Young People Aged 4 to 18 Years, 1997*. Office for National Statistics Social Survey Division, Medical Research Council Centre for Human Nutrition Research, Ministry of Agriculture, Fisheries and Food (MAFF), and Department of Health. Colchester, Essex, UK Data Archive (UKDA) [distributor]; 25 January 2001. SN: 4243.

U.S. FDA. *Agency Response Letter GRAS Notice No. GRN 000109 [Tuna oil – Clover Corporation].* U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety, Washington, DC. Available from: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153918.htm [Dec. 4, 2002].

U.S. FDA. Chapter IV.C.4.a. Subchronic Toxicity Studies with Rodents. In: *Toxicological Principles for the Safety Assessment of Food Ingredients: Redbook 2000*. U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), College Park, Maryland, 2003. Available from: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/ucm078345.htm [Nov. 2003].

U.S. FDA. Substances affirmed as generally recognized as safe: Menhaden oil (Final rule - 21 CFR Part 184) [Docket no. 1999P-5332]. *U.S. Federal Register* 2005, 70:14530-14532.

U.S. FDA. Substances affirmed as generally recognized as safe: Menhaden oil (Final rule) [21 CFR, Part 184, Docket No. 86G-0289]. *U.S. Federal Register* 1997, 62:30751-30757.

Villac MC, Roelke DL, Villareal TA, Fryxell GA. Comparison of two domoic acid-producing diatoms: a review. *Hydrobiologia* 1993, 269/270:213-224.

Van Goor SA, Smit EN, *et al.* Letter to the Editor: Milk of women with lifetime consumption of the recommended daily intake of fish fatty acids should constitute the basis for the DHA contents of infant formula. *J Perinat Med* 2008, 36:548-549.

Woodcock AH. Note concerning human respiratory irritation associated with high concentrations of plankton and mass mortality of marine organisms. *Journal of Marine Research* 1948, 7:56-62.

B Eerste beoordeling / Initial assessment

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

OPINION ON AN APPLICATION UNDER THE NOVEL FOOD REGULATION FOR A DHA AND EPA RICH OIL FROM THE MICROALGAE SCHIZOCHYTRIUM

Applicant	**Martek Biosciences**
Responsible Person	**Rodney Gray**
EC Classification	**2.2**

1. An application has been submitted by Martek Biosciences for the use of a Docosahexaenoic acid (22:6(n-3), DHA) and Eicosapentaenoic acid (20:5(n-3), EPA) rich algal oil as a novel food ingredient.

2. This is the third application made by Martek for an oil rich in polyunsaturated fatty acids obtained from the microalgae *Schizochytrium sp.* This oil differs from the one described in the previous applications[1] in that it contains significant quantities of EPA as well as DHA, more closely resembling the composition of fish oil. The applicant proposes that the oil should be used in a similar range of foods to those that are permitted for the original oil. The minor amendments to the proposed level of use in certain products are a reflection of the amounts that would be needed to support a health claim linked to the consumption of polyunsaturated fatty acids (PUFAs); in line with recent opinions from the European Food Safety Authority (EFSA).

3. For the purposes of this opinion the novel ingredient will be referred to as **DHA-O**, which is the name used in the application dossier. Reference to **DHA-S** (both here and in the dossier) applies to the company's DHA rich algal oil which has previously been authorised.

I Specification of the Novel Ingredient (NI)

Dossier pp 6-14

4. The applicant has provided a specification for DHA-O that is consistent with the approved specification for DHA-S, apart from a lower level of DHA (not less than 22.5%, instead of not less than 32%), and a minimum level of 10% for EPA This specification is detailed below and in Tables 3 and 4 of the dossier, which also sets out the analytical results for three batches of DHA-S, each being within specification. In each case the measurable level of DHA is significantly higher than 22.5% and the applicant has advised that this is to allow for standardisation of the algal oil with vegetable oil (see Section XI).

[1] 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 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 (2009/778/EC)

Proposed Specification of 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%

5. DHA-O contains a range of fatty acids, of which DHA and EPA, together with palmitic acid, are the most abundant (Dossier, Table 7). The applicant also provides details of the unsaponifiable component (Dossier, Table 8) noting that the sterols present in the product are commonly found in the diet.

6. The applicant also provides results of analyses of heavy metals, protein and residual solvents, which are consistent with those seen for DHA-S (Dossier, Table 5). Levels of polycyclic aromatic hydrocarbons, dioxins, acrylamide and pesticide residues have also been examined and all were found to comply with published limits (see Dossier, Tables 9, 10, 11, pp 12-14).

