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Application for the Authorization of DHA-rich Algal Oil from *Schizochytrium* sp. to Additional Food Groups

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

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Table of Contents

List of Tables

List of Appendices

EXECUTIVE SUMMARY AND CONCLUSIONS

This application seeks to extend the food categories to which docosahexaenoic acid (DHA)-rich oil derived from the microalgae *Schizochytrium* sp. (hereinafter DHA-rich algal oil) can be added under EU Novel Foods Regulation No 258/97 (European Parliament and the Council of the European Union, 1997).

With this application, Martek[1] specifically requests authorization to add DHA-rich algal oil the following use groups:

(i) bakery products, breads and rolls;
(ii) nutrition bars; and
(iii) non-alcoholic beverages, milk-based drinks and dairy analogue drinks.

Proposed Use Groups	**Maximum Use Level of DHA**
Bakery products Breads and rolls	200 mg/100 g 200 mg/100 g
Nutrition bars	500 mg/100 g
Non-alcoholic beverages Milk-based drinks Dairy analogue drinks	60 mg/100 mL 60 mg/100 mL 60 mg/100 mL

Adding categories to those already authorized will provide European Union (EU) consumers a broader choice of foods to obtain the benefits of DHA omega-3 from a vegetarian source.

Having regard to the above, the following summary information is presented for consideration:

- National agencies in the EU recommend an increase of consumption of long-chain omega-3 fatty acids given the existing deficiencies in the population, in general. Consumption of long-chain omega-3 fatty acids is generally recognized as having considerable heart health benefits. In particular, the European Food Safety Authority (EFSA) recognized intake recommendations by some authorities (such as the UK, the Netherlands, France and the Nordic Countries) of long-chain omega-3 fatty acids [eicosapentaenoic acid (EPA) and docosohexaenoic acid DHA] in adults for cardio-protective effects (200 to 500 mg/day). Further, specific population groups such as pregnant and nursing women are advised to consume DHA (200 to 300 mg/day). The unique role of DHA in maternal health is recognized by national agencies in France and Belgium and recently by the EU research project PeriLip, charged by the European Commission for the development of recommendations on dietary fat intake during pregnancy and lactation.

[1] On April 25 2002, Martek Biosciences Corporation announced that it had acquired OmegaTech, Inc., whereby OmegaTech became a wholly owned subsidiary of Martek Biosciences Corporation (Martek).

- Foods containing DHA at maximum use level will deliver approximately 100 mg DHA per serving. Recognizing that serving portions vary throughout Europe, use levels are presented as DHA per 100 g (or mL) of food.

- Based on intake recommendations by EU expert bodies, individuals are expected to consume 2 to 5 foods containing DHA-rich algal oil per day. Given this scenario, meaningful increases of 200 to 500 mg DHA per day will be introduced into an individual's diet; anticipated exposure of DHA from DHA-rich algal oil is 200 to 500 mg per day.

- Intakes are appropriately controlled due to the very unlikely scenario that individuals will consume more than 5 food servings containing DHA-rich algal oil per day (*i.e.*, intake is self-limiting and therefore controlled).

- In the estimation of maximum intake, care has been exercised to assure that the sources of data are representative of intake across Europe by not only using the well-recognised United Kingdom (UK) National Diet and Nutrition Survey (NDNS) data but also by using market data based on per capita intakes across Europe. The estimated intake using either of these data sources, for both authorised and proposed uses, resulted in the same calculated intake of 1.7g/day.

- Maximum estimated intake is based on the assumption that all of the designated food groups will contain DHA from DHA-rich algal oil at the maximum level. An individual who consumes approximately 13 portions of food containing DHA-rich algal oil will ingest approximately 1.7 g of DHA per day.

- Clinical studies in over 1200 adults, children, and pregnant/lactating women demonstrate that ingestion of DHA-rich algal oil is not associated with any serious adverse experiences and no adverse experiences other than minor fishy "burps" [reflux, an organoleptic experience associated with other long-chain polyunsaturated fatty acids (LCPUFA)]. Clinical studies support safe use of DHA-rich algal oil up to 7.2 g per day (which equates to approximately 2.7 g DHA per day).

- DHA from DHA-rich algal oil is the only vegetarian version of this omega-3 fatty acid that is commercially available to meet the needs of persons that may be vegans, vegetarians or allergic to other sources.

- Comments expressed at the time of the submission of the original application are considered and addressed.

Further, and considering that:

(i) In its 2002 assessment, having led to the issuance of the authorization Decision 2003/427, the UK Advisory Committee on Novel Foods and Processes (ACNFP) concluded that the addition of DHA-rich algal oil to all foods covered in the original list, which importantly included the proposed food categories that are the subject of this application,, is "safe for use" (Commission of the European Communities, 2003);

(ii) The Commission Decision 2003/427 acknowledges in Recital 5 that "it is established that DHA-rich oil from the microalgae *Schizochytrium* sp. complies with the criteria laid down in Article 3(1) of the Regulation",[2]

(iii) Intake data in the present application show that consumption is appropriately controlled, taking into consideration the highly unlikely scenario of consumption of all food categories combined; and

(iv) DHA-rich oil, subject of the present application, is the same ingredient that has previously been reviewed in the original application.

It is therefore respectfully requested that review of this application be expedited.

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[2] Article 3 §1 of the Novel Food Regulation provides that "[f]oods and food ingredients falling within the scope of this Regulation must not:
— present a danger for the consumer,
— mislead the consumer,
— differ from foods or food ingredients which they are intended to replace to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer."

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 DHA-rich oil produced from *Schizochytrium* sp. (hereinafter "DHA-rich algal oil"), a marine microalgae, 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 current authorized uses for DHA-rich algal oil under this decision (as detailed in its Annex 2) are reproduced in Table 1.

Table 1 Authorized Uses of DHA-rich Algal Oil 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 the initial assessment report, the United Kingdom's ACNFP came to the conclusion that DHA-rich algal oil is safe for human consumption. More specifically, it stated:

> "The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by OmegaTech that DHA Gold® [DHA-rich oil from the microalgae *Schizochytrium* sp.] is safe for use as a nutritional food ingredient, for the types of uses as described in the application dossier, subject to the labelling requirements described above".

The Commission forwarded the initial assessment report to all Member States, opening the second stage of the procedure and the 60-day period laid down in Article 6(4) of Regulation (EC) No 258/97 (European Parliament and the Council of the European Union, 1997).

During this period, a number of comments regarding the marketing of the product were raised. In response to those comments, and most importantly, in the interest of timely progress to approval of some applications, OmegaTech amended the food applications of the DHA-rich oil.

With this application, Martek requests authorization of DHA-rich algal oil to an extended range of foods. More specifically, Martek hereby submits for authorization, additional food categories and use levels for DHA from DHA-rich algal oil as detailed in Table 2, below.

Table 2 Proposed Food Categories and Use Levels

Proposed Food Category Use Groups	**Maximum Use Level of DHA**
Bakery products Breads and rolls	200 mg/100g 200 mg/100g
Nutrition bars	500 mg/100g
Non-alcoholic beverages Milk-based drinks Dairy analogue drinks	60 mg/100 mL 60 mg/100 mL 60 mg/100 mL

This application has been prepared in accordance with the EU recommendation of 29 July 1997, where relevant (Commission of the European Communities, 1997). Consistent with the original application of 2001, Sections IV to VIII of the EU recommendation are not applicable to DHA-rich algal oil.

1. ADMINISTRATIVE DATA

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

Address of the applicant is as follows:

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2. GENERAL DESCRIPTION

Consistent with the original application submitted in 2001, DHA-rich algal oil is classified as Class 2.2, *i.e.*, "complex Novel Food From non-GM Source", the source of the NF has no history of use in the Community.

3. IDENTIFICATION OF THE ESSENTIAL INFORMATION REQUIREMENTS

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

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

Unless necessary to the discussion for the present application, reference is made to the full dossier submitted in 2001.

I SPECIFICATION OF THE NOVEL FOOD

The specification for DHA-rich algal oil is specified in Annex 1 to the approval Decision 2003/427/EC and is presented in Table 3 below. No changes in the specification of the product are made (Commission of the European Communities, 2003).

Table 3 Specification of DHA-rich Algal Oil Established by Decision 2003/427/EC

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 32,0%

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

No changes have been made to the original application.

III HISTORY OF SOURCE ORGANISM

No changes have been made to original application.

IX ANTICIPATED INTAKE/EXTENT OF USE

As stated above, during its initial assessment, the United Kingdom's ACNFP came to the conclusion that DHA-rich algal oil is safe for human consumption. The UK competent authority did not restrict or limit food categories in their opinion and concluded that *"the Advisory Committee on Novel Foods and Processes is satisfied by the evidence … that DHA Gold*® (DHA-rich oil from the microalgae *Schizochytrium* sp.) *is safe for use as a nutritional food ingredient, for the types of uses as described in the application dossier* [which included the food categories authorized under Decision 2003/427/EC and also the food categories that are the object of the present petition]*, subject to the labelling requirements described above"* (Commission of the European Communities, 2003)*.*

IX.1 Anticipated Intake

Consumption of long-chain omega-3 fatty acids is generally recognized as having considerable heart health benefits. In particular, EFSA recognized intake recommendations by some (such as the UK, the Netherlands, France and the Nordic Countries) authorities of long-chain omega-3 fatty acids (EPA and DHA) in adults for cardio-protective effects (200 to

500 mg/day). Further, specific population groups such as pregnant and nursing women are advised to consume DHA (200 to 300 mg/day). Based on marketed use of DHA-rich algal oil in food products around the world, it is reasonable to assume that food manufacturers will formulate food products containing 100 mg or less DHA per serving. Therefore, it is anticipated that most foods containing DHA will deliver maximally 100 mg DHA per food serving. Given recommendations to increase consumption of long-chain omega-3 fatty acids, individuals are expected to consume 2 to 5 foods containing DHA-rich algal oil per day. Given this scenario, meaningful increases of 200 to 500 mg DHA per day will be introduced into an individual's diet. **Anticipated exposure of DHA from DHA-rich algal oil is 200-500 mg per day.**

IX.2 Maximum Estimated Intake

In this section we present 2 ways of estimating maximum intake levels. Firstly we estimate maximum intake based on the well-recognised National Diet and Nutrition Survey (NDNS) data from the UK. This is generally recognised as being the most accurate calculation of intake but it is accepted that high-level exposure estimates represent significant overestimation and by far the worst-case scenario, because it makes the assumption that all foods are replaced with those dosed with a maximum level of DHA. It does, however, take into consideration eating patterns, allowing for a consumer who may consume breakfast cereal but might not consume soft drinks in a given day, as example. Secondly, we estimate maximum intake using real market data, which is based on per-capita intakes across Europe, to calculate and compare the dietary habits of individuals within member states and to show their similarities. Again this technique makes significant over-estimates based on simple summation of intakes from the various foods. It is also difficult to remove specific foods within the groups that would not be targets for DHA addition, for example due to technological reasons.

