# Application for the Approval of DHA-Rich Algal Oil from *Schizochytrium* sp. (DHA-B) as a Novel Food Ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 Concerning Novel Foods and Novel Food Ingredients

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# Application for the Approval of DHA-Rich Algal Oil from *Schizochytrium* sp. (DHA-B) as a Novel Food Ingredient for Use under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 Concerning Novel Foods and Novel Food Ingredients

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## EXECUTIVE SUMMARY

Martek Biosciences Corporation [Martek, now DSM Nutritional Products (DSM)] has previously gained approval for docosahexaenoic acid (DHA)-rich oil produced from *Schizochytrium* sp. (hereinafter "DHA-S"), for general use as a nutritional ingredient in foods. Martek also has developed an improved strain, from another species of *Schizochytrium* microalgae. This strain produces an oil which contains DHA as in DHA-S, along with an eicosapentaenoic acid (EPA) content that is approximately half that of the DHA concentration. This DHA and EPA-rich oil from *Schizochytrium* sp. (hereafter "DHA-O") has a fatty acid profile that more closely represents that of other sources of long chain omega-3 oils. Martek has gained approval to market DHA-O as a novel food ingredient for similar categories to those currently approved for DHA-S, but with minor modifications to use levels to reflect recent developments in recommended daily intakes for DHA and EPA. Historically, since the mid-1990s Martek's DHA-rich oil from *Crypthecodinium cohnii* (DHASCO®) has been used safely in infant and follow-on formula. DHASCO® is now approved throughout the world as a source of DHA for infant and follow-on formula and is used by nearly all manufacturers.

DSM has now developed another strain of the *Schizochytrium* species (*i.e.*, the same species of *Schizochytrium* sp. from the production strain for which DHA-O has been derived) to produce a new oil which is rich in DHA and is primarily being targeted as a replacement/alternative to DHASCO® in infant and follow-up formula but also as an alternative to DHA-S and DHA-O in conventional foods, Foods for Particular Nutritional Use (PARNUTS), and food supplements. This new DHA-rich microalgal oil is given the abbreviation "DHA-B".

DSM is hereby presenting its application for the approval of DHA-B as a novel food ingredient under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients* (European Parliament and Council of the European Union, 1997)[1]. Under Article 1, point 2, DHA-B would be classified under group "(d) foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae".

[1] (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090807:EN:PDF)

The specification of DHA-B is well-defined with the principal composition being not less than 35% DHA. Oxidative stability is assured by the inclusion of acid value and peroxide value and other contaminants are confirmed by extensive independent analyses. Detailed fatty acid and sterols analyses reveal a profile of DHA similar to those of DHA-S and DHASCO® and with no new components that are not already present in the diet.

The production of DHA-B is tightly controlled using standard fermentation, recovery, and purification techniques. Aqueous separation allows the use of a solvent-free production process, which is considered an important goal for the infant and follow-on formula industry. Safe, suitable and approved antioxidants are used and, for commercial reasons, DHA content may be standardised using high oleic sunflower oil.

The proposed use of DHA-B in infant formula and follow-on formula would be exactly as currently used for current sources of DHA and as regulated under Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC (Commission of the European Communities, 2006a)[2] Annex I Section 5.7 (infant formula) and Annex II, Section 4.7 (follow-on formula). Specifically:

"Long-chain (20 and 22 carbon atoms) polyunsaturated fatty acids (LCP) may be added. In that case their content shall not exceed:

- 1% of the total fat content for n-3 LCP, and
- 2% of the total fat content for n-6 LCP (1% of the total fat content for arachidonic acid (20:4 n-6))

The eicosapentaenoic acid (20:5 n-3) content shall not exceed that of docosahexaenoic (22:6 n-3) acid content.

The docosahexaenoic acid (22:6 n-3) content shall not exceed that of n-6 LCP."

The other proposed uses of DHA-B are identical to currently approved and proposed uses for DHA-O in the EU. As such, intakes of DHA-B are anticipated to be similar to DHA-S, DHA-O, DHASCO®, and to fish oil(s).

In addition to the extensive safety database already available on *Schizochytrium* sp. algal biomass, on DHA-S, DHA-O, and on fish oil, DSM has conducted supporting confirmatory pre-clinical studies on DHA-B, which include a 90-day rat study preceded by an *in utero* phase and a suite of genotoxicity studies. These studies follow the protocols that have been accepted previously for the approval of novel arachidonic acid rich oils for use in infant and follow-on formula. Results of these studies show no adverse effects at the maximum doses/concentrations tested. In the 90-day rat study, the no-observed-adverse-effect level (NOAEL) for DHA-B was equivalent to 3,278.9 and 3,788.4 mg/kg body weight/day for male and female rats respectively, the highest doses tested. For infants (5 kg body weight), this is

[2] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:401:0001:0033:EN:PDF

equivalent to 18.9 g per day of DHA-B. For a 70 kg adult this equates to approximately 265 g per person per day of DHA-B.

The absence of significant levels of protein in DHA-B and extensive history of safe consumption of DHASCO® and DHA-S indicate there is no significant risk for allergenicity. DHA-B is therefore proposed as a safe and suitable vegetarian and sustainably produced alternative to fish oil for use in infant and follow-on formula and in foods, PARNUTS and food supplement products as a source of the important LCP DHA.

# ADMINISTRATIVE DETAILS

## Name and Contact Details for Correspondence

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For the purpose of regulatory filings DSM Nutritional Products considers the marked specific data herein to be proprietary.

# INTRODUCTION

Martek Biosciences Corporation [Martek, now DSM Nutritional Products (DSM)] has previously gained approval for docosahexaenoic acid (DHA)-rich oil produced from *Schizochytrium* sp. (hereinafter "DHA-S"), for general use as a nutritional ingredient in foods. Under *Commission Decision of 5 June 2003 authorising the placing on the market of oil rich in DHA 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), DHA-S was authorised for use in the foods outlined in Table 1.

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

| Proposed Food Category Use Groups | 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 |

DHA = docosahexaenoic acid

In December 2007, Martek applied for additional use categories for DHA-S, which resulted in the following additional approval: *2009/778/EC Commission Decision of 22 October 2009 concerning the extension of uses of algal oil from the micro-algae Schizochytrium sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council* (Commission of the European Communities, 2009). The additional authorised uses for DHA-S under this decision (as detailed in its Annex) are reproduced in Table 2.

**Table 2 Authorised Uses of DHA-rich Algal Oil (DHA-S) Pursuant to Decision 2009/778/EC**

| Proposed Food Category Use Groups | Maximum Use Level of DHA |
|---|---|
| Bakery products (Breads and rolls) | 200 mg/100 g |
| Cereal bars | 500 mg/100 g |
| Non-alcoholic beverages (including milk based beverages) | 60 mg/100 mL |

DHA = docosahexaenoic acid

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

In December 2010, Martek made a further application for approval under novel foods for an improved strain from another species of *Schizochytrium* microalgae. This strain produces an oil which contains a similar amount of DHA as in DHA-S, along with an eicosapentaenoic acid (EPA) content that is approximately half that of the DHA concentration. This DHA and EPA-rich oil from *Schizochytrium* sp. (hereafter called DHA-O) has a fatty acid profile that more closely represents that of common sources of long chain omega-3 oils. Martek applied to market DHA-O for similar categories to those currently approved for DHA-S, but with minor modifications to use levels to reflect recent developments in recommended daily intakes for DHA and EPA. Approval for uses in biscuits (cookies) and cooking oils also were sought, along with clarification of the Foods for Particular Nutritional Use (PARNUTS) categories approved to include baby foods (but not infant and follow-on formula). On July 6, 2012, DHA-O was authorised for uses listed in Table 3.