Discussion *The Committee was satisfied that the composition of DHA-O did not give rise to any safety concerns.*

II Effect of the production process applied to the NI

Dossier pp15-21

7. The production process used to produce DHA-O is very similar to that used for the production of DHA-S. The process involves the fermentation of algae from the genus *Schizochytrium sp* in a pure culture, heterotrophic fed-batch fermentation process followed by an oil recovery stage.

8. Once sufficient cell mass is available the oil recovery stage begins, involving either fresh broth or reconstituted dried algae. The broth is first treated with antioxidants, followed by heating and pH adjustment, prior to homogenisation to induce cell lysis and to release the oil. The resulting broth is cooled and isopropyl alcohol is added to form an emulsion. The applicant then separates the oil from the aqueous phase by centrifugation. The oil phase is dried and then refined using methods commonly used by the vegetable oil industry to obtain clear oil. The oil recovery process is significantly different from the one used for DHA-S, which relied on solvent (hexane) extraction of oil from the dried biomass prior to refining.

Discussion *The Committee noted that the production process was similar to that used for the production of DHA-S and, although the differences in the extraction procedure were noted, Members were content that they did not give cause for concern.*

III History of the organism used as the source of the NI

Dossier, pp

9. The alga used in the production of DHA-O is a previously unpublished member of the genus *Schizochytrium* which was selected by the applicant following a strain selection process. The production strain has not been genetically modified. The strain was selected for its ability to produce EPA and further improvements in productivity were obtained by optimisation of the fermentation process.

10. The applicant provides a detailed overview of algal toxin production noting that, based on both published and unpublished studies, there have been no reports of toxic compounds, or association with toxic compounds, produced by Thraustochytrids (the order to which *Schizochytrium* belongs). The company also notes that most of the toxic compounds produced by microalgae are produced by blue-green algae or dinoflagellates, which lie in a separate kingdom to *Schizochytrium.* Two toxic compounds, domoic acid and prymnesin, are known to be produced in the Chromista, the Kingdom to which *Schizochytrium* sp. belongs. However, these toxins are largely restricted to two genera (*Pseudonitzschia* and *Prymnesium*) which are in a separate class (Prymnesiophyceae*)*) and phylum, respectively, from the Thraustochytrids. Additional tests carried out by the applicant confirm that neither domoic acid nor prymnesin are present in *Schizochytrium* sp. (Dossier, Appendix 3a).

Discussion *The Committee accepted that Schizochytrium sp had previously been used to produce DHA rich oils and although DHA-O was produced from a newly characterised member of the genus, as there were no reports of toxins being produced by any members of the Class which includes the genus Schizochytrium, the use of the organism as a source of the oil did not give cause for concern. The Committee also accepted that the test results confirming the absence of domoic acid and prymnesin offered additional reassurance in this regard.*

IX Anticipated intake and extent of use of the NI

Dossier, pp

11. DHA-S is currently permitted in a range of food categories and the applicant proposes a similar list of uses for DHA-O. However, the applicant proposes certain changes in order that they, like fish oil producers, can provide products that supply the recommended daily intakes of PUFAs. The applicant notes that these amendments are relatively minor and in line with a recent EFSA opinion regarding the reference intake values for n-3 and n-6 PUFAs[2]. This opinion concludes that there is evidence of a relationship between intake of PUFAs (EPA, DHA) and cardiovascular health at 250mg per day and this claim is now permitted under the relevant health claims legislation.

12. In addition, the applicant also proposes a high dose supplement (450mg/day) for pregnant and lactating women, referring to recommendations from a number of Government bodies and expert groups (including the EFSA report at Annex B) that pregnant and nursing women should consume at least 450 mg EPA and DHA per day (200mg DHA) in order to compensate for increased metabolic

[2] http://www.efsa.europa.eu/en/efsajournal/pub/1461.htm

demands associated with pregnancy and lactation. This recommendation takes account of accumulation in the foetus or infant and the requirements for cardiovascular health.

