Food Standards Agency
food.gov.uk

Mr Andreas Klepsch
European Commission
By email

Reference: NFU 786

9 December 2011

INITIAL OPINION: **A DHA AND EPA RICH OIL FROM THE MICROALGAE SCHIZOCHYTRIUM**

Dear Mr Klepsch,

On 31 January, the UK Competent Authority accepted an application from Martek Biosciences for a Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) rich algal oil as a novel food ingredient, in accordance with Article 4 of regulation (EC) 258/97.

The Advisory Committee on Novel Foods and Processes (ACNFP) reviewed this application and their opinion is attached. I apologise for the delay in submitting this opinion as the ACNFP's evaluation was extended while we obtained additional information from the applicant.

In view of the ACNFP's opinion, the UK Competent Authority considers that this DHA and EPA rich algal oil, at levels not exceeding the maximum use levels described, meets the criteria for acceptance of a novel food defined in Article 3(1) of regulation 258/97.

I am copying this letter, and the ACNFP's opinion, to the applicant.

Yours sincerely,

(By e-mail only)

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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
Cooking Fats		360 mg/100 g (NEW)
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.

December 2011