Maximum estimated intake is based on the assumption that all of the designated food groups will contain DHA from DHA-rich algal oil. The calculation of intake, based on both NDNS survey data for an all-user individual in the 97.5th percentile, and those on *per capita* consumption is estimated to be 1.7 g/day. This should be considered the maximum possible intake for a dedicated DHA consumer. The intake of the average consumer should clearly be much less.[3]

Realistically, intakes will be controlled due to the very likely scenario that individuals will consume less than 5 food servings containing DHA-rich algal oil per day. (*i.e.*, consumption is self-limiting).

We discuss these aspects in more detail below.

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[3] Calculations of anticipated exposure, based on 100% market penetration must of necessity exceed actual exposures. Such calculated estimates can only represent the consumption of extremely high percentile population groups.

IX.2.1 Consumption Estimates Based on UK National Diet and Nutrition Survey Data

In the first approach to estimate intake of DHA from DHA-rich algal oil, food consumption data collected as part of the UK Food Standards Agency's, Dietary Survey Programme (DSP) were used. The mean and high-level (97.5th percentile) all-user intakes were calculated for each of the individual authorized and proposed food categories based on highest use level.

This type of intake methodology is generally considered to represent the highest possible consumption scenario as a result of the assumptions made. It is assumed that all food products within a food category contain DHA-rich algal oil at the maximum specified use level and that all food categories are consumed within a given day.

Food consumption data collected as part of the United Kingdom (UK) Food Standards Agency's, Dietary Survey Programme (DSP) were used as a basis for the estimates for the intake of DHA from DHA-rich algal oil in the EU. The mean and high-level (97.5th percentile) all-person and all-user intakes, and percent consuming were calculated for each of the individual authorized and proposed food-uses. Similar calculations were used to determine the estimated total intake of DHA from DHA-rich algal oil from all authorized and proposed food-uses combined. In both cases, per person and per kilogram body weight intakes were reported for the following population groups:

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

A full description of the databases used and report on the estimated daily intake of DHA from DHA-rich algal oil using UK NDNS data is found in Appendix 1.

Table 4 summarizes the estimated total intake of DHA from DHA-rich algal oil (mg/person/day) from all authorized and proposed food-uses in the EU by UK population group, while Table 5 presents the data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of users was high among all age groups evaluated in the current intake assessment as would be expected for a 7-day survey. As a consequence, only the all-user intake results will be discussed in detail.

Male adults were determined to have the greatest 97.5th percentile all-user intakes, with a value of approximately 1.7 g/person/day (Table 4). Estimated daily intakes of DHA from DHA-rich algal oil (Table 4) generally increased with age in all groups; however, these values were lower in females relative to males.

Table 4 Summary of the Estimated Daily Intake of DHA from DHA-rich Algal Oil from All Authorized and Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children	1½-4½	98.8	1,628	418	648	724	829	419	647	725	853
Young People	4-10	99.6	834	662	972	1,107	1,222	662	972	1,107	1,222
Female Teenagers	11-18	97.8	436	667	1,047	1,165	1,278	665	1,038	1,144	1,250
Male Teenagers	11-18	99.5	414	871	1,316	1,489	1,607	869	1,312	1,489	1,607
Female Adults	16-64	94.1	901	619	960	1,108	1,230	626	962	1,119	1,231
Male Adults	16-64	94.8	726	795	1,247	1,502	1,664	802	1,250	1,502	1,662

On a body weight basis, female adults had the lowest mean and 97.5th percentile intakes, at 9 and 19 mg/kg body weight/day, respectively (Table 5).

Table 5 Summary of the Estimated Daily Per Kilogram Body Weight Intake of DHA from DHA-rich Algal Oil from All Authorized and Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-Users Consumption			
				Mean (mg/kg)	Percentile (mg/kg)			Mean (mg/kg)	Percentile (mg/kg)		
					90	95	97.5		90	95	97.5
Children	1½-4½	98.8	1,628	29	45	51	57	29	45	51	57
Young People	4-10	99.6	834	26	38	43	49	26	38	43	49
Female Teenagers	11-18	97.8	436	12	20	23	26	13	20	23	26
Male Teenagers	11-18	99.5	414	16	25	28	31	16	25	28	31
Female Adults	16-64	94.1	901	9	15	17	19	9	15	17	19
Male Adults	16-64	94.8	726	9	15	18	21	10	16	18	21

IX.2.2 *Per capita* Consumption Estimates

The second approach to estimate maximum intakes from foods containing DHA-rich algal oil is presented herein. Food categories for specific DHA addition have been identified (See Table 6). Consumption of DHA is calculated by estimating the EU-wide average daily intake

of these foods together with their maximum DHA content. For reasons discussed below, it is believed that this represents the maximum possible daily intake of DHA.[4]

(a) Is this intake level protective of heavy consumers of the selected food groups? **Yes**. While a given individual may consume more than the average amount of a particular food group every day, it is highly improbable that this will occur for each designated food category. If some persons consume one or two food groups at higher than the mean, they are likely to consume others at less than mean levels.

(b) Does this intake level adequately protect the consumer who selects DHA-containing foods? **Yes**. It is unlikely that even a dedicated consumer of DHA will religiously select only the DHA-containing food groups every day. By assuming that he will, the DHA-pattern of his daily intake has been exaggerated and compensates for the fact that his DHA intake of some foods may exceed the calculated averages.

*IX.2.2.1 Estimate of Added Intake of DHA (***Per capita** *Consumption Approach)*

Table 6 below shows:

(i) the estimated DHA intake from the authorized food categories at the maximum level established by Decision 2003/427 and

(ii) the estimated DHA intake from the proposed food categories at the maximum proposed use level (Commission of the European Communities, 2003).

The mean daily intake of DHA from each food category is calculated by multiplying the DHA concentration in that food category (Column 2) by the mean daily intake of that food category (Column 3). The result is given in the last column (Column 4). Average daily intake of food (Column 3) is based on EU-wide population databases described in more detail below.

[4] Combined intakes of DHA and EPA have remained fairly steady both in the United States (200 mg/d) over the recent past (see Kris-Etherton *et al.*, ref cited). The impact of DHA and EPA supplements on the true population intake of DHA is not easily measured, but it must be small overall because there is still little market penetration, *i.e.*, relatively few manufacturers add DHA to foods and relatively few people consume foods with added DHA. The different nature of the DHA intake estimates must be kept in mind. DHA prescriptive estimates like the one in this application assume 100% market penetration and thereby overestimate the actual consumption of the ingredient. Actual retrospective population estimates of DHA intake, like those of Kris Etherton *et al.,* determine the content of the DHA in foods actually consumed by individuals and have consistently obtained average levels in the 200 mg/d range.

Table 6 Estimated Maximum Intake of DHA in Designated Food Categories			
1. Authorized Use Groups and Levels			
Authorized Food Categories Containing DHA	**Maximum DHA Use Levels in Food Category**	**Average Daily Intake of Food Category**	**Daily Intake of DHA per Food Category**
Dairy products (except milk-based drinks)	200 mg/100 g 600 mg/100 g (for cheese products)	58 g 36 g	116 mg 216 mg
Dairy analogues* (except drinks)	200 mg/100 g 600 mg/100 g (for cheese analogue products)	58 g 36 g	--- ---
Spreadable fat and dressings	600 mg/100 g	10 g	60 mg
Breakfast cereals	500 mg/100 g	31 g	155 mg
Food Supplements	200 mg/capsule	--	200 mg
Dietary foods for special medical purposes**	As required	---	[<2 grams/day]
Foods intended for use in energy restricted diets for weight reduction**	200 mg/meal replacement	One replacement meal /day	[200 mg]
* Dairy analogues are assigned intakes equal to the real dairy products and perfect substitution is assumed. **The intake of special dietary foods and meal replacements is considered separately.			
Sub-total (Current, except special dietary foods and meal replacement)			**747 mg**
2. Proposed Use Groups and Use Levels			
Proposed DHA Food Categories	**Maximum DHA Use Levels in Food Category**	**Average Daily Intake of Food Category**	**Daily Intake of DHA per Food Category**
Bakery products Breads and rolls	200 mg/100 g 200 mg/100 g	35 g 137 g	70 mg 274 mg
Nutrition bars*	500 mg/100 g	50 g	250 mg
Non-alcoholic beverages Milk and milk-based drinks Dairy analogue drinks	60 mg/100 mL 60 mg/100 mL 60 mg/100 mL	360 mL 240 mL 240 mL	216 mg 144 mg ----
* The intake of nutrition bars is estimated at one 50 g bar every day.			
Sub-total (Proposed)			**954 mg**
Total DHA in designated food categories 1+2			**0.747 g + 0.954 g = 1.70 g**

The following points are emphasized:

1. Care has been exercised to assure that the sources of data are representative of intake across Europe by not only using the well-recognised NDNS data but also by using market data based on *per capita* intakes across Europe.

2. There are a number of food categories to which DHA-rich algal oil may be added. Some of these *e.g.*, non-alcoholic beverages are broad categories and contain

subgroups. It is very unlikely than many individuals will consume all the food types represented by these food groups daily. As stressed above, it is assumed that all of the food in each category will contain DHA. In practice, within these designated food types only a few manufacturers will actually add DHA. Therefore, the DHA intake estimate is considerably exaggerated.

3. The maximum estimated DHA intake using either NDNS survey data approach or EU *per capita* consumption approach result in the same calculated intake of 1.7 g/d including both proposed applications and authorized uses of DHA-rich algal oil.