**Table 3 Authorised Uses of DHA and EPA-rich Algal Oil (DHA-O) (ACNFP, 2012a)**

| Proposed Food Category Use Groups | Maximum Use Level (mg EPA+DHA/100 g) Unless Otherwise Stated |
|---|---|
| Food Supplements | 250 mg per daily dose as recommended by the manufacturer for normal population; 450 mg per daily dose as recommended by the manufacturer for pregnant and lactating women |
| Foods intended for use in energy-restricted diets for weight reduction | 250 mg/meal replacement |
| Other foods for particular nutritional uses (PARNUTS), as defined in Directive 2009/39/EC (European Parliament and the Council of the European Union, 2009) excluding infant and follow on formula | 200 |
| Bakery Products, Breads and Rolls | 200 |
| Breakfast Cereals | 500 |
| Cooking Fats | 360 |
| Dairy products except drinks | 600 mg/100 g for cheese; 200 mg/100 g for soy and imitation milk products (excluding drinks) |
| Dairy Products | 600 mg/100 g for cheese; 200 mg/100 g for milk products (including milk, fromage frais, and yogurt products) excluding drinks |
| Non-alcoholic beverages (including dairy analogue and milk-based drinks) | 80 |
| Nutrition Bars | 500 |
| Spreadable Fat and Dressings | 600 |

DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

In early 2013 an extension of the uses of DHA and EPA-rich oil from microalgae *Schizochytrium* sp. was submitted to the UK on the basis of recent scientific opinions on DHA and EPA health claims related to triglyceride and blood pressure maintenance published by the European Food Safety Authority (EFSA). Consequently, the maximum level of DHA + EPA in food supplements was proposed to be increased to 3,000 mg per daily dose, as recommended by the manufacturer, 450 mg per daily dose for food

supplements for pregnant and lactating women, 250 mg per daily servings for meal replacements, and the addition of sweet biscuits to the baked goods category.

DHA already is approved for use as a long chain polyunsaturated fatty acid (LC PUFA) in infant and follow-on formula as per the Commission Directive 2006/141/EC (Commission of the European Communities, 2006a). Under Annex IV of the Directive, nutrition claims may be made on infant formula containing DHA at not less than 0.2% of the total fatty acid content. There are two predominant sources of DHA that are not considered novel: fish oil (usually tuna oil), and DHASCO®. DHASCO® (also developed by Martek) is a DHA-rich oil produced from *Crypthecodinium cohnii*.

DSM has now developed another strain of the *Schizochytrium* species (*i.e.*, the same species of *Schizochytrium* sp. from which the production strain for DHA-O was derived) to produce a new oil which is rich in DHA and is primarily being targeted as a replacement/ alternative to DHASCO® and fish oil in infant and follow-up formula, but also as an alternative to DHA-S and DHA-O in conventional foods, PARNUTS, and food supplements. This new DHA-rich microalgal oil is given the abbreviation "DHA-B".

A summary of the DHA- and EPA-rich oils that are currently marketed or considered for approval as a novel food is presented in the table below.

**Table 4 Summary of DHA- and/or EPA-rich oils**

| Name | Description | Authorisation |
|---|---|---|
| Fish oil | Fish oil | Not novel |
| DHASCO® | DHA-rich single cell oil produced from *Crypthecodinium cohnii* | Not novel |
| DHA-S | DHA-rich oil produced from *Schizochytrium* sp. | 2003/427/EC; 2009/778/EC (Commission of the European Communities, 2003, 2009) |
| DHA-O | DHA and EPA-rich oil produced from *Schizochytrium* sp. | ACNFP (2012a,b) |
| DHA-B | DHA-rich oil produced from *Schizochytrium* sp. | Current dossier |

ACNFP = Advisory Committee on Novel Foods and Processes; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

As per Regulation (EC) No 258/97, DHA-B would be classified under "(d) foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae". Following Recommendation 97/618/EC (Commission of the European Communities, 1997), DHA-B also belongs to Class 2 "complex novel food from non-genetically modified sources" and sub-class (2) "the source of the novel food has no history of food use in the Community".

The dossier presented herein follows the structured sections which are required to establish the safety of a Class 2(2) novel food ingredient:

I Specification of the novel food
II Effect of the production process applied to the novel food
III History of the organism used as the source of the novel food
IX Anticipated intake/extent of use of the novel food
X Information from previous human exposure to the novel food or its source

XI Nutritional information on the novel food
XII Microbiological information on the novel food
XIII Toxicological information on the novel food

Sections IV to VIII are not applicable to DHA-B, as no genetic modification (GM) is involved in the production of the ingredient.

We have included Section IX because there are a number of similar oils already with a history of consumption.

A glossary is provided at the end of the document to explain the abbreviated terms referred to in the dossier.

# I SPECIFICATION OF THE NOVEL FOOD

Based on the Commission Recommendation 97/618/EC structured schemes the following information must be provided pertaining to the specification of the novel food (Commission of the European Communities, 1997):

a. “Is there an appropriate specification (including species, taxonomy *etc*. for living organisms) to ensure that the novel food marketed is the same as that evaluated?”

b. “Is the information representative of the novel food when produced on a commercial scale?”

c. “…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?”

These points are addressed in the section that follows.

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

The product specifications for DHA-B are presented in Table I.A-1 below. Production lots of DHA-B may be standardised to approximately 40% DHA prior to packaging. This is accomplished by using a safe and suitable vegetable oil, specifically high oleic sunflower oil (HOSO).

**Table I.A-1 Specifications for DHA-B**

| Parameter | Specification | Test Method |
|---|---|---|
| Free fatty acids (%) | Not more than 0.4 | AOCS Ca 5a-40 |
| Peroxide value (meq/kg) | Not more than 5.0 | AOCS Cd 8-53 |
| Unsaponifiables (%) | Not more than 3.5 | AOCS Ca 6b-53 |
| DHA (%) | Not less than 35 | AOCS Ce 1b-89 |
| Trans fatty acids (%) | Not more than 2.0 | AOCS Cd 14-95 |
| Arsenic (mg/kg) | Not more than 0.1 | AOCS Ca 17-01 |
| Cadmium (mg/kg) | Not more than 0.1 | AOCS Ca 17-01 |
| Copper (mg/kg) | Not more than 0.1 | AOCS Ca 17-01 |
| Lead (mg/kg) | Not more than 0.1 | AOCS Ca 17-01 |
| Mercury (mg/kg) | Not more than 0.4 | AOAC 977.15 |

AOCS = American Oil Chemists' Society; DHA = docosahexaenoic acid.

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

DHA-B is a complex triglyceride oil which contains a number of fatty acids, predominantly DHA. A series of batch analyses has been conducted to ensure consistency between production lots of DHA-B. The following sections summarise data on the specification parameters, fatty acid profile, and unsaponifiables profile to support that the manufacturing process produces a consistent product compliant with product specifications established by DSM.

### I.B.1 Compositional Information

Compositional data for 3 batches each of DHA-B are provided in Table I.B.1-1 below. See Appendix 1 for reports of analysis and for details on the method of analysis. The analytical data demonstrate that the ingredient complies with the product specifications, and is comparable to those values obtained from recent batches of DHA-S for reference.