Food use	DHA-S (Max level of DHA)[1]	DHA-O (Max level of DHA+EPA)
Dairy Products except milk based drinks	200mg/100g; 600mg/100g for cheese	Unchanged
Dairy Analogues except drinks	200mg/100g; 600mg/100g for cheese analogues	Unchanged
Spreadable Fats and Dressings	600mg/100g	Unchanged
Breakfast Cereals	500mg/100g	Unchanged
Foods for Particular Nutritional Uses as defined in Commission Directive 2009/39/EC, but excluding infant and follow on formula	In accordance with the nutritional requirements of the persons for whom the products are intended	Unchanged
Foods Intended for use in energy restricted diets for weight reduction	200mg/meal replacement	250mg/day
Bakery Products, Breads and rolls	200mg/100g	Unchanged
Nutrition Bars	500mg/100g	Unchanged
Non-alcoholic beverages	60mg/100g	80mg/100g
Milk Based Drinks	60mg/100g	80mg/100g
Food Supplements	200mg/daily dose	250mg/day
Food Supplements for pregnant and lactating women	-	450mg/day (NEW)

[1] As listed in Commission Decisions 2003/427/EC and 2009/778/EC

Estimated intake

13. The applicant has calculated the mean and 97.5th percentile “all user” intakes for each of the authorised and proposed food categories. This methodology assumes highest possible consumption as it is assumed that all products within a category contain the maximum level of the NI. (The "all user" description indicates that the distribution of intakes is obtained by considering only those individuals who consume the relevant foods, discounting individuals who do not consume them).

14. The results of this analysis indicate that male teenagers potentially have the greatest 97.5th percentile all-user intake of DHA+EPA at 1.72g per day. By body weight, the highest consumers are children (97.5th percentile all-user intake at 62mg) (See table below). These estimates are broadly similar to those seen for DHA-O in which greatest 97.5th percentile all-user intake was for male adults with a consumption of 1.66g/day and, by kilogram body weight children (57mg).

Summary of the Estimated Daily Intake of DHA-O (as DHA+EPA) from all Proposed Food Categories in the U.K. by Population Group – based on 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 Adult	16-64	94.1	901	0.6	0.95	1.10	1.21	0.60	0.96	1.12	1.23
Male Adult	16-64	94.8	726	0.76	1.23	1.45	1.66	0.77	1.23	1.45	1.65

Summary of the Estimated Daily Intake of DHA-O (as DHA+EPA) from All Proposed Food Categories in the U.K. by Population Group – based on NDNS Data											
Population Group	**Age Group (Years)**	**% User**	**Actual # of Total Users**	**All-Person Consumption**				**All-Users Consumption**			
				Mean (mg/kg bw)	**Percentile (mg/kg bw)**			**Mean (mg/kg)**	**Percentile (mg/kg bw)**		
					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	97.3	436	13	21	24	26	13	21	24	26
Male Teenager	11-18	99.3	414	16	26	28	32	16	26	28	32
Female Adult	16-64	91.6	901	8	14	16	19	9	14	16	19
Male Adult	16-64	91.4	726	9	15	17	20	9	16	18	20

15. **Food Supplements.** The applicant proposes to increase the level of PUFAs from 200mg to 250mg per day and to market a separate 450mg supplement specifically for pregnant and nursing mothers. The applicant is of the view that, as supplements are consumed as an alternative to fortified food products, these products will not significantly affect levels of intake. The applicant also notes that fish oil supplemented products are widely available, and DHA-O is a direct replacement for these products.

Discussion *The Committee was content that the minor changes to the use levels would not lead to an increase in the level of consumption amongst the general population. Members noted the high dose supplements which are targeted at pregnant and nursing mothers were also in line with a recent health claim request*

that had recently been evaluated by EFSA and noted that this may lead to an increase in gestation periods (See Discussion Section XIII).

XI Nutritional information on the Novel Food

Dossier p43-

16. The applicant again refers to the rationale for the changes in use categories (see above) and also refers to a 2009 novel food authorisation for a DHA+EPA rich oil from Antarctic Krill (*Euphasia superba),* which has use categories that are consistent with those that have been approved for DHA-S. The applicant also compares the profile of DHA-O with a range of oils including both krill oil, salmon and cod liver oil (Dossier Table 12). Blending with vegetable oils (see Section I above) will enable DHO-O to be formulated in such a way that it closely resembles the composition of existing fish oils, so that it can be used as a direct substitute in manufacturers' recipes.

17. In the previous application the applicant noted that the DHA-S oil is to be added into a range of existing foods, either as a partial replacement for the fat component of the food or as a direct replacement for fish oil (added as an ingredient). The applicant therefore did not envisage that the addition of DHA-S would change the nutritional profile of the food as consumed and they illustrated this by comparing a milk based drink fortified with the NI and with fish oil. Although this information was not repeated in this application, the same reasoning would apply to DHA-O.