4. The recent EFSA opinion of the Panel on food additives, flavourings, processing aids and materials in contact with food deals (NDA panel) related to nutrition claims concerning omega-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat (EFSA, 2005) provided additional data on the mean intake of DHA in France, Sweden, and Germany. This data shows that the mean population consumption of DHA is 273 mg/d in France, 240 mg/d in Sweden, and 140-210 mg/d in Germany.

5. The total maximum estimated DHA intake including both proposed applications and authorized uses of DHA and the background level is approximately 1.7 g /day + 0.2-0.3 g/d = 1.9-2.0 g/day. This level is within guidance upper levels (based on nutritional benefit and not safety) established in France, Belgium, Australia, New Zealand and is well below the 3 g/day Generally Recognized as Safe (GRAS) level for DHA and EPA in the United States.

IX.3 Specific EU Intake Estimates

Data representative of the *per capita* consumption of commodities from various European countries was obtained from Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT). Consumption of various food categories based on household budget surveys was obtained from the Data Food Networking (DAFNE) databases. Additionally, data on beverage consumption across several EU Member States was obtained from Zenith International (See Appendix 2 for consumption data). These different estimates were compared when duplicate data was available to ensure a reliable estimate of the daily intake for the food categories proposed for DHA addition. The results are given in Table 6. The last column gives the daily intake of DHA for each food category. The total DHA intake from the listed approved uses, when formulated with the maximum use levels of DHA (second column), is estimated at 747 mg. Similarly, the total DHA intake from the proposed food categories is estimated at 954 mg. [Note: In the dairy categories, intake is estimated either as that from the dairy product or from the dairy analogue since complete substitution of one category for the other is assumed and the estimated intake figures for each category is the same.] Dietary foods for special medical purposes are excluded from the total, as individuals consuming such foods will not be consuming all the other given foods at the indicated levels; their diets have to be considered separately. The estimated total DHA consumption from both current and proposed uses is thus 747 g/d + 954 g/d =

1.7 g/day. As indicated above, this estimate assumes that the average individual will consume food from each food category every day and further that selection will be only of those brands that contain DHA. Either of these prospects is remote, and therefore the consumption estimate is highly exaggerated. We describe below the details of the intake calculations for the proposed categories of foods (1.7 g/day would represent 17 servings of foods delivering 100 mg of DHA per serving, which is clearly unrealistic in practical terms).

IX.3.1 Non-alcoholic Beverages

The detailed breakout of the EU consumption pattern for non-alcoholic beverages for the EU from 2000-2010 is given in the Appendix 3.[5] The list includes the consumed annual volume in litres of bottled water, carbonates, dilutables, fruit juices, tea, coffee, soy and protein beverages, and other hot beverages. Milk-based drinks are excluded from the list, as is water from the tap. The total estimated volume of non-alcoholic beverages consumed in the 27 countries of the EU in 2007 is 201,772 million litres or approximately 202,000 million litres.

Two major categories of non-alcoholic beverages are not candidates for added DHA: (1) hot beverages (87,000 million litres) and, (2) bottled water (51,000 million litres). Hot beverages are made either at home or in restaurants with tap water; their contribution to the total non-alcoholic consumption designated DHA addition is therefore excluded. Bottled water, *i.e.,* table drinking water containing only small amount of minerals or CO_2, is also not intended for DHA addition. This reduces the total volume of non-alcoholic beverages designated for DHA addition to 64,000 million litres.

The total population in the EU in 2006 per the Statistical Office of the European Communities (EUROSTAT) information found was 492,964,961 individuals (Eurostat, 2006).[6] Since the EU population has increased at approximately 1.5 million per year in recent years, the current 2007 population is estimated at 494,500,000 individuals. Therefore the annual *per capita* intake of non-alcoholic beverages is [64 x 10^9 litres] / [494.5 x 10^6] = 0.129 x10^3 = 130 litres/year. The *per capita* daily intake is: [130 L/yr]/[365 d/yr] = 0.36 L/person /day.

In Table 6, a use level of DHA in non-alcoholic beverages at 60 mg/100 mL is proposed. The designated food categories include: flavoured water, carbonates, dilutables, and fruit juice drinks. If the anticipated consumption is 360 mL/day, the total daily intake expected from this use is 216 mg (360 mL x 60 mg/100 mL = 216 mg). The EU (27) consumption data in this food category is reasonably complete and lends confidence to the intake estimate.

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[5] Non-carbonated beverage intake data from Zenith International

[6] This Eurostat information although dated 2006 covers the 27 Member States; See http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1996,39140985&_dad=portal&_schema=PORTAL&screen=detailref&language=en&product=Yearlies_new_population&root=Yearlies_new_population/C/C1/C11/caa10000

IX.3.2 Milk-based Drinks

The DAFNE 2006 average milk consumption for 15 selected Member States of the EU was approximately 240 mL (Appendix 3). It is also reasonable to expect some substitution between milk and milk-based drinks. We therefore use the liquid milk number of 240 mL/day as a reasonable surrogate for the category of milk-based drinks. The estimated daily intake of DHA from this use is 144 mg.

IX.3.3 Bakery Products

Baked goods containing DHA were included in the original DHA-rich algal oil application dossier. The German Competent Authority opened a discussion during the 60-day period regarding trans-fat content and stability during processing for this use group. The following additional information is presented to address these subjects prior to discussion of the DHA intake estimate.

IX.3.3.1 Trans-fat Content and Stability During Processing

Independent analysis of the trans-fat content of DHA-rich algal oil was conducted at the Mylnefield Laboratories at the Scottish Crop Research Institute (SCRI), under the supervision of Dr. William Christie. The results (see Appendix 4) show almost the complete absence of C18 trans isomers. This is not surprising given that the total C18 fatty acid content in DHA-rich algal oil is negligible (see Table I-3 of original dossier).

To address general concerns over oxidative stability during processing, Martek conducted a trial to demonstrate the organoleptic, physical, and chemical stability of DHA-rich algal oil in white bread. This study was designed to show the stability of DHA-rich algal oil in fortified white bread compared to control bread produced with vegetable shortening. The DHA-rich algal oil replaced part of the vegetable shortening commonly used in table bread formulations in order to obtain 32 g slices of bread fortified with 50 mg DHA (approximately 150 mg DHA/100 g serving of bread). Commercial loaf breads were produced following the sponge dough procedure with the aim of obtaining samples for organoleptic evaluation, chemical analyses, crumb colour and texture analyses for 14 days storage at room temperature, the typical commercial shelf-life of table bread. Bread produced using DHA-rich algal oil resulted in a 32 g slice with 50 mg DHA that had almost identical properties compared to the control bread produced using vegetable shortening. Addition of DHA-rich algal oil did not cause any significant difference in trans-fat or sterol content compared to the control. Addition of DHA-rich algal oil did not significantly affect optimum water absorption but reduced mix time between 10 to 15%. All breads changed in texture as expected, the report in Appendix 5).

Specific trans-fatty acid results generated on bread from this trial, from Mylnefield Laboratories, document that the total trans-fatty acids (C18:1 and C18:2) were significantly higher in the control bread (175.2 mg/100 g serving) compared to the DHA-rich algal oil fortified bread (144.3 mg /100 g serving). Lower amounts of trans-fatty acids are expected in

the DHA-rich algal oil bread due to the fact that some of the partially hydrogenated shortening used in the control was replaced by DHA-rich algal oil in the DHA fortified bread.

IX.3.3.2 Intake Estimate

Bakery products include bread and rolls, pies, cakes, pastries, pizza, bagels, pretzels, doughnuts and many other specialized varieties. Bread and rolls constitute the largest subcategory of baked goods common to each Member State and are totalled separately. The *per capita* consumption of bread and rolls in the EU has been fairly stable, from 37.4 kg in the UK to 84.4 kg in Germany, or from 103 g/p/d to 230 g/p/d. The DAFNE database gives 137 g/p/d and this is used in Table 6. The consumption for other bakery products is given as 34.96 g in DAFNE (see Appendix 6) The number of products in the baked goods category is very large and varied with some different products in different Member States. The DAFNE database defines two broad categories "Breads and rolls" and "Other bakery products". Use of approximately 172 g as the intake estimate for the combined category gives a total for DHA of 70 + 274 mg or 344 mg from the bakery products category.

IX.3.4 Nutrition Bars

Nutrition bars were included in the original application dossier for DHA-rich algal oil. This use group was accepted throughout both 90-day and 60-day periods and was carried forward to the Standing Committee on the Food Chain and Animal Health meeting. It was mentioned that, at the last minute, there was some confusion regarding the definition of "Nutrition Bars" specifically concerning whether this definition was sufficient to exclude pure confectionery products such as "Mars bars". Martek is resubmitting this food group in the present application and is specifically excluding the addition of DHA–rich algal oil to confectionery bars.

Nutrition bars are a fairly new and specific food type and unlike bakery products or non-alcoholic beverages, this category of foods is not broadly consumed by the entire population. Nutrition bars may be consumed frequently by special populations, young people, athletes, hikers, *etc.* Accordingly, it would be a gross underestimate of consumption for those individuals who are "eaters" to compute the *per capita* intake and divide by the entire population. Accordingly, it is assumed that a typical bar is 50 g and that the average dedicated consumer of nutrition bars may eat as much as one bar each day. This gives an estimated average daily intake of 50 g of nutrition bars per day for those individuals who are "eaters". Appendix 7 provides some examples of the types of Nutrition Bars requested. The estimated daily intake of DHA from this food category is 250 mg.

IX.4 Product Categories and Nutrient Profiles

In addition to information presented regarding combined consumption of all food categories and mean and high-level intake groups, consideration should also be given to the fact that only certain types of products within each food group/category will actually be suitable for the inclusion of DHA-rich algal oil. The addition of DHA-rich algal oil to fortified food products will involve health and/or nutrition claims being made (there would be no point in adding

DHA-rich oil if this were not the case), and as such would be subject to the risk management principles laid down in *Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods* (European Parliament and the Council of the European Union, 2006). As part of this regulation, nutrient profiles will be introduced to control the types of food to which functional ingredients such as DHA-rich algal oil may be added. As a consequence DHA-rich algal oil will be added to "healthier option" foods. This regulation will further limit intake, making the above considerations even more conservative.

IX.5 Guidance Upper Levels in Certain Member States

Guidance upper levels have been established for consumption of long-chain omega-3 fatty acids [LCPUFA, including DHA, EPA and docosapentaenoic acid (DPA)] in the EU. These levels are based on nutritional benefit, specifically levels beyond which no additional benefit can be shown.