The level of lead in each batch of DHA-B is lower than the maximum level established for fats and oils, including milk fat, established in Commission Regulation (EC) No 1881/2006 (Commission of the European Communities, 2006b).

| Table I.B.I-1 Results of Quality Control Testing for DHA-B (and Compared to DHA-S and DHA-O) | | | | | | |
|---|---|---|---|---|---|---|
| **Tests** | **Spec.** | **DHA-B Batch No** | | | **DHA-S REFERENCE ONLY** | **DHA-O REFERENCE ONLY** |
| | | **R223** | **080000 6586** | **080000 6592** | **Mean of 2 batches[1]** | **Mean of 3 batches[2]** |
| Free fatty acids (%) | Not more than 0.4 | 0.07 | 0.05 | 0.07 | N/A | N/A |
| Peroxide value (meq/kg) | Not more than 5.0 | 0.35 | ND | ND | 0.33 | 2.5 |
| Unsaponifiables (%) | Not more than 3.5 | 0.97 | 0.96 | 0.78 | 1.76 | 1.13 |
| DHA (%) | Not less than 35 | 44.35 | 42.65 | 41.23 | 45.8 | 33.7 |
| Trans fatty acids (%) | Not more than 2.0 | <1.0 | <1.0 | <1.0 | <2.0 | <2.0 |
| Arsenic (mg/kg) | Not more than 0.1 | <0.1 | <0.1 | <0.1 | <0.2 | <0.2 |
| Cadmium (mg/kg) | Not more than 0.1 | <0.1 | <0.1 | <0.1 | <0.04 | N/A |
| Copper (mg/kg) | Not more than 0.1 | <0.02 | <0.02 | <0.02 | <0.05 | <0.02 |
| Lead (mg/kg)[a] | Not more than 0.1 | <0.1 | <0.1 | <0.1 | <0.20 | <0.01 |
| Mercury (mg/kg) | Not more than 0.4 | <0.01 | <0.01 | <0.01 | <0.20 | <0.04 |

DHA = docosahexaenoic acid; N/A = not available; ND = not detected; Spec.= specifications.
[a] Meets limits set for "Fats and oils, including milk fat" of 0.10mg/kg wet weight, set in *Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs* (Commission of the European Communities, 2006b)[3]
[1] Calculated as a mean of batch numbers 95-5913 and 95-5767F
[2] Calculated as a mean of batch numbers 98-5807, 98-5814E, and 98-5828E.

## I.B.2 Stability of the Novel Food Ingredient

Quality assurance personnel routinely monitor the stability of oils at DSM. Summaries of the results of stability testing of DHA-B under frozen and room temperature storage conditions are presented in Table I.B.2-1. The results demonstrate that DHA-B conforms to specifications for DHA concentration and peroxide value for up to 12 months. Inclusion of testing under frozen conditions represents the range of conditions to which DHA-B will be exposed to during shipment and storage. The results demonstrate that DHA-B conforms to specifications for DHA concentration and peroxide value for up to 12 months.

[3] (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF)

| Table I.B.2-1 Stability of DHA-B under frozen storage conditions | | | | |
|---|---|---|---|---|
| **Lot Number** | **Specifications** | **Time (months)** | | |
| | | **0** | **6** | **12** |
| **DHA (mg/g)** | | | | |
| 9800005999 | Min. 35 | 40.7 | 42.5 | 40.1 |
| 0800006592 | | 38.5 | N/A | 40.9 |
| **Peroxide Value (meq/kg)** | | | | |
| 9800005999 | Max. 5 | <0.1 | <0.1 | <0.1 |
| 0800006592 | | <0.1 | N/A | <0.1 |

DHA = docosahexaenoic acid; N/A = not available.

## I.B.3 Additional Compositional Analysis

### *I.B.3.1 Heavy Metals*

Additional compositional analyses include testing for heavy metals. The results of heavy metal analysis for 3 non-consecutive lots of DHA-B are summarized in Table I.B.3.1-1. Certificates of analyses are provided in Appendix 1.

| Table I.B.3.1-1 Additional Quality Control Testing for DHA-B (and Compared to DHA-S and O) | | | | | |
|---|---|---|---|---|---|
| **Tests** | **DHA-B Batch No** | | | **DHA-S REFERENCE ONLY** | **DHA-O REFERENCE ONLY** |
| | **R223** | **080000 6586** | **080000 6592** | **Mean of 2 batches[1]** | **Mean of 3 batches[2]** |
| Chromium (mg/kg) | <0.1 | <0.1 | <0.1 | N/A | N/A |
| Iron (mg/kg) | 0.04 | 0.02 | 0.03 | 0.15/ND | 0.02 |
| Manganese | <0.01 | <0.01 | <0.01 | N/A | N/A |
| Molybdenum (mg/kg) | <0.05 | <0.05 | <0.05 | N/A | N/A |
| Nickel (mg/kg) | <0.1 | <0.1 | <0.1 | N/A | N/A |
| Phosphorus(mg/kg) | 15 | 21 | 20 | N/A | N/A |
| Silicon (mg/kg) | 2 | 1 | <1 | N/A | N/A |
| Sulphur (mg/kg) | 7 | 3 | 8 | N/A | N/A |

ND, not detected; N/A, not available.
[1] Calculated as a mean of batch numbers 95-5913 and 95-5767F
[2] Calculated as a mean of batch numbers 98-5807, 98-5814E, and 98-5828E.

### *I.B.3.2 Fatty Acid Profile*

The fatty acid profiles for 3 batches each of DHA-B are presented in Table I.B.3.2-1 below. Analysis was conducted at Eurofins Central Analytical Laboratories (Metairie, LA). Certificates of Analysis are provided in Appendix 1. All of the fatty acids detected are present already in the diet from a variety of vegetable and animal sources.

| Table I.B.3.2-1 Fatty Acid Composition of DHA-B (and Compared to DHA-S) | | | | |
|---|---|---|---|---|
| **Fatty Acid Content mg free fatty acids (% w/w oil) Oil** | **DHA-B Batch Number** | | | **DHA-S FOR REFERENCE ONLY** |
| | **R223** | **080000 6586** | **080000 6592** | **Mean of 2 batches[1]** |
| 14:0 Myristic | 1.30 | 1.15 | 1.04 | 7.24 |
| 14:1 Myristoleic | ND | ND | ND | 0.07 |
| 15:0 Pentadecanoic | 0.25 | 0.23 | 0.23 | 0.25 |
| 16:0 Palmitic | 13.95 | 13.06 | 13.43 | 16.77 |
| 17.0 Heptadecanoic (Margaric) | ND | ND | ND | 0.15 |
| 18:0 Stearic | 1.64 | 1.68 | 1.72 | 0.41 |
| 18:1(n-9)* Oleic | 24.52 | 26.47 | 27.96 | 1.67 |
| 18:1(n-7)* cis-vaccenic | 0.22 | 0.27 | 0.28 | 0.12 |
| 18:2 Linoleic | 2.05 | 2.15 | 2.01 | 0.31 |
| 18:4 Octadecatetraenoic | N/A | N/A | N/A | 0.28 |
| 20:0 Eicosanoic (Arachidic) | 0.32 | 0.32 | 0.33 | 0.07 |
| 20.1 Eicosenoic acid | 0.14 | 0.14 | 0.14 | 0.00 |
| 20:3(n-6) Eicosatrienoic | ND | ND | ND | 0.32 |
| 20:4(n-6) Arachidonic | 0.67 | 0.7 | 0.63 | 1.02 |
| 20:3(n-3) Eicosatrienoic | ND | ND | ND | 0.10 |
| 20:4(n-3) Eicosatetraenoic | N/A | N/A | N/A | 0.70 |
| 20:5(n-3) Eicosapentaenoic | 5.90 | 6.10 | 6.10 | 0.93 |
| 22:0 Docosanoic (Behenic) | 0.32 | 0.38 | 0.39 | 0.06 |
| 22:1(n-11) Cetoleic | N/A | N/A | N/A | 0.00 |
| 22:4(n-6) Docosatetraenoic | N/A | N/A | N/A | 0.08 |
| 22:5(n-6) Docosapentaenoic | 2.63 | 2.65 | 2.26 | 13.56 |
| 22:5(n-3) Docosapentaenoic | 0.55 | 0.63 | 0.95 | 0.41 |
| 24:0 Tetracosanoic (Lignoceric) | 0.12 | 0.14 | 0.15 | 0.20 |
| 22:6(n-3) Docosahexaneoic | 44.35 | 42.65 | 41.23 | 34.80 |
| Minor components (individual fatty acids <0.005 mg FFA/g) | 0.47 | 0.6 | 0.54 | 0.13 |
| **TOTAL FATTY ACIDS** | **99.40** | **99.32** | **99.39** | 79.65 |