Discussion *The Committee accepted that the nutritional information provided was appropriate and the non-fat nutritional profile of a product containing the novel ingredient would not be significantly different when compared with an equivalent product fortified with fish oil. The Committee also noted that the fatty acid profile of the product was broadly comparable with existing fish oil derived products and, as such, would be unlikely to give rise to safety concerns. The Committee also noted that the applicant does not discuss the nutritional profile of the product in terms of its composition as a fat but, as it is almost entirely composed of triglycerides, a caloric value of 9 kcal will therefore be used on nutritional labels, as is currently used for DHA-S.*

XII Microbiological Information

Dossier p46

18. The applicant notes that DHA-O is a lipid with little water activity and would not support the growth of microorganisms. The company may elect to pasteurise the cell biomass and the solvent recovery stage also requires the application of heat and would kill any vegetative cells present. The applicant has included a specification for the presence of microorganisms (Dossier, p46, Table 19) and also shows the results for three individual batches of the oil, each of which were within the specification.

Discussion *The Committee accepted the data provided in the application although Members regarded the possibility of contamination by Cyanobacteria to be one that should not be discounted. In regard to this point, Members were reassured by the quality control regime and confirmation from the applicant that that the fermentation*

proceeds in the absence of light under axenic[3] conditions. The Committee accepted that these measures were sufficient to ensure that any risk of Cyanobacterial contamination was no greater than for any other closed system fermentation process used in food production.

XIII Toxicological information

Dossier p.72-77

19. In addition to the toxicological studies carried out on DHA-O (see below), the applicant notes that its traditional counterpart, fish oil, is widely used both in food supplements and in fortified foods in the EU without restriction. The applicant also highlights the absence of algal toxins and the broad similarity between DHA-O and DHA-S, meaning that the toxicological studies carried out in support of the earlier product have some relevance to DHA-O. These data are not supplied again in the current application, but are summarised in the Committee's 2002 initial opinion on DHA-S[4].

20. **14 day dose ranging study**. This study, carried out according to OECD guidelines, indicated that doses up to 60,000 mg/kg/day should be administered to rodents in the 90-day repeat dose toxicity study. Food efficiency changes were viewed to be non-adverse and toxicologically insignificant. A single reported death was viewed to be as a result of anaesthesia.

21. **90 day toxicity study.** Carried out in accordance with relevant OECD guidelines. DHA-O (0 – 5% in the diet) was administered to Sprague-Dawley rats for the duration of the study with a fish oil being used as a control. Although a number of statistically significant changes were observed *e.g*. body weight gain, food consumption and food efficiency, these were attributed to high dietary fat concentrations, in general, and not specifically to DHA-O. The administration of DHA-O at levels of 0.5%, 1.5% and 5% resulted in a dose-dependent increase in DHA levels in plasma, liver, and brain. DHA levels were generally higher in females than males. With a few exceptions, and in all groups, EPA plasma and liver concentrations were generally lower compared to DHA concentrations, and were generally higher in females. Plasma EPA concentrations were higher than those seen in the liver.

22. There were no adverse changes in haematology, clinical chemistry, coagulation, or urinalysis parameters in male or female rats that were attributable to the administration of DHA-O. Statistically significant findings in red cell mass and clinical chemistry were seen but these were of small magnitude and, as similar effects have been historically observed with high fat diet diets, they were considered to be non-adverse and toxicologically insignificant. There were no macro- or micro-scopic findings related to administration of DHA-O. Incidental histological findings included masses involving the penis that corresponded to abscesses or duct ectasia involving the preputial glands, unilateral masses of the epididymides that corresponded to sperm granulomas, hepatodiaphagmatic nodule and fluid-filled uteri/fallopian tubes.

[3] axenic: a pure culture of a single organism.
[4] http://www.acnfp.food.gov.uk/assess/fullapplics/60694

23. An increased incidence of alveolar histiocytosis in the lungs of males and females in two groups was related to the unintended aspiration of the test substance (fish oil or DHA-O) into the lungs, in association with aspiration of food meal. A single, benign mammary gland fibroadenoma in one high-dose female was most likely a spontaneous neoplasm, not associated with the administration of the test substance. In general, the absolute and relative liver (males and females) and kidney (females) weights were significantly increased. However, these values were significantly lower than in the fish oil control group.

24. Incidental findings included absolute adrenal (female) and testicular (male) weight changes which were not attributable to DHA-O. Changes in kidney weight were considered incidental without notable clinical chemistry changes, while increases in liver weight (males and females) are considered secondary to high fat diet intake, as similar effects were observed with fish oil.