It is worth noting that Food Agencies in certain Member States, such as the French Food Safety Agency (AFSSA – Agence Française de Sécurité Sanitaire des Aliments) and the Belgian Food Experts Group of the Superior Council of Hygiene (CSH - Conseil Supérieur d'Hygiène, 2004) agree on a maximum recommended nutritional level at about 2 g/day, underlining that they DO NOT consider this recommended nutritional level as an upper safety limit (AFSSA, 2003).

Other competent authorities in countries, such as in Australia and New Zealand and the US, consider it safe to ingest long-chain omega-3 fatty acids at higher daily intake and/or have granted broad allowance for the addition of DHA-rich algal oil in foods. More specifically:

- *Food Standards Australia New Zealand* (FSANZ) allows DHA-rich oil from *Schizochytrium* sp. in all food categories without limitation or restriction. Notably novel food approval in Australia and New Zealand includes (i) bakery products, breads and rolls; (ii) nutrition bars; and (iii) non-alcoholic beverages, milk-based drinks and dairy analogue drinks. FSANZ considers DHA-rich oil to be similar to other traditional sources of omega-3 LCPUFA and raised no safety concern (Commonwealth of Australia, 2002)

- The *US Food and Drug Administration (FDA)* allows DHA-rich oil from *Schizochytrium* sp. in a broad range of food categories at defined levels including (i) bakery products, breads and rolls; (ii) nutrition bars; and (iii) non-alcoholic beverages, milk-based drinks and dairy analogue drinks. The FDA established a "generally recognized as safe" GRAS value of 3 g of DHA and EPA per day (FDA, 1997, 2004a).

- *Health Canada* allows DHA-rich algal oil from *Schizochytrium* sp. in a broad range of food categories providing that no more than 100 mg total omega-3 LCPUFA is added per food serving. The variety of products to which Health Canada has permitted the

addition of DHA-rich oil, as a novel food ingredient, is substantially broader than that sought in the EU and includes (i) bakery products, breads and rolls; (ii) nutrition bars; and (iii) non-alcoholic beverages, milk-based drinks and dairy analogue drinks Moreover, the authorized level to which DHA may be included in these products is, in general, consistent with those proposed in this request for EU approval.

Should intake concerns surface at EU level regarding consumption of DHA from DHA-rich algal oil, then it is submitted that equivalent restrictions and controls would be just as appropriate for all sources of omega-3 LCPUFA, including fish oil. The following points are respectfully submitted for consideration:

- Restricting the addition of DHA-rich algal oil in foods on the grounds that there are cumulative intake concerns is inconsistent with the approach retained by the European legislature to authorize the addition of vitamins and minerals in a broad range of applications, such as fortified foods, food supplements, foods for specific nutritional uses (PARNUTS), keeping in mind that some of those nutrients are also permitted as technological food additives. All these nutrients may - at certain doses and in case of cumulative intakes - raise concerns. However, the European legislature has appropriately not restricted the addition of such nutrients to specific food categories.

- There is no objective reason to treat DHA-rich algal oil and fish oil differently. As the European Court of Justice ruled in a long-standing jurisprudence, it is a violation of EU law to treat 2 similar products differently when such a difference of treatment is not objectively justified. Indeed, the principle of non-discrimination requires that comparable situations must not be treated differently and different situations must not be treated alike unless such treatment is objectively justified.[7]

- In this regard, there is a complete absence of scientific evidence to objectively support any suggestion that DHA-rich algal oil is less suitable for use in foods than is fish oil. In such circumstances, imposing restrictions on the addition of DHA-rich oil to additional food categories, despite scientific evidence supporting the safety of these foods and while not imposing equivalent restrictions on fish oil, would constitute unfair and unjustified discrimination against DHA-rich algal oil vis-à-vis fish oil. As a result, such restrictions could create trade barriers contrary to EU law and would furthermore be arguably inconsistent with international standards.

- Furthermore, there are specific groups of the population that do not consume fish and fish-derived products such as fish oil, either because of allergenicity reasons or because of diet choices (such as vegans or vegetarians). Other groups, such as

[7] See in particular: Case 15/83 *Denkavit Nederland* [1984] ECR 2171, paragraph 22; Joined Cases 201/85 and 202/85 *Klensch and Others* [1986] ECR 3477, paragraph 9; Joined Cases T-466/93, T-469/93, T-473/93, T-474/93 and T-477/93 *O'Dwyer and Others* v *Council* [1995] ECR II-2071, paragraph 113; and Case T-119/95 *Hauer* v *Council and Commission* [1998] ECR II-2713, paragraph 63); Case T-373/94 *R.W. Werners v Council of the EU and Commission of the EC*, paragraph 98

pregnant women, nursing women and infants, have increased needs for consumption of DHA. Such categories of consumers should not be deprived of the health and nutritional benefits of DHA from sources such as DHA-rich algal oil, in particular in a context where it is recommended to have a varied diet. The authorization of DHA-rich algal oil in additional use groups would allow adequate consumption of DHA while maintaining a varied and balanced diet.

Having regard to the above[8], Martek has addressed herein the questions raised during the 60-day period of the original application for approval of DHA-rich algal oil.

[8] Also, it should be noted that, in contrast to fish and fish oils, DHA-rich algal oil does not contain contaminants. From an economic standpoint, it is also worth pointing out that the implementation of the requirements of Regulation 183/2005 regulating feed hygiene by fish oil industry seems to raise serious difficulties to such an extent that there are serious concerns of shortage in fish oil supply in the EU after 31 October 2007, the deadline for compliance of feed businesses with the 2005 hygiene rules. See press release from Food Navigator.com/Europe of 10 July 2007. Because of these difficulties, the Commission and Member States within the Standing Committee on the Food Chain and Animal Health that met on 19 June 2007 agreed to postpone the date of entry into force of hygiene rules to 31 October 2008. Given, however, that most crude fish oil is mainly imported from Peru, Morocco and Chile and that very little is intended for human consumption, despite the postponement of the date, there is little incentive for companies in these countries to invest to meet the new EU hygiene rules, so that the risk of shortage will possibly exist even after 31 October 2008.

X INFORMATION ON PREVIOUS HUMAN EXPOSURE

X.1 DHA-rich Algal Oil

The safety of DHA-rich algal oil is supported by its extensive use as a dietary supplement and as a food ingredient in several countries around the world.

In the United States, DHA-rich algal oil has been marketed as a dietary supplement and was the subject of a New Dietary Pre-market Notification submitted to the FDA in December 1997 for SeaGold™ DHA-rich oil (FDA, 1997), the same oil that is the subject of the current application. The use of DHA-rich algal oil as a nutritional food ingredient was previously determined to be GRAS through scientific procedures following review by qualified experts. This conclusion was reviewed by the US FDA as part of GRAS notification GRN 000137, with no objections (FDA, 2004b).

In the United States DHA-rich algal oil has been added to food products including yoghurts, dairy products and analogues, non-alcoholic beverages, nutrition bars and baked goods. Examples of these products are shown in Appendix 8.

DHA-rich algal oil is also approved for use as a novel food ingredient in the European Union 2003/427/EC (Official Journal of the European Union, 2003). In Europe, DHA-rich algal oil has been added to food products including yoghurts, dairy products and analogues. Examples of these products are shown in Appendix 9.

The Australia New Zealand Food Authority (ANZFA) considers DHA-rich algal oil (derived from *Schizochytrium* sp.) and the microorganism itself to be safe for use as a novel food ingredient in various foods including (i) bakery products, breads and rolls; (ii) nutrition bars; and (iii) non-alcoholic beverages, milk-based drinks and dairy analogue drinks (Commonwealth of Australia, 2002).

DHA-rich algal oil was approved by Health Canada for use in a broad range of foods (including bakery products, breads and rolls; nutrition bars; and non-alcoholic beverages, milk-based drinks and dairy analogue drinks) in 2006 (Health Canada, 2006), was approved for use in China by the Ministry of Health in a broad range of foods (including bakery products, breads and rolls; nutrition bars; and non-alcoholic beverages, milk-based drinks and dairy analogue drinks) in 2007 (Chinese Ministry of Health, 2007) and is allowed for use in foods in Mexico following consultation with competent authorities in all food categories proposed herein (Mexico, 2007).

X.2 DHA and EPA Exposure - NDNS Survey Data

Previous DHA and EPA human exposure data from the National Diet and Nutrition Survey (NDNS) 1986/1987 were reviewed extensively in the initial application dossier (UKDA, 1991). The principal sources of EPA and DHA in the diet are fatty fish. However, only 35% of UK adults regularly consume fatty fish. In the absence of fatty fish in the diet, average

intakes of EPA and DHA would be only 33 mg/day and 54 mg/day, respectively, for the same group of consumers (UKDA, 1991)[9].

Human exposure data from the latest NDNS (Henderson *et al.*, 2002) of adults aged 19 to 64 years shows that the mean consumption of oily fish has increased from 34 g to 53 g/week (97 to 151 mg/day DHA/EPA - from about a quarter to a third of a portion) since the last survey of this age group in 1986/1987. Increased salmon consumption largely accounts for the increase. The intake data has been recently reviewed by the Fish Inter-Committee Sub-Group (FICS) of the UK Scientific Advisory Committee on Nutrition (SACN). This subcommittee has presented recommendations to increase LCPUFA (DHA/EPA) consumption to 450 mg/day (SACN, 2004).

The 2003 AFSSA report on omega-3 fatty acids, referred to previously, recognizes that omega-3 fatty acid intakes are insufficient since the average consumption of fish products by the French population is only to 35 g/day/person. This insufficient intake of omega-3 fatty acids from fish results in the promotion of foods to which they are added (AFSSA, 2003).[10]

The Belgian Superior Council of Hygiene, strongly relying on the comments of the French Agency in the 2003 report, recommends increasing intake of omega-3 fatty acids by pregnant and nursing women, advising the consumption of approximately 250 mg (200 to 300 mg) of DHA on a daily basis.