FFA = free fatty acids; ND, not detected; N/A, not available.
* the source of oleic acid is from the high oleic sunflower oil.
[1] Calculated as the mean of batch numbers 95-5913 and 95-5767F

The DHA-B oil profile (DHA:EPA ratio) is very similar to that of other algal oils and fish oils that are used in infant formula, *e.g.*, tuna oil. Table I.B.2.2-2 provides a comparison of the oil profile of DHA-B to DHASCO®, DHA-S, and tuna oil. The profile of sunflower oil is provided as this is the predominant vegetable oil used to standardise DHA-B.

| Table I.B.3.2-2 Fatty Acid Composition of DHA-B (*Schizochytrium* sp.), and Comparable Oils as Percentage (%) of Total Fatty Acids | | | | |
|---|---|---|---|---|
| **Fatty Acid** | **DHA-B[1]** (***Schizochytrium* sp.**) | **DHA-S[2]** (***Schizochytrium* sp.**) | **Tuna Oil[3]** | **Sunflower oil** |
| 12:0 | N/A | N/A | N/A | N/A |
| 14:0 | 1.2 | 9.3 | 3 | 0.1 |
| 16:0 | 13.6 | 21 | 22 | 5.9 |
| 16:1 | N/A | 0.3 | 3 | N/A |
| 18:0 | 1.69 | 0.5 | 6 | 4.5 |
| 18:1:00 (total n-7+9) | 26.7 | 2 | 21 | 19.5 |
| 18:2 | 2.1 | 0.4 | 1 | 65.7 |
| 18:3 | N/A | 0.8 | 1 | 0.4 |
| 18:4 | ND | 0.3 | 1.9 | N/A |
| 20:3 (n-3+6) | ND | 0.5 | N/A | N/A |
| 20:4 | ND | 1.2 | 2 | N/A |
| 20:5 | 6.1 | 1 | 6 | N/A |
| 22:4 | ND | 0.1 | N/A | N/A |
| 22:5 (total n-3+6) | 3.2 | 17 | 2 | N/A |
| 22:6 (n-3) | 43.0 | 43 | 22 | N/A |

ND = not detected; N/A = not available.
[1] Average of 3 batches of DHA-B corrected to % of total fatty acids (results are higher than those in Table I.B.2.2-1, which are expressed as % of total oil)
[2] Average of 3 recent batches of DHA-S
[3] Handbook of Lipid Research

### *I.B.3.3 Unsaponifiables*

The unsaponifiable content for 3 batches each of DHA-B is presented in Table I.B.3.3-1 below. Testing was also carried out at Eurofins Central Analytical Laboratories and the certificates of analysis presented in Appendix 1. All of the sterols detected are present already in the diet from a variety of vegetable and animal sources.

| Table I.B.3.3-1 Unsaponifiable Composition of DHA-B (and Compared to DHA-S, DHA-O, and DHASCO®) | | | | | |
|---|---|---|---|---|---|
| **Sterol (% w/w of total oil)** | **DHA-B** | | | **DHA-S FOR REFERENCE ONLY** | **DHA-O FOR REFERENCE ONLY** |
| | **Lot No. 08-6530** | **Lot No. 08-6586** | **Lot No. 08-6592** | **Mean of 2 batches[1]** | **Mean of 3 batches[2]** |
| Cholesterol | 0.009 | 0.073 | 0.062 | 0.140 | 0.182 |
| Cholestanol | NA | NA | NA | 0.000 | 0.000 |
| Brassicasterol | 0.007 | 0.009 | 0.006 | 0.035 | 0.008 |
| 24-Methylene cholesterol | 0.007 | 0.010 | 0.007 | 0.006 | 0.006 |
| Campesterol | 0.011 | 0.009 | 0.010 | 0.004 | 0.005 |
| Campestanol | 0.007 | 0.009 | 0.006 | 0.000 | 0.000 |
| Stigmasterol | 0.360 | 0.309 | 0.359 | 0.252 | 0.505 |
| Δ-7-Campesterol | 0.002 | 0.002 | 0.002 | 0.022 | 0.002 |
| Δ-5,23-stigmastadienol | 0.006 | 0.004 | 0.004 | 0.108 | 0.003 |
| Chlerosterol | 0.009 | 0.008 | 0.009 | 0.004 | 0.015 |
| β-sitosterol | 0.057 | 0.058 | 0.059 | 0.043 | 0.033 |
| Sitostanol | 0.003 | 0.003 | 0.003 | 0.016 | 0.001 |
| Δ-5-avenasterol | 0.010 | 0.009 | 0.016 | 0.007 | 0.008 |
| Δ-5,24-stigmastadienol | 0.002 | 0.003 | 0.002 | 0.014 | 0.003 |
| Δ-7-stigmastenol | 0.010 | 0.010 | 0.010 | 0.054 | 0.003 |
| Δ-7-avenasterol | 0.002 | 0.002 | 0.002 | 0.020 | 0.001 |
| **TOTAL STEROLS (% w/w of total oil)** | 0.56 | 0.51 | 0.55 | 0.725 | 0.775 |

NA = not available.
[1] Calculated as the mean of batch numbers 95-5913 and 95-5767F.
[2] Calculated as the mean of batch numbers 98-5807, 98-5814E, and 98-5828E.

## I.B.4 Is appropriate analytical information available on the potential toxic inherent constituents, external contaminants and nutrients?

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

- Dioxins – See Section I.B.4.1 below
- Polycylic aromatic hydrocarbons (PAHs) – See Section I.B.4.2 below
- Pesticides – See Section I.B.4.3 below
- Acrylamide – See Section I.B.4.5 below
- Algal Toxins – See Section XII below
- Microorganisms – See Section XII below

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

### *I.B.4.1 Dioxins*

The analytical results for dioxins and dioxin-like polychlorinated biphenyls (PCBs) in DHA-B are presented in Appendix 2. Full certificates of analysis and details of test methods also are provided in Appendix 2. All analytical data meet published acceptance criteria for polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) and equivalent criteria for PCBs. The method used has been validated and published after peer review. Each batch of samples analysed incorporates at least one of several reference materials (RMs), for which results are compared with certified or assigned data and laboratory performance (indicative) data. Results for the batch RM must fall within the acceptable range. Each batch of samples analysed includes a full reagent blank extract. The contribution from the batch blank should be negligible. The analytical performance of the laboratory in international inter-comparison studies, using essentially the same method, has been adjudged to be acceptable or better.