25. The applicant has concluded that there was no toxicity related to administration of DHA-O in male or female rats. Under the conditions of this study and based on the toxicological endpoints evaluated, the no-observed-adverse-effect level (NOAEL) for DHA-O in the diet was judged to be 5% (50,000 mg/kg) for male and female rats, equivalent to 3149 and 3343 mg/kg body weight/day, for male and female rats respectively.

26. **Genotoxicity studies.** The applicant viewed the results of a reverse mutation (Ames) assay, carried out to OECD Guidelines, to indicate that DHA-O was non-mutagenic. An *in vitro* mammalian chromosome aberration test and an *in vitro* mouse micronucleus test did not report any unusual findings.

27. The applicant concludes that these studies demonstrate that the intake of DHA-O arising from consumption in the proposed food categories does not give rise to any safety concerns noting that their NOAEL value equates to consumption of approximately 200g of DHA-O per day for a 60kg adult.

28. The Committee asked that the applicant provide reassurance that its proposal to target a high dose supplement at pregnant and nursing women was supported by available safety data, noting that there have been reports of increased gestation in women who consumed a high fish oil diet. The applicant's response noted that a meta-analysis of trials involving the supplementation of up to 3g n-3 PUFAs in women with high risk pregnancies reported a reduced risk of pre-term delivery, while other trials report decreased maternal adverse events during labour and delivery together with decreased infant morbidity. Although the applicant acknowledged that a consequence of extended gestation could be an increase in post-term births, in their view, this does not appear to be borne out by an analysis of the available data which do not appear to identify an increase in post-term births compared with the reported national averages.

Discussion

The Committee concluded that the range of the toxicological studies carried out by the applicant were sufficient to assure the safety of the product at the proposed levels of use. Members noted that concerns related to post-date births had not been addressed by the applicant's response. Members disagreed with the applicant's

conclusions regarding reviews by Makrides at al. in 2006[5] and 2010[6], noting that the latter paper provided evidence that there is a valid concern in relation to post-date births and high intakes of n-3 fats. However the Committee accepted that any increase in gestation periods was a generic issue that had previously been taken into account both by EFSA and the UK Scientific Advisory Committee on Nutrition when setting recommended intake levels for long chain polyunsaturated fatty acids in pregnant and lactating women, but suggested that possible effects of increased gestation should be taken into account when considering the levels at which the novel ingredient is used, and when monitoring possible adverse events following its widespread introduction into the diet.

Allergenicity and Labelling

29. The level of residual protein in DHA-O is less than 0.02%, measured by the Kjeldahl method (Dossier Table 5). The applicant notes that DHA-S is produced from very similar source materials and also contains low levels of protein (<0.1%), and has not been associated with any serious adverse events. The applicant also notes that reports of respiratory and dermatologic responses (including allergy) to microalgae have been restricted to human exposure to blue-green algae.

30. The applicant does not make any proposal for the labelling of this ingredient. The authorisation for the existing product DHA-S requires it to be labelled as "DHA-rich oil from the microalga *Schizochytrium sp".*

Discussion *The Committee agreed that DHA-O was not an allergenic risk and that labelling similar to that of DHA-S adequately describes the product.*

Overall Discussion

The Committee concluded that the applicant had provided sufficient scientific data to assure them that the proposed additional uses of the DHA-O did not give rise to specific concerns over safety when consumed at the proposed levels of use. The Committee highlighted that current policy in the UK is to encourage the intake of long chain n-3 polyunsaturated fatty acids and that this product may help consumers with low intakes to increase their consumption of n-3 fatty acids[7].

Concerns have been raised during the previous assessments of novel PUFA-rich algal oils about the impact that long term, high-level consumption of these products may have on health. Members noted that this should be kept under review and intakes of DHA should monitored at national and/or EU level. However, the Committee reiterated their view that this uncertainty was not solely related to the extension of use of this DHA and EPA rich oil "DHA-O" and any studies that looked at the impact of consumption of foods fortified with n-3 long chain polyunsaturated

[5] Makrides M, et al., 2006. Database of Systematic Reviews. Issue 3, Article No. CD003402
[6] Makrides M, et al. 2010. JAMA 304:1675-1683.
[7] Advice on fish consumption: Benefits and Risks; SACN/COT 2004

fatty acids should address all dietary sources and different age groups, particularly children.

Conclusion

The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by the applicant, Martek Biocsciences that the range of uses for the novel ingredient (DHA and EPA rich algal oil from *Schizochytrium* sp., DHA-O) is acceptable.

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