In the report from the Health Council of the Netherlands of 18 December 2006, experts recommend that the amount of fish fatty acids in the diet be increased substantially in order to meet the norm for these fatty acids of 450 mg/day (Health Council of the Netherlands, 2006).[11] Clearly this has proved difficult to achieve in the EU from fish intake alone, and this fact supports the need for algal oil, a more acceptable, reliable, sustainable, and convenient dietary source of omega-3 DHA.

[9] National Dietary and Nutrition Surveys (NDNS) of adults aged 16 to 65 (1986/87) which was commissioned jointly by the UK Ministry of Agriculture Foods and Fisheries (MAFF) and Department of Health (DH).
[10] See p. 22 of that report
[11] See in particular p. 13 of the Dutch report entitled “Guidelines for a healthy diet 2006”, N° 2006/21

XI NUTRITIONAL INFORMATION

XI.1 Nutritional Profile of DHA-rich Algal Oil

DHA-rich algal oil is essentially 100% fat (principally in the triglyceride form) and as such has a caloric value of approximately 9 kcal per g. The nutritional profile of DHA-rich algal oil is shown in Appendix 10.

XI.2 Nutritional Profile of Example Foods Containing DHA-rich Algal Oil

The nutritional profile of a food containing DHA-rich algal oil will depend on both the food product and the amount of oil added to the food product. It is highly likely that a food manufacturer will simply use DHA-rich algal oil to replace some or all of the added fish or vegetable oil; therefore, there will be little, if any, change to the nutritional profile of the food product. In the case of food fortification, a food product with 100 mg of DHA from added DHA-rich algal oil will deliver 286 mg of oil or 2.6 kcal to the food product. In the case of food fortification in which DHA-rich algal oil is used to replace all of the fish oil, no change in nutritional profile of the food will be observed. A few examples of foods in which DHA-rich algal oil may be used to replace fish oil are as follows.

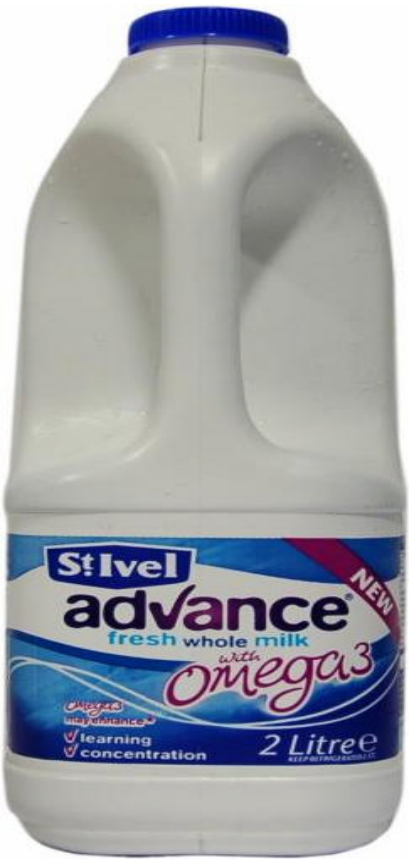
XI.2.1 Milk-based Drink

Table 7 provides a comparison of the nutrition profile of milk-based drink, in which DHA-rich algal oil is used to replace fish oil as a source of DHA/EPA. It can be clearly seen that the overall profile will not be changed.

Table 7 Nutritional Comparison of Milk-based Drink Fortified with Omega-3 from Fish Oil or DHA-rich Algal Oil

Nutrient	**Milk-based Drink with Fish Oil**	**Milk-based Drink with DHA Algal Oil**
Typical Values	Per 100 mL	Per 100 mL
Energy	206 Kj/49Kcal	206 Kj/49Kcal
Protein	3.4 g	3.4 g
Carbohydrates	5.0 g	5.0 g
Of which: sugars	5.0 g	5.0 g
Fat	1.7 g	1.7 g
Of which: saturates	1.0 g	1.0 g
Of which: monounsaturates	0.4 g	0.4 g
Of which: polyunsaturates	0.07 g	0.08 g
Of which: Omega-3 (DHA and EPA)	0.025 g	0.025 g
Fibre	Nil	Nil
Sodium	0.057 g	0.057 g
Salt	122 mg	122 mg
Ingredients Listing:	Milk-based Drink with Fish Oil Ingredients: Fresh Semi Skimmed Milk, Omega-3 (Derived from Fish Oil), Milk Protein	Milk-based Drink with DHA Algal Oil Ingredients: Fresh Semi Skimmed Milk, Omega-3 (DHA-rich oil from the microalgae *Schizochytrium* sp.), Milk Protein

Example of a Milk-based Drink (St. Ivel Advance)
(63 mg DHA/EPA per 250 mL serving from fish oil)

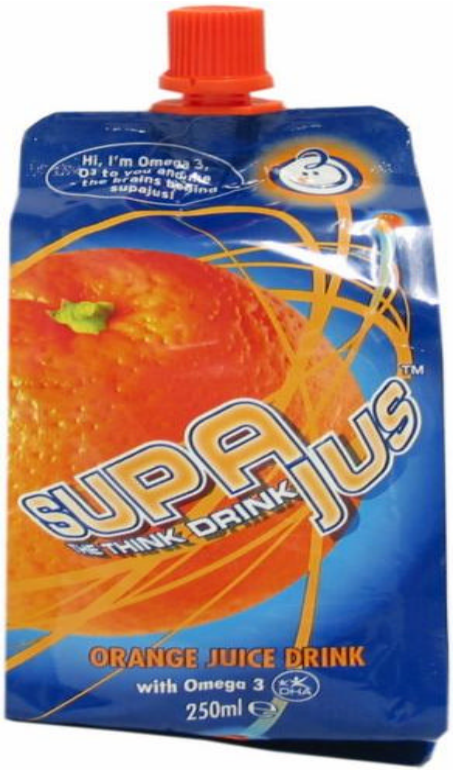


XI.2.2 Juice Drink

Table 8 provides a comparison of the nutrition profile of a juice drink, in which DHA-rich algal oil may be used to replace tuna oil as a source of DHA/EPA. It can be seen that the overall profile will not be changed.

Table 8	**Nutritional Comparison of a Juice Drink with DHA Omega-3 from Tuna Oil or DHA-rich Algal Oil**	
	Orange Juice Drink with Tuna Oil	**Orange Juice Drink with DHA-rich Algal Oil**
Typical Values	Per 100 mL	Per 100 mL
Energy	41 kcal	41 kcal
Protein	0.4 g	0.4 g
Fat	0.2 g	0.12 g
Of which saturated	0.05 g	0.04 g
Of which monounsaturated	0.04 g	0.01 g
Of which polyunsaturated	0.07 g	0.06 g
Of which Omega 3 DHA	0.04 g	0.04 g
Carbohydrate	9.4 g	9.4 g
Of which sugar	9.2 g	9.2 g
Sodium	0.04 g	0.04 g
Vitamin E	8 mg	8 mg
Ingredients Listing:	Orange juice 50%, water sugar, stabiliser (pectin), flavouring, citric acid, refined Pacific tuna oil (source of Omega-3 DHA), antioxidants	Orange juice 50%, water sugar, stabiliser (pectin), flavouring, citric acid, DHA-rich oil from the microalgae *Schizochytrium* sp. (source of Omega-3 DHA), antioxidants

Example of a Juice Drink (Supajus)
(40 mg DHA per 100 mL serving from tuna oil)



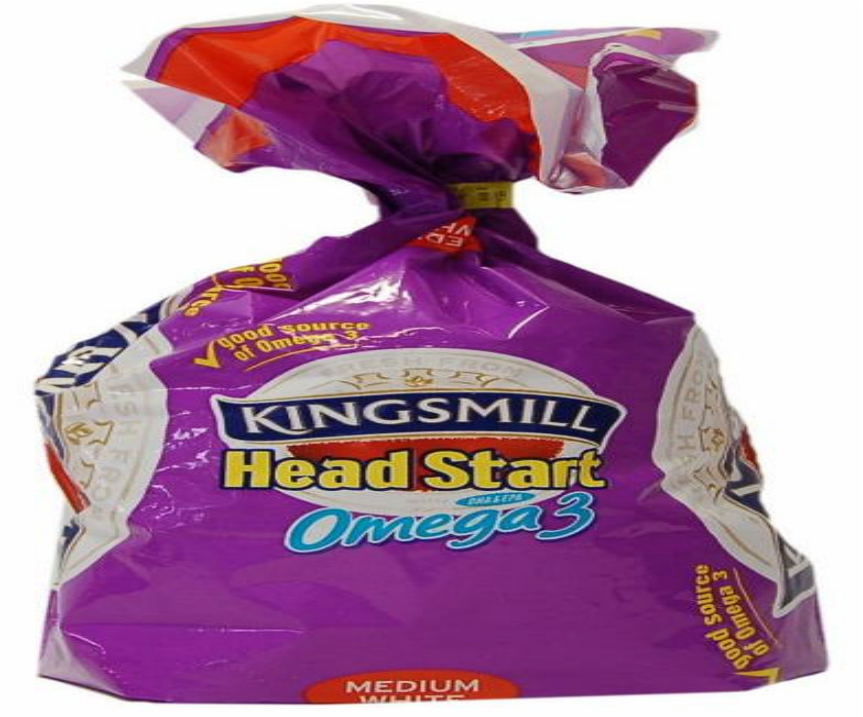
XI.2.3 Bread

Table 9 provides a comparison of the nutrition profile of bread, based on DHA-rich algal oil replacing fish oil as a source of DHA Omega-3. It can be clearly seen that the overall profile will not be changed.