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

The levels of dioxins in DHA-B are lower than the maximum levels established in Commission Regulation (EC) No 1881/2006 for vegetable oils and fats (*i.e.*, 0.75 pg/g fat for sum of dioxins and 1.25 pg/g fat for sum of dioxins and dioxin-like PCBs) and Commission Regulation (EC) No. 1259/2011 – European Commission, 2011a (*i.e.,* 40 ng/g for the sum of PCB28, PCB52, PCB1010, PCB138, PCB153, and PCB180 of 40 ng/g fat).

### *I.B.4.2 Polycyclic Aromatic Hydrocarbons (PAH)*

The analytical results for polycyclic aromatic hydrocarbons (PAH), including full details of test methodology and certificates of analysis, are provided in Appendix 2.

The levels of benzo(a)pyrene and the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluroanthene, and chrysene in DHA-B are lower than the maximum level established for oils and fat intended for direct human consumption or use as an ingredient in food, as per Commission Regulation (EU) No 835/2011 amending Regulation (EC) No 1881/2006 (European Commission, 2011b).

[4] (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF)

*I.B.4.3 Pesticides*

DHA-B is not an agricultural product, as the oil is not extracted from a plant source. As such, pesticide residues are not anticipated to be present in the oil. The results of comprehensive pesticide analyses conducted on a batch of DHA-B is summarised in Table I.B.4.3-1 and confirms the absence of pesticide residues in the oil. The certificate of analysis is provided in Appendix 2.

**Table I.B.4.3-1 Pesticide Analyses in DHA-B**

| **Pesticide** | **DHA-B Lot No. 08-6592** |
|---|---|
| **Organochlorine Pesticides** | |
| Benzene hexachloride (mg/kg) | < 0.01 |
| Alachlor (mg/kg) | < 0.02 |
| Aldrin (mg/kg) | < 0.01 |
| Benfluralin (mg/kg) | < 0.02 |
| Binfenox (mg/kg) | < 0.03 |
| Boscalid (mg/kg) | < 0.02 |
| Bromacil (mg/kg) | < 0.02 |
| Captafol (mg/kg) | < 0.01 |
| Captan (mg/kg) | < 0.02 |
| Chlordane (total) (mg/kg) | < 0.01 |
| Chlorfenapyr (mg/kg) | < 0.01 |
| Chlorobenzilate (mg/kg) | < 0.04 |
| Chlorthalonil (mg/kg) | < 0.02 |
| Cyanazine (mg/kg) | < 0.02 |
| Dacthal (chlorthal) (mg/kg) | < 0.02 |
| DDD (mg/kg) | < 0.01 |
| DDE (mg/kg) | < 0.01 |
| DDT (mg/kg) | < 0.01 |
| Dichlobenil (mg/kg) | < 0.01 |
| Dichlone (mg/kg) | < 0.05 |
| Dicloran (mg/kg) | < 0.01 |
| Dicofol (mg/kg) | < 0.02 |
| Dieldrin (mg/kg) | < 0.01 |
| Endosulfan beta (mg/kg) | < 0.01 |
| Endosulfan, alpha (mg/kg) | < 0.01 |
| Endosulfan-sulphate (mg/kg) | < 0.01 |
| Endrin (mg/kg) | < 0.01 |
| Ethalfluralin (mg/kg) | < 0.01 |
| Fenhexamid (mg/kg) | < 0.02 |
| Folpet (mg/kg) | < 0.02 |
| Hexachlorbenzene (mg/kg) | < 0.01 |
| Heptachlor (mg/kg) | < 0.01 |
| Heptachlor epoxide (mg/kg) | < 0.01 |
| Indoxacarb (mg/kg) | < 0.02 |

| Table I.B.4.3-1 Pesticide Analyses in DHA-B | |
|---|---|
| **Pesticide** | **DHA-B Lot No. 08-6592** |
| Iprodione (mg/kg) | < 0.02 |
| Lidane (mg/kg) | < 0.01 |
| Linuron (mg/kg) | < 0.10 |
| Methoxychlor (mg/kg) | < 0.02 |
| Metribuzin (mg/kg) | < 0.01 |
| Mirex (mg/kg) | < 0.01 |
| Myclobutanil (mg/kg) | < 0.01 |
| Oxadiazon (mg/kg) | < 0.02 |
| Oxyfluorfen (mg/kg) | < 0.01 |
| Pendimethalin (mg/kg) | < 0.01 |
| Pentachloraniline (mg/kg) | < 0.01 |
| Pentachloronitrobenzene (mg/kg) | < 0.01 |
| Perthane (mg/kg) | < 0.01 |
| Procymidone (mg/kg) | < 0.01 |
| Profuralin (mg/kg) | < 0.02 |
| Pronamide (mg/kg) | < 0.02 |
| Propanil (mg/kg) | < 0.01 |
| Pyrethrins (total) (mg/kg) | < 0.02 |
| Tetradifon (mg/kg) | < 0.02 |
| Toxaphene (campheclor) (mg/kg) | < 0.01 |
| Trifloxystrobin (mg/kg) | < 0.01 |
| Triflumizole (mg/kg) | < 0.01 |
| Trifluralin (mg/kg) | < 0.02 |
| Vegadex (mg/kg) | < 0.02 |
| Vinclozolin (mg/kg) | < 0.02 |
| **Organophosphorus Pesticides** | |
| Azinphos-methyl (mg/kg) | < 0.02 |
| Carbophenothion (mg/kg) | < 0.02 |
| Chlrofenvinphos (mg/kg) | < 0.02 |
| Chlorpyrifos (mg/kg) | < 0.02 |
| Coumaphos (mg/kg) | < 0.02 |
| Diazinon (mg/kg) | < 0.02 |
| Dibrom (naled) (mg/kg) | < 0.02 |
| Dicrotophos (mg/kg) | < 0.02 |
| Dimethoate (mg/kg) | < 0.02 |
| Disulfoton (mg/kg) | < 0.02 |
| EPN (mg/kg) | < 0.02 |
| Ethion (mg/kg) | < 0.02 |
| Ethoprop (mg/kg) | < 0.02 |
| Fenamiphos (mg/kg) | < 0.02 |
| Fenitrothion (mg/kg) | < 0.02 |
| Fenthion (mg/kg) | < 0.02 |
| Fonofos (mg/kg) | < 0.02 |

| Table I.B.4.3-1 Pesticide Analyses in DHA-B | |
|---|---|
| **Pesticide** | **DHA-B Lot No. 08-6592** |
| Isofenphos(mg/kg) | < 0.02 |
| Malathion (mg/kg) | < 0.02 |
| Methyl parathion (mg/kg) | < 0.02 |
| Mevinphos (mg/kg) | < 0.02 |
| Omethoate (mg/kg) | < 0.02 |
| Parathion (mg/kg) | < 0.02 |
| Phorate (mg/kg) | < 0.02 |
| Phosalone (mg/kg) | < 0.02 |
| Phosmet (mg/kg) | < 0.02 |
| Phosphamidon (mg/kg) | < 0.02 |
| Pirimiphos-methyl (mg/kg) | < 0.02 |
| Profenofos (mg/kg) | < 0.02 |
| Propetamphos (mg/kg) | < 0.02 |
| Ronnel (mg/kg) | < 0.02 |
| Sulprofos (mg/kg) | < 0.02 |
| Tetrachlorvinphos (mg/kg) | < 0.02 |
| Thionazin (mg/kg) | < 0.02 |
| **Pyrethroid Pesticides** | |
| Bifenthrin (mg/kg) | < 0.02 |
| Cyfluthrin (mg/kg) | < 0.02 |
| Cyhalothrin lambda (mg/kg) | < 0.02 |
| Cypermethrin (mg/kg) | < 0.02 |
| Deltamethrin (mg/kg) | < 0.02 |
| Esfenvalerate (mg/kg) | < 0.02 |
| Fenpropathrin (mg/kg) | < 0.02 |
| Fluvalinate (mg/kg) | < 0.02 |
| Permethrin (mg/kg) | < 0.02 |
| Tralomethrin (mg/kg) | < 0.02 |

DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; DDT = dichlorodiphenyltrichloroethane; EPN = O-ethyl O-(4-nitrophenyl) phenylphosphonothioate.