Table 9 Nutritional Comparison of White Bread with Fortified with Omega-3 From Fish Oil or DHA-rich Algal Oil

	White Bread with Fish Oil	**White Bread with DHA Algal Oil**
Typical Values	Per 100 g	Per 100 g
Energy	984 Kj/232Kcal	984 Kj/232Kcal
Protein	8.5 g	8.5 g
Carbohydrates	45.0 g	45.0 g
Of which sugars	3.4 g	3.4 g
Fat	2.0 g	1.9 g
Of which: saturates	0.4 g	0.4 g
Of which: monounsaturates	0.9 g	0.8 g
Of which: polyunsaturates	0.7 g	0.7 g
Of which: Omega-3 (DHA and EPA)	68 mg	68 mg
Fibre	2.7 g	2.7 g
Sodium	0.50 g	0.50 g
Salt	1.25 g	1.25 g
Ingredients Listing	Wheat Flour, Water, Yeast, Salt, Soya Flour, Vegetable Fat and Vegetable Oil, Refined Fish Oil, Emulsifier: E472e; Fish Gelatin, Preservative: Calcium Propionate (added to inhibit mould growth); Flavourings, Flour Treatment Agent: Ascorbic Acid (Vitamin C). Contains Wheat, Gluten, Soya, Fish Oil and Fish Gelatin	Wheat Flour, Water, Yeast, Salt, Soya Flour, Vegetable Fat and Vegetable Oil, DHA-rich Oil from the microalgae *Schizochytrium* sp.), Emulsifier: E472e; Fish Gelatin, Preservative: Calcium Propionate (added to inhibit mould growth); Flavourings, Flour Treatment Agent: Ascorbic Acid (Vitamin C). Contains Wheat, Gluten, Soya and Fish Gelatin

Example of a Bread (Head Start)
(68 mg DHA/EPA per 100g – 2-3 slices)



XII MICROBIOLOGICAL INFORMATION

This issue was discussed and addressed in the initial submission.

XIII ADDITIONAL TOXICOLOGICAL AND HUMAN SAFETY INFORMATION

XIII.1 Overview

Fifteen clinical studies in over 1200 adults, children, and pregnant/lactating women demonstrate that ingestion of DHA-rich algal oil is not associated with any adverse experiences unrelated to oil organoleptic properties. *(In some cases ingestion of DHA-rich algal oil capsules is associated with a fishy like "burp"/reflux, an organoleptic property inherent to any LCPUFA oil consumed at high concentrated doses.)* The product has been evaluated in children, adults with and without cardiac disease and diabetes and pregnant and lactating women. There are no signs of any safety concern in any population. Demonstrating confidence in the safety of DHA-rich algal oil, the US National Institutes of Health is currently supporting a study which involves the use of DHA-rich algal oil in pregnant/nursing women at a dose of 800 mg DHA/day. Martek is unaware of any study or investigation that has been terminated due to safety issues with DHA-rich algal oil. A recent EU recommendation noted that pregnant and lactating women should ingest at least 200 mg per day of DHA with up to one gram per day as appropriate (Koletzko *et al.*, 2007). As the algal source of DHA does not contain mercury or environmental contaminants there is no issue of over-exposure to toxins from this source.

This overview surveys clinical data from 15 studies for which Martek has at least some safety information available. Martek conducts some clinical trials itself; however, most clinical trials are conducted by independent investigators/universities or agencies and therefore, Martek does not always have access to full data sets or information is incomplete. The following section outlines these studies with the current information available. To date, there is no safety signal detected that in any way suggests a safety issue with the use of DHA-rich algal oil in subjects, including the especially vulnerable populations of children and pregnant women.

For the purpose of this summary, adverse experiences and serious adverse experiences are defined as follows:

- ***Adverse experiences****—any unfavourable and unintended sign, symptom, or disease temporally associated with the use of DHA-rich algal oil whether or not it is related to the product.*

- ***Serious adverse experiences****—any untoward medical occurrence that at any dose of DHA-rich algal oil results in death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability, is a congenital anomaly/birth defect, or is an important medical event which may jeopardize the subject and may require medical or surgical intervention to prevent one of the above outcomes.*

XIII.2 Clinical Studies Using DHA-rich Algal Oil

Clinical studies using DHA-rich oil derived from the microalgae *Schizochytrium* sp. are attached in Appendix 11 (presented as summary tables of clinical safety). The following sections describe both completed and ongoing clinical studies (unpublished) during which subjects received moderate to high levels of DHA from DHA-rich algal oil, listed as grams DHA consumed per day, and the resulting adverse experiences summary. These studies are from the US and Australia as well as the EU. One study provides preliminary data from a yet incompletely reported study of women consuming DHA-rich algal oil during the second and third trimesters of pregnancy utilizing a manufactured nutrition bar. Three additional studies provide data from children consuming DHA-rich algal oil in both food form (orange juice) and capsules. The estimated consumption was in the 20 mg DHA/kg body weight range for the majority of children, which translates into approximately 1.4 g DHA/day in an adult. The other studies involved non-pregnant adults. While studies evaluated a number of potential clinical outcomes, only the safety portion of the study is reported here.

As reported below, no significant product-related adverse or serious adverse experiences have been attributed to DHA-rich algal oil in any of the studies. The highest intake of oil during these studies was approximately 7.2 g oil/day (2.7 g DHA/day) for 12 weeks.

XIII.2.1 DHA Supplementation and the Prevention of Premature Labour in Pregnant Women Using a Functional Food Source of DHA (McGregor *et al.*, 1999)

Investigational Product: Nutritional bars containing 0, 300, or 600 mg of DHA from 0, 0.86, and 1.7 g DHA-rich algal oil, respectively.

Subject Population: A target sample size of 1200 women (400/treatment) ≥18 years of age in their 16 to 18th week of pregnancy (based upon obstetric assessments and dates) with no known risk factors for premature delivery.

Study Design: Randomised, double-blind, placebo-controlled study with enrolment beginning between 16 and 18 weeks and supplementation beginning between 20 and 22 weeks of pregnancy. Subjects receive 30 nutritional bars each month and are asked to consume 1 bar per day. Subjects are advised to avoid rich sources of omega-3 fatty acids throughout the study period.

Safety Outcomes: The study is incomplete and a final report has not been received by Martek. A University Data Safety Monitoring Board performs periodic reviews of all safety data related to this study. The last estimate of exposure was 442 women receiving active product. At this time no safety concerns have been raised by the investigators. The overall incidence of non-serious adverse events was 24.4%, 36.7%, and 35.3% for the control, 300 mg and 600 mg groups, respectively. All serious adverse experiences (AEs) were considered unrelated to the clinical trial material.

XIII.2.2 Memory Improvement with Docosahexaenoic Acid Study (MIDAS) – A Randomised, Double-blind, Placebo-controlled Study to Evaluate the Effects of DHA on Cognitive Functions in the Elderly (Yurko-Mauro, 2007)

Investigational product: DHA is provided as soft-gelatin capsule containing 300 mg DHA (DHA-rich algal oil). Subjects are instructed to take (3) capsules/day. Total daily dose is therefore 900 mg DHA per day. Matched placebo.

Subject population: Males or females, aged 55 or greater have a subjective memory complaint and have a Logical Memory subtest (of the Wechsler Memory Scale - III [WMS-III]) raw score one standard deviation or greater below the mean of a younger population

Study Design: DHA-rich oil Treatment, Randomised, Double-Blind, Placebo Control, Parallel Assignment, Number of arms in study: 2. Treatment period 6 months

Safety Conclusion: Trial is ongoing, as of October 9, 2007 320 of 465 subjects enrolled, therefore estimated exposure is 160 subjects to 900 mg DHA/day for 6 months. Treatment is still blinded to investigators. A total of 5 serious AE reported, including one death due to chronic obstructive pulmonary disease. Treatment group blind has not been broken by the investigator for any of these cases.

XIII.2.3 Effect of Supplemental DHA on High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) Subclasses in Patients with Low HDL and High Non-HDL Cholesterol (Barringer *et al.*, 2003)

Investigational Product: Orange drink fortified with 1.5 g of DHA from approximately 4 g DHA-rich algal oil or a non-fortified orange drink placebo.

Subject Population: Forty-one non-smoking, mildly overweight men and women over 25 years old with low HDL cholesterol. To qualify for entry, subjects' HDL cholesterol must have been $\leq$ 45 mg/dL or $\leq$ 50 mg/dL with non-HDL > 160 mg/dL.

Study Design: This study was a randomised, double-blind, placebo-controlled design with two parallel treatment arms. Subjects were randomly assigned to receive the drinks containing 1.5 g DHA or placebo oil daily for 12 weeks while continuing their usual diet. Subjects visited the clinic at baseline and after 12 weeks of treatment to provide fasting blood samples for analysis.

Safety Outcomes: There were no statistically significant differences between the treatment and placebo groups in the incidence of any adverse event. Although there was a higher overall incidence of persons experiencing at least one adverse event in the treatment group compared to the placebo group, this was largely due to an increase in colds (nasopharyngitis) and infections that were not considered related to the treatment. The only adverse experiences that were classified as related or possibly related to the study

supplements were dyspepsia (upset stomach, in both placebo and treatment groups) and oesophagitis (reflux).

XIII.2.4 Evaluation of the Effects of DHASCO-S on the Minimal Erythema Dose Under Static Conditions (Berg *et al.*, 2006)

Investigational Product: DHA-rich algal oil capsules delivering 1.2 g DHA/day (200 mg DHA capsules x 6 capsules per day) or matching placebo.

Subject population: Healthy adults, aged 18-50 with acceptable skin colour, exposure was 23 on placebo and 23 on DHA-rich algal oil.

Study design: Double blind, placebo controlled

Treatment period: 4 weeks

Safety conclusion: "The product was generally well tolerated". There were no serious adverse experiences.

XIII.2.5 ABCD-E Trial; Attention and Behaviour in Children on a Dietary Supplement (ABCD-Efficacy) - A Randomised, Double-Blind, Placebo-Controlled Study to Evaluate the Effects of DHA on Cognitive Functions in Preschool (Blue *et al.*, 2007)

Investigational product: DHA-rich algal oil delivering 400 mg DHA/day, provided from two chewable softgel capsules (200 mg DHA per capsule x 2 capsules per day) containing bubblegum flavoured oil administered once daily during the 4-month double-blind treatment period. Matched placebo.

Subject population: Normal male and female children, 4 years 0 months to 4 years 8 months of age at the Baseline visit. 202 subjects enrolled, 102 (53 male and 49 female) received DHA and 100 (50 male and 50 female) received placebo. All 202 subjects were included in the safety analysis population

Study Design: Multi-centre, randomised, double-blind, placebo-controlled study to assess the safety and efficacy of 400 mg DHA/day provided from chewable softgel capsules containing bubblegum flavoured microalgal oil (DHA-rich algal oil). Exposure was for 4 months.

Safety Outcome: Docosahexaenoic acid supplementation (400 mg DHA/day for 4 months in 4-year old children) provided from chewable softgel capsules containing bubblegum flavoured oil demonstrated a favourable safety profile and was well-tolerated in preschool children. The overall AE profiles for the DHA and placebo treatment groups were similar. There were no serious adverse experiences (SAEs).

XIII.2.6 Nutritional Intervention to Prevent Diabetes, Pilot Study (Chase and Clare-Salzler, 2007)

Investigational product: DHA-rich algal oil capsules delivering 800 mg DHA/d (200 mg DHA per capsule x 4 capsules per day) or placebo.

Subject population: Pregnant females in the last trimester or nursing mothers. Mother, father or sibling must have type I diabetes for mother to be eligible to enrol in the study.

Study design: Pilot study, double-blind, placebo-controlled, includes product not prepared by Martek and for which Martek receives no information. National Institute of Health (US) sponsored study.

Treatment period: Last trimester of pregnancy and during breast feeding period

Safety conclusions: At the time of this report the study is ongoing. A treatment allocation schedule not available to Martek. All AE reports blinded. Seven serious AE reported.

XIII.2.7 Supplementation of Orange Juice with DHA Improves Plasma Phospholipid DHA Content of Children (Hawthorne *et al.*, 2007)

Investigational product: Orange juice containing either 50 or 100 mg DHA per 180 mL per day. DHA source is from DHA-rich algal oil.

Subject population: Children ages 4 to 12 (divided 4-6, n=16 and 7-12, n=15) total n=31.

Treatment period: 6 weeks

Safety Conclusion: “In both age groups, orange juice 180 mL/day supplemented with 50 or 100 mg DHA was well tolerated”

XIII.2.8 OmegaSoy Study - Combined Benefits of Soy Isoflavones and DHA-rich Algal Oil (Howe *et al.*, 2002)

Investigational Product: Capsules delivering an olive oil placebo or 2.7 g DHA/day from approximately 7.2 g/day DHA-rich algal oil. These products were tested in combination with a soy and control cereal.

Subject Population: Forty subjects divided between 2 groups (20/group). Men and women over 40 years of age were recruited to enter into the dietary intervention study if they met the following criteria: elevated LDL cholesterol (>3.5 mmol/L); elevated triglyceride concentrations (>1.6 mmol/L); and, preferably, mildly elevated blood pressure (>135/85).

Study Design: Subjects were randomised to take six 1.2 g capsules/day of either olive oil or DHA-rich algal oil for 12 weeks. Within each oil treatment group, subjects consumed a daily 45 g serving of breakfast cereal (containing either a placebo or >75 mg of isoflavones) for 6 weeks then crossed over to the alternative for another 6 weeks.

Safety Outcomes: No serious adverse events were reported during the study. Two subjects voluntarily withdrew from the study stating that they did not like "the taste" of the dietary supplements.

XIII 2.9 ABCD Pilot Trial Attention and Behaviour in Children on a Dietary Supplement: A Pilot Study (Jensen and Heird, 2005)

Investigational product: Chewable capsules containing DHA-rich algal oil (providing 200 mg DHA/capsule), bubblegum or orange flavoured.

Subject populations: Normal children ages 4 to 6. 42 children exposed to product.

Study Design: Open label evaluation of dose response of 200 mg DHA/day S (n=20) *vs*. 400 mg DHA/day (n=22) over a 4-week period. No placebo utilized.

Safety Outcome: "The chewable product was well tolerated by all children. No serious adverse experiences were recorded."

XIII.2.10 A Pilot Study Assessing the Cognitive and Mood Effects of 8 Weeks of Supplementation with 400mg or 1000mg of DHASCO-S in Healthy Children Aged 10 to 12 years (Kennedy, 2007)

Investigational product: DHA-rich algal oil capsules delivering 400 mg DHA/day or 1000 mg DHA/d with matching placebo.

Subject population: Normal children, males or females age 10 to 12 years old, exposure was 30 subjects per group.

Study design: Double-blind, placebo-controlled, children with no history of behavioural problems or taking medications for any neuro-psychiatric condition. Study conducted at the Northumbria University, New Castle, UK.

Treatment period: 8 weeks

Safety conclusions: "There were no serious adverse events related to taking the active treatments. No participant withdrew from the study due to intolerance of their treatment, and in general the study treatment was well tolerated."

XIII.2.11 A Double-Blind, Randomised, Parallel, Controlled Clinical Trial to Evaluate the Effects of a DHA-Containing Capsules on Serum Lipids in Men and Women with Below-Average HDL Cholesterol Levels (Maki *et al.*, 2005)

Investigational Product: Capsules delivering an olive oil placebo or 1.52 g DHA/day from approximately 4 g DHA-rich algal oil per day.

Subject Population: Sixty subjects were randomised equally to 2 treatment arms (30/arm). To qualify for entry into the study, men and women had to be between the ages of 21 and 80

years with a fasting HDL cholesterol concentration less than the sex-specific median value (≤44 mg/dL for men, ≤54 mg/dL for women).

Study Design: This study was a randomised, double-blind, controlled design with 2 parallel treatment arms. There were 6 clinic visits: 2 for screening (Weeks -2 and -1), 1 at baseline (Week 0), and 3 treatment visits (Weeks 3, 5, and 6). Subjects participated in a 6-week treatment period. Subjects were told to exclude all other rich sources of omega-3 fatty acids from their diet, but otherwise normal diets were maintained.

Safety Outcomes: There were no statistically or clinically important differences in the incidence of adverse experiences from baseline to the end of each treatment. Those experiences deemed to be possibly product related were consistent with published reports of marine-based dietary supplements and included abdominal discomfort, flatulence, and taste perversion.

XIII.2.12 A Bioequivalence Study Testing Blood Levels of DHA Following Supplementation with DHASCO or DHA-rich Algal Oil Capsules or Nutrition Bars (Arterburn *et al.*, 2007)

Investigational Product: (1) Corn/soy oil placebo capsules; (2) Capsules delivering 200 mg, 600 mg, or 1000 mg DHA/day from either DHA-rich algal oil (~0.57, 1.7, or 2.9 g oil/day) or DHA oil derived from *Crypthecodinium cohnii* (3) Nutritional bar delivering 600 mg DHA/day from approximately 1.7 g DHA-rich algal oil.

Subject Population: Ninety-six healthy male and female adults, age 18 to 70 years.

Study Design: An 8-arm, prospective, randomised, parallel group, bioequivalence study in healthy adults using three doses of DHA-rich algal oil (200, 600, or 1000 mg DHA in capsules per day) or from other sources of DHA. Only the DHA-rich algal oil data is reported here, from capsules or nutritional bars. The capsule arms of the study were double-blind and placebo-controlled. Subjects received the supplements over a 4-week period.

Safety Outcomes: There were no serious or clinically significant adverse events reported for any subject during the supplementation period. No subject discontinued because of an AE. All AE were evaluated by the investigator as mild to moderate in severity. Only eructation was significantly associated with supplementation.

XIII.2.13 Assessment of the Safety of DHA-S oil (*i.e.*, DHA-rich algal oil), an Algal Source of Docosahexaenoic Acid in Human Subjects (Sanders *et al.*, 2006)

Investigational Product: Capsules delivering an olive oil placebo or 1.5 g/day DHA from approximately 4 g/day DHA-rich algal oil.

Subject Population: Eighty healthy, free-living male and female adults recruited from among the staff and students of King's College London. Subjects were non-smoking, normotensive and normolipidemic.

Study Design: This was a randomised, double-blind, placebo-controlled, parallel group study. There was a brief run-in period with limited LCPUFA intake followed by 4 weeks of supplementation.

Safety Outcomes: A total of 79 subjects completed the study, with one subject withdrawing for personal reasons. Haematology, liver function tests, C-reactive protein, creatinine kinase, and plasma glucose were not affected by treatment. Factor VII coagulant activity was significantly higher following DHA treatment compared with placebo. However, the values were within the normal laboratory range. There were no other significant changes in haemostatic factors between treatment groups. Total, HDL, and LDL cholesterol levels increased but remained within the normal range with no change in the LDL/HDL ratio. There was a non-significant reduction in triglycerides, and a non-significant trend toward reduced systolic blood pressure in the DHA treatment group. The incidence of adverse events did not differ between treatment groups, and the symptoms reported were mild. In conclusion, DHA-rich algal oil was well tolerated with no adverse effects on liver function, cardiac enzymes, glucose metabolism, haematology, or markers of inflammation or haemostatic function.

XIII.2.14 The Triglyceride Lowering Effects of a Modest Dose of DHA Alone Versus in Combination with Low Dose EPA in Patients with Coronary Artery Disease and Elevated Triglycerides (Schwellenbach *et al.*, 2006)

Investigational product: DHA-rich algal oil capsules (providing 1000 mg DHA/day) or commercial fish oil capsules (providing 1000 mg DHA/day + 252 mg EPA/day (fish oil capsules). No information on the source of fish oil.

Study population: Adults with known cardiac risk factors attending a clinic for subjects with known risk factors, over 90% taking a lipid lowering drug-statin or aspirin. All had elevated triglycerides. Over one-third had a diagnosis of diabetes. Exposure was 57 on DHA-rich algal oil and 50 on fish oil. No placebo was used in the study.

Study design: Double blind, randomised study. Study conducted by a health care provider corporation in US, Kaiser Clinic.

Safety conclusion: “Overall the omega-3 fatty acids were well tolerated”. A greater proportion of subjects in the fish oil group reported a fishy taste.

XIII.2.15 The Influence of Docosahexaenoic Acid Supplementation on Vascular Function: A Randomised Controlled Trial (Abstract publication-ISSFAL)-Study currently ongoing, interim report (Singhal *et al.*, 2006)

Investigational product: DHA-rich algal oil providing 1.65 g DHA/day in capsules and matching placebo.

Subject Population: Healthy young adult volunteers, 18 to 36 years old.

Study Design: Double blind, placebo controlled.

Treatment Period: 16 weeks

Safety Conclusion: None specifically reported, 128 subjects included in the report, estimate that 64 exposed to DHA-rich algal oil for 16 weeks, with no notation of safety problems.

XIII.3 Summary of Clinical Exposure to DHA-rich Algal Oil

Estimated exposure from DHA-rich algal oil clinical studies is shown in Table 10. Estimated numbers are utilized as some studies are still not finished and the treatment codes are still blinded to Martek. None of these studies has been the subject of a hold or even questioned by a committee for safety reasons.