### *I.B.4.4 Acrylamide*

Results for acrylamide analysis are provided in Table I.B.4.4-1 and in Appendix 2. No maximum levels have been established and one would not expect there to be acrylamide formation from the process and results are below the detection limit for all batches. The batch sample was extracted with hot water. The aqueous extract was brominated, solvent extracted, concentrated then analysed by gas chromatography with mass spectrometry detection (GC-MS). $^{13}$C-Acrylamide was used as an internal standard, which gives an implicit correction for recovery. This method is based on a method validated in-house[5] and

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

widely used by international researchers. There are currently no EU regulations governing acrylamide analysis.

**Table I.B.4.4-1 Acrylamide Levels in DHA-B**

| | Maximum Limit | DHA-B Lot No. | | |
|---|---|---|---|---|
| | | 08-6530 | 08-6586 | 08-6592 |
| Acrylamide level (mcg/kg) | N/A | <50 | <50 | <50 |

N/A = not available.

# II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees the following questions must answered pertaining to the production process of the novel food (Commission of the European Communities, 1997):

a. “Does the novel food undergo a production process?”

b. “Is there a history of use of the production process for the food?” If no, “does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?”

c. “Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?”

d. “Are the means identified for controlling the process to ensure that the novel food complies with its specification?”

e. “Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?”

f. “After processing is the novel food likely to contain microorganisms of adverse public health significance?”

These points are addressed in the section that follows.

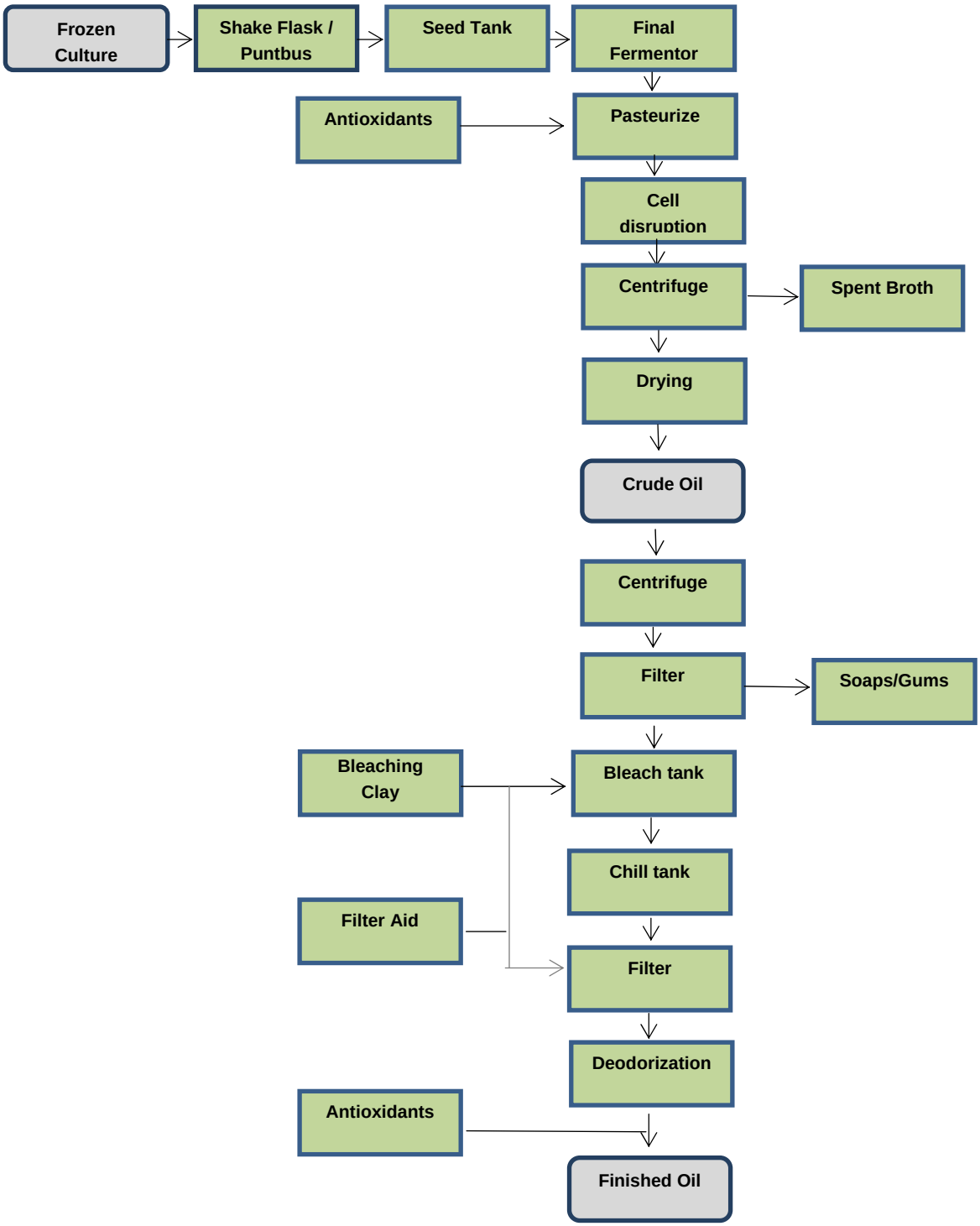
## II.A Does the Novel Food undergo a production process?

The manufacturing process used to produce and recover DHA-B is described in the following section.

### II.A.1 Overview of the Manufacturing Process

The production process of DHA-B consists of 3 distinct stages (contained fermentation, oil recovery, and oil purification), which are described in detail in Sections II.A.1.1 to II.A.1.3 below. DHA-B is produced using an aerobic fermentation process to cultivate a *Schizochytrium* species to produce high quality omega-3 algal oil. The DHA-B process uses a wild-type *Schizochytrium* organism (see Section III for more detail on the source organism) that has been through a classical strain screening program to obtain the production strain. The omega-3 oil accumulates within the cells primarily as a triglyceride. The oil recovery process involves an aqueous extraction process (see Section II.A.1.2). A general overview of the manufacturing process for DHA-B is represented in Figure II.A.1-1 below.

**Figure II.A.1-1 General Overview of the Manufacturing Process of DHA-B**



*II.A.1.1 Contained Fermentation*

DHA-B is produced *via* a self-contained fermentation process. This fermentation process uses media containing carbon and nitrogen sources, bulk nutrients, trace minerals, and vitamins. This is a fed-batch process for carbon and nitrogen, where a portion of the carbon and nitrogen is added during the initial fill along with the bulk nutrients, trace metals and vitamins. The remaining portion of carbon and nitrogen is added throughout the fermentation.

*II.A.1.2 Oil Recovery and Purification*

Crude oil is obtained from the fermentation broth *via* aqueous extraction, using standard operating procedures after the broth is first pasteurised. Following cell disruption and breakage, the resultant material is centrifuged and dried to yield crude oil. The crude oil is treated using standard refining, bleaching, chill filtration and deodorizing operating procedures to produce the DHA-B oil.

The finished oil is standardized to 40% (by weight).