Table 10 Estimated Exposure to DHA from DHA-rich Algal Oil in Clinical Studies Known to or Reported to Martek as of October, 2007

Subjects	Estimated Number*	Amount of DHA	Exposure Length (approximate)
Pregnant Pregnant or nursing women	442 22	300 or 600 mg/day 800 mg	16+ weeks 16+ weeks
Children	31 21 154 30	50-100 mg/day 200 mg/day 400 mg/day 1000 mg/day	6 weeks 4 weeks 4-16 weeks 8 weeks
Adults	1035	200 mg/day -2700 mg/day	4-26 weeks

*DHA-rich algal oil exposure only, no placebo subjects included, if study still treatment blinded, estimate of exposure based on 50:50 allocation schedule.

As reported above, no significant product-related adverse or serious adverse experiences have been attributed to DHA-rich algal oil in any of the studies. The highest intake of oil during these studies was approximately 7 g oil/day (2.7 g DHA/day) for 12 weeks.

XIII.4 Human Safety Information - Review of Clinical Studies

The review by Kroes *et al.* considers the existing clinical studies demonstrating that the safe level of DHA may be substantially higher than 3 g/day (Kroes et al., 2003). In a 90-day clinical trial dietary supplementation with 6 g marine algal-derived DHA per day was reported not to affect bleeding times or platelet aggregation in 6 healthy males (Nelson *et al.*, 1997). Other studies, with doses at high as 9 g/day, reported no adverse effects, but focused mainly on efficacy, *e.g.,* LDL cholesterol changes. Other efficacy–related clinical studies that were conducted at lower dose levels, in the 0.5 to 1.5 g DHA range, also found no adverse affects.

In their review, Kroes concluded that when viewed in its entirety, the scientific evidence both from toxicological studies of algal derived DHA oils and DHA containing fish oils, under the conditions of intended used in food, would not be expected to produce adverse effects in human health.

XIII.5 Adverse Event Monitoring

XIII.5.1 Overview

Martek Biosciences Corporation has a written regulatory, medical, and clinical group procedure for obtaining, evaluating, and reporting AEs and SAEs occurring in clinical trials and in reports from marketed use of its products. Martek monitors product AEs during sponsored clinical studies or during investigator-initiated studies for which product is supplied. Also monitored are AEs reported to Martek through an established toll free (800) phone number and website. However, Martek does not formally monitor AEs reported to manufacturers of infant formulas containing Martek oils since these are considered the responsibility of the infant formula manufacturer.

There is an active component and a passive component to the AE monitoring program. All reports of AEs and SAEs are referred to Martek's medical affairs personnel for evaluation, reporting and recording. Medical affairs and regulatory groups are jointly responsible for collecting and maintaining an AE database for each product.

XIII.5.2 Active Monitoring (ongoing clinical studies)

Martek continues to sponsor clinical studies utilizing products and also supplies products to investigators in investigator-initiated studies. In both cases, Martek describes procedures for collecting AEs and SAEs and, in the case of investigator-initiated studies, outlines criteria for reporting AEs and SAEs to Martek in a research agreement. Clinical study personnel at the study site who are in contact with clinical trial subjects are responsible for collecting AE information. A physician, usually the Principal Investigator (PI), is responsible for: identifying and evaluating the severity and clinical importance of the AE; taking appropriate medical action; notifying Martek within 24 hours of learning of serious or unexpected AEs; and notifying the Institutional Review Board/Ethical Review Board (IRB/ERB). The Study Coordinator or Study Nurse is responsible for collecting AE information from the subject at each scheduled clinic visit or during routine telephone calls to the subjects. During the course of the study, complete reports of all AEs are entered in the subject's clinic source document and on the appropriate AE page of the study case report forms (CRFs). Each Study Protocol defines AEs and SAEs and instructs the PI and study personnel to evaluate and record the occurrence of all AEs occurring during the study. The following information is recorded: subject's ID (treatment allocation number); description of the AE; date AE started; date AE ended (or ongoing); maximum severity of the AE; action taken; and PIs opinion of the relationship of the AE to the study product. Martek clinical research personnel who monitor the study are responsible for explaining the procedures of reporting and evaluating AEs to the PI and all study personnel who come in contact with the subjects. Study personnel are instructed to notify Martek's study monitor immediately (within 24 hours) of any serious or unexpected AE. This is forwarded to Martek's medical director, who works with the PI to assess the significance. Martek's medical director together with the PI prepares an assessment report and submits it to Martek's regulatory department, to the study monitor, the PI and to the IRB/ERB. Martek's study monitor reviews all completed

CRF data and compares CRF entries with information recorded in the source documents. Any discrepancies or omissions in either data source are discussed with the study coordinator/study nurse who makes appropriate corrections to the documents. All AEs are reported to Martek's medical affairs department at the end of the study.

XIII.5.3 Passive Monitoring (Worldwide Marketed Product Experience)

Reports of AEs for marketed products may be received directly by Martek's medical affairs department from physicians in the field or from product consumers. Reports of AEs received by any Martek employee are referred directly to medical affairs personnel. A report of an AE to a Martek employee is recorded on a form including name and address of the consumer, physician, or other reporter, and a brief description of the AE. The Martek employee informs the consumer or physician that a Martek medical affairs person will contact him/her with a follow-up call for additional information. Martek medical affairs will collect information on the AE, including age, sex of the consumer/patient, a complete description of the AE including severity, date started, date ended (or continuing), concurrent medication, concurrent diseases, and relationship of the AE to the Martek product and usage of the Martek product.

Martek medical affairs and regulatory groups collect and maintain an AE database for each Martek product. The database consists of all AEs reported worldwide from 1) the various clinical trials of the product; 2) use of marketed product; and 3) reports from marketing or licensing partners of AEs related to marketed product. Martek medical affairs group prepares summary reports and determines if any trend toward clustering or any unusual increase in the type of AE is apparent. If so, a product investigation by medical affairs, regulatory and manufacturing groups may be conducted.

XIII.6 Discussion of the Safety of DHA and EPA

France and Belgium – The French working group revised recommended nutritional levels for intake of long-chain omega-3 fatty acids. The group decided to establish levels based on daily intake beyond which the nutritional benefit of long-chain omega-3 fatty acids can no longer be shown. The group emphasised that that this level should not be considered a safety limit, *i.e.*, a level of intake beyond which there are health risks.

The Belgian authorities of the Superior Council of Hygiene agree with this position, recalling the French Agency expressly mentions in its 2003 report mentioned above that the 2 g/day recommendation is not a safety limit.

FDA- The FDA has accepted as GRAS, oils whose levels of daily intake of EPA and DHA are estimated at less than 3 g/day.

There exists a strong scientific argument that daily intakes of DHA/EPA up to 3 g/day are safe. This analysis was recently re-affirmed both by FDA and by independent scientific reviewers in the EU.

In 1997, FDA affirmed as GRAS menhaden oil as a direct food ingredient with specific limitations to assure that the total daily intake of EPA and DHA would not exceed 3 g per person per day (FDA, 2007). In 2004, in a subsequent consideration of a Health Claim Petition, FDA reiterated its conclusion that 3 g DHA/EPA per day is GRAS (FDA, 2004a).

Known or suspected risks of high levels of EPA, DHA and other omega-3 fatty acids include the following which are discussed individually below:

- Reduced platelet aggregation, decreased bleeding times and consequent increased bleeding.
- Increased LDL cholesterol levels
- Reduced glycaemic control among diabetics

Bleeding time - In the preamble to the 1997 Federal Register Notice, FDA thoroughly reviewed the extant data on menhaden oil and its principal active components EPA and DHA (20% by weight) (FDA, 1997). The principal safety issue with the menhaden oil is increased bleeding times. The increased bleeding time (apparently produced by reduced platelet aggregation) was attributed solely to the presence of the two major long-chain omega-3 fatty acids present in the menhaden oil. This bleeding occurred in some clinical subjects when menhaden oil was fed at levels which delivered more than 3 g/p/day of these LCPUFAs. Increased bleeding times are not necessarily of clinical significance, but it is certainly reasonable to treat it as a risk factor when a substance is consumed by a large and diverse population. Because of the potential safety concerns noted above and because there were not, at the time, any food oils or other products in the food supply containing significant amounts of added DHA and EPA, FDA examined more than 50 scientific reports on fish oil

that included data on bleeding times (The Mitre Corporation, 1989). These studies are reviewed in the cited Federal Register Document. The totality of the scientific evidence demonstrated that when consumption of fish oil is limited to 3 g/p/day or less of EPA and DHA, there was no significant risk from increased bleeding time beyond the normal range. The other noted adverse effects were also negligible at doses of 3 g or less. FDA therefore concluded that for levels of 3 g/p/day or less, the 2 polyunsaturated fatty acids (PUFAs) are GRAS.

FDA's conclusions have been more recently confirmed in studies reviewed by Kroes *et al*., (2003). In one 90-day clinical trial (Nelson *et al.*, 1997) with dietary supplementation of 6 g marine algal-derived DHA/day, no effect on bleeding time was observed.

Glycaemic Control - Some studies on non-insulin-dependent diabetics have reported increased plasma glucose when large amount of fish oils (4.5-8.0 g/p/d) were used in the diet. After examining the available studies, FDA concluded that the consumption of fish oils at 3 g/p/d of EPA and DHA had no clinically significant effect on glycaemic control, although higher amounts (4.5 g /p/d) remain of concern.

LDL Cholesterol - In 1997 FDA concluded that there seemed to be a trend toward increased LDL cholesterol values with increased fish oil consumption in all population subgroups in most studies, with the effects being greater and more reliable in populations with diabetes, cardiovascular disease, and hypertension. These effects occurred primarily at high levels of use and FDA concluded that, so long as the maximum use levels would be less than 3 g of DHA and EPA per day from all the authorized food categories, there would be no adverse effect on LDL cholesterol. Kroes *et al.* (2003) reviewed the clinical trials conducted up to 2001 and concluded that DHA provided in fish or marine-derived oils alone or in combination with DHA and/or EPA at intake up to 6 g DHA /person/day would not produce significant adverse effects on the parameters identified by FDA. In healthy subjects, dietary supplementation with up to 9.9 g DHA/day for periods of up to 13 weeks was well tolerated and was reported not to produce significant effects on LDL cholesterol levels. (Conquer and Holub, 1996.)

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