**II.A.2 Is there a history of use of the production process for the food?**

The production process of DHA-B uses unit operations found in traditional vegetable oil processing.

**II.A.3 Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from the use of the process?**

Details of the analysis of contaminants are provided in Section I above. The absence of algal toxins is specifically discussed in Section III.D.1. The absence of microbiological contamination is confirmed in Section XII.

**II.A.4 Are the means identified for controlling the process to ensure that the novel food complies with its specification?**

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

### II.A.5 Has the process the potential to alter the levels of Substances with an adverse effect on public health, in the Novel Food?

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

### II.A.6 After processing is the Novel Food likely to contain micro-organisms of adverse public health significance?

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

# III HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD

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

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

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

c. “Is the source organism characterised?”

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

These points are addressed in the section that follows.

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

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

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

The source of DHA-B is not derived from GM technology.

## III.C Is the source organism characterised?

*Schizochytrium* sp. microalgae is obtained using a classic screening program that utilised well-accepted techniques commonly employed in industrial strain improvement programs.

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

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

### *III.C.1 Production Strain for DHA-B*

The microalgae was isolated from the Intertidal coastline, west coast of U.S. near Seattle, in 2007. Preliminary examination of the organism indicated the microalgae is a thraustochytrid. Subsequent detailed examination of the microalgae indicated that it possessed the definitive characteristics of the genus *Schizochytrium* and was a previously unpublished member of that genus.

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

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

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

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

### III.D.1 Algal Toxins

It has long been known that some species of microalgae produce toxic substances. The occurrence of these toxins in microalgae has been reviewed extensively (Collins *et al.*, 1981; Tu, 1988; Granéli and Moreira, 1990). All of the species known to produce toxins are found in just 6 of the approximately 76 known orders of microalgae and algae-like microorganisms. The majority of toxins produced in microalgae occur in the species of dinoflagellates (kingdom Protozoa, phylum Dinophyta) and blue-green algae (kingdom Eubacteria, phylum Cyanobacteria).

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

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

#### *III.D.1.1 Domoic acid*

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

In the phylum Heterokonta, the thraustochytrids are in a separate subphylum and class from the diatoms, so one would not expect to find domoic acid in *Schizochytrium* sp. Confirmatory testing of *Schizochytrium* sp. dried microalgae for domoic acid using a domoic acid ELISA (Biosense) did not detect this compound.

The analysis report is presented in Appendix 3.

*III.D.1.2 Prymnesins*

The other toxins found in a member of the Chromista (Stramenopilia) are limited to two species of *Prymnesium* (*P. parvum* and *P. patelliferum*). These toxins (called prymnesins by some) exhibit a broad spectrum of activity including lethal effects on gill breathing animals, cytotoxic effects on erythrocytes, nucleated mammalian cells, protozoa and bacteria.

The major economic impact of prymnesin toxins for humans to date has been related to fish kills in aquaculture ponds (mostly occurring in Israel) and in coastal waters associated with intensive aquaculture production (Scandinavia). All gill breathing animals tested to date have proven sensitive to prymnesin toxins. As a result, a sensitive toxicity test for prymnesin toxins has been developed using nauplii of the brine shrimp Artemia (Larsen *et al.*, 1993). The $LC_{50}$ values for *Artemia* sp. in 24 hour exposures to toxic strains of *Prymnesium* sp. are only 3,000 to 5,000 cells/ml (Larsen *et al.*, 1993). Due to the unavailability of authentic standards *Schizochytrium* sp. dried microalgae have not been directly analyzed for the presence of prymnesin toxins. However, a bioassay for prymnesin has been developed (Vanhaecke *et al*., 1981) utilising *Artemia* nauplii as the test organism. The overall experiment conducted was based on two previously published papers (Granéli and Johansson, 2003; Houdan *et al.*, 2004). Results of this bioassay on *Schizochytrium* sp. dried microalgae indicate normal growth of Artemia culture, indicating the absence of prymnesin toxin.

The analysis report is presented in Appendix 3.

*III.D.1.3 Conclusions on Algal Toxins*

Based on existing published and unpublished scientific data, it is concluded that:

1) there are no known reports on toxic compounds produced by thraustochytrids;
2) most of the toxic compounds produced by microalgae are produced by bluegreen algae or dinoflagellates, and *Schizochytrium* sp. is in a separate kingdom from both of these types of microalgae;
3) the two toxic compounds known to be produced in the Chromista (to which *Schizochytrium* sp. belongs) are largely restricted to two genera (domoic acid in Pseudonitzschia and prymnesin in Prymnesium spp.) which are in a separate class and phylum, respectively, from the thraustochytrids;
4) chemical tests indicate that domoic acid is not present in *Schizochytrium* sp. microalgae;
5) biological assay for prymnesin toxin is negative; and
6) a subchronic dietary toxicity study in rats and a battery of cytotoxicity/mutagenicity tests have been completed with no effects attributed to algal toxins (see Section XIII).

# IX ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD

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

a. "Is there information on the anticipated uses of the novel food based on its properties?"

b. "Is there information to show anticipated intakes for groups predicted to be at risk?"

c. "Will introduction of the novel food be restricted geographically?"

d. "Will the novel food replace other foods in the diet?"

These points are addressed in the section that follows.

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

DHA-B is a close alternative to other currently used DHA sources used in infant formula, follow-on formula and conventional foods, and is from sustainable and vegetarian sources. It also is important to note at this point that there are clear limits to which such oils can be added to foods due to sensory and economic issues. Even without restrictions, there would be no realistic possibility that significant bolus doses could arise that would have any impact on safety.

In this application we wish to apply for the same uses and use-levels currently used for DHASCO® and fish oil (infant formula) and recently proposed[6] extension of uses approved for DHA-S and DHA-O. The proposed uses and use-levels are listed in Table IX.A-1.

**Table IX.A-1 Summary of the Individual Proposed Food Uses and Use-Levels for DHA from DHA-B in the EU**

| Food Category | Proposed Use-Level (mg DHA/100 g unless otherwise stated) | Status for DHA-S | Status for DHA-O |
|---|---|---|---|
| Food Supplements | 3,000 mg DHA + EPA per daily dose as recommended by the manufacturer | Proposed | Proposed |
| | 450 mg per daily dose as recommended by the manufacturer for pregnant and lactating women | Proposed | Approved |
| Foods intended for use in energy-restricted diets for weight reduction | 250 mg per meal replacement | Proposed | Approved |
| Other foods for particular nutritional uses (PARNUTS), as defined in Directive 2009/39/EC (European Parliament and the Council of the European Union, 2009) excluding infant and follow-on formula | 200 | Proposed (clarification/tidy only) | Approved |
| Dietary foods for special medical purposes | In accordance with the particular nutritional requirements of the persons for whom the products are intended | Approved | Approved |
| Bakery Products, Breads and Rolls, Sweet Biscuits | 200 | Proposed | Approved |
| | 200 | Approved | Approved |
| | 200 | Approved | Approved |
| Breakfast Cereals | 500 | Approved | Approved |
| | 500 | Approved | Approved |
| Cooking Fats | 360 | Proposed | Approved |

[6] http://acnfp.food.gov.uk/assess/fullapplics/dhaepaalgoil

| Table IX.A-1 Summary of the Individual Proposed Food Uses and Use-Levels for DHA from DHA-B in the EU | | | |
|---|---|---|---|
| **Food Category** | **Proposed Use-Level (mg DHA/100 g unless otherwise stated)** | **Status for DHA-S** | **Status for DHA-O** |
| Dairy Analogues (except drinks) | 600 | Approved | Approved |
| | 200 | | |
| Dairy Products (except milk-based drinks) | 600 | Approved | Approved |
| | 200 | | |
| Non-alcoholic Beverages (including dairy analogue and milk-based drinks) | 80 | Approved | Approved |
| | 80 | Approved | |
| | 80 | Approved | |
| | 80 | Approved | |
| | 80 | Approved | |
| | 80 | Approved | |
| Nutrition Bars | 500 | Approved | Approved |
| Spreadable Fats and Dressings | 600 | Approved | Approved |
| Food for Infants and Young Children | Used in accordance with Commission Directive 2006/141/EC[1] | | |
| | Used in accordance with Commission Directive 2006/141/EC[1] | | |
| | Used in accordance with Commission Directive 2006/125/EC[2] | | |

DHA = docosahexaenoic acid.
[1] The DHA content shall not exceed that of n-6 long chain polyunsaturated fatty acids in accordance with Commission Directive 2006/141/EC (Commission of the European Communities, 2006a).
[2] The lipid content shall not exceed 0.8 g/100 kJ in accordance with Commission Directive 2006/125/EC (Commission of the European Communities, 2006c).

### IX.A.1 Infant Formulae and Follow-On Formulae

DHA-B has been predominantly developed as an alternative or replacement to DHASCO® and fish oil which are currently used in infant formulae and follow-on formulae. The proposed use of DHA-B would be exactly as currently used for DHASCO® and fish oil and as regulated under Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC (Commission of the European Communities, 2006a)[7] Annex I Section 5.7 (infant formula) and Annex II, Section 4.7 (follow-on formula).

It should be noted that a health claim pertaining to DHA and normal visual development of infants has been authorised in the EU under Regulation (EC) 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). The conditions

[7] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:401:0001:0033:EN:PDF

of use of this claim are that “Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 100 mg of DHA. When the claim is used on follow-on formula, the food shall contain at least 0,3 % of the total fatty acids as DHA.”

### IX.A.2 Food Supplements

DHA-B is proposed to be used as an alternative to fish oil supplements. The main purpose of this proposed intake is to enable the daily advised intake of long chain omega-3 fatty acids of 250 mg per day for the general population (EFSA, 2010a).

However, EFSA recently issued 2 scientific opinions related to DHA and EPA and health claims related to triglyceride maintenance and blood pressure maintenance that recommend daily doses of between 2 and 3 g (EFSA, 2010b, 2011)

In support of such levels, on 27th July 2012 EFSA published its Scientific Opinion on the Tolerable Upper Intake Level of EPA, DHA, and docosapentaenoic acid (DPA), which concluded as follows:

“The Panel considers that supplemental intakes of EPA and DHA combined at doses up to 5 g/day …do not raise safety concerns for the adult population.” (EFSA, 2012)

The triglyceride and blood pressure claims have now been adopted into the Register, *via Commission Regulation (EU) No 536/2013 of 11 June 2013 amending Regulation (EU) No 432/2012 establishing a list of permitted health claims made on foods* (European Commission, 2013) “The claims shall not be used for foods targeting children” and “when the claim is used on food supplements and/or fortified foods information shall also be given to consumers not to exceed a supplemental daily intake of 5 g of EPA and DHA combined.

DSM recently proposed to extend the use of DHA-O to up to a maximum DHA and EPA content of 3,000 mg per daily dose as recommended by the manufacturer for normal population; 450 mg per daily dose as recommended by the manufacturer for pregnant and lactating women”. The same use level is sought for DHA-B. This use level does not exceed that of currently available fish oil supplements (*e.g.*, for vegetarians).

The conditions laid down for labelling and presentation under food supplements legislation would prevent involuntary excessive dosing. Specifically these conditions are laid down in Article 6, point 3 of *Directive 2002/46/EC on food supplements* (European Parliament and the Council of the European Union, 2002)[8], as follows:

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

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

[8] (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:183:0051:0057:EN:PDF)

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

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

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

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

### IX.A.3 Conventional Foods

DHA-B is proposed to be used as an alternative to DHA-S, DHA-O, or fish oils in conventional foods providing 80 to 600 mg DHA per 100 g food. These levels are consistent with levels already permitted (DHA-S and DHA-O).

Claims pertaining to DHA and maintenance of normal brain function and normal vision were authorised in the EU under Regulation (EC) 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). For both claims, the claim may be used only for food which contains at least 40 mg of DHA per 100 g and per 100 kcal. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 250 mg of DHA. The levels of DHA that will be provided from the proposed uses of DHA-B meet the conditions of use of the aforementioned authorised health claims.

### IX.A.4 Foods Intended for Use in Energy-Restricted Diets for Weight Reduction

The proposed maximum inclusion level of 250 mg reflects the reference daily advisory level for omega-3 polyunsaturated fatty acids (EFSA, 2010a). These products are controlled under the requirements of Commission Directive 96/8/EC of 26 February 1996 on foods intended for use in energy-restricted diets for weight reduction (Commission of the European Communities, 1996)[9] specifically with regard to the labelling and delivery of daily servings.

### IX.A.5 Other Foods for Particular Nutritional Uses (PARNUTS), as Defined in Directive 2009/39/EC (European Parliament and the Council of the European Union, 2009)

DHA-B is proposed to be used as a source of DHA in processed cereal-based foods and baby foods for infants and young children; foods intended to meet the expenditure of intense muscular effort, especially for sportsmen; foods for people with gluten intolerance; and miscellaneous PARNUTS under Article 11, as defined in Directive 2009/39/EC. Its use level of 200 mg/100g is in agreement with the reference daily advisory level for omega-3 polyunsaturated fatty acids (EFSA, 2010a).

[9] (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1996L0008:20070620:EN:PDF

## IX.B Consumption Estimates

As mentioned, DHA-B is proposed for use as a replacement or alternative to DHA-containing oils already permitted and /or proposed for use (DHASCO®, DHA-S, and DHA-O), at levels consistent with those already approved and/or proposed. As the proposed uses and use-levels of DHA-B are aimed to be harmonised to those recently approved/in process of approval for DHA-O (with the exception of infant formula and follow-on formula), the consumption estimates generated in support of the DHA-O novel food application are applicable to DHA-B.

Estimates of consumption of DHA from DHA-B were provided in the application for approval of the novel food dated December 15, 2010 (Appendix IV). Briefly, estimates for the intake of DHA-O were based on use-levels and food consumption data collected as part of the United Kingdom (UK) Food Standards Agency's Dietary Survey Programme (DSP). Three surveys from the UK Data Archive (UKDA) for the National Diet and Nutrition Survey (NDNS) were utilised in the generation of intake estimates: Adults Aged 16 to 64 years collected in 2000-2001 (NDNS 2000-2001) (Office for National Statistics, 2005); the National Diet, Nutrition and Dental Survey of Children Aged 1½ to 4½ Years, 1992-1993 (NDNS 1992-1993) (UKDA, 1995); and the National Diet and Nutrition Survey: Young People aged 4 to 18 Years (NDNS 1997) (UKDA, 2001). Calculations for the mean and high-level (95$^{th}$ percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for DHA-B.

The estimated consumption of DHA from DHA-B, expressed in g/person/day and in g/kg body weight/day, is summarised in Tables IX.B-1 and IX.B-2.

**Table IX.B-1 Summary of the Estimated Daily Intake of DHA from DHA-B from Approved Food Categories in the UK by Population Group (NDNS Data)**

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

DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; NDNS = National Diet and Nutrition Survey; UK = United Kingdom.

**Table IX.B-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of DHA from DHA-B from Approved Food Categories in the UK by Population Group (NDNS Data)**

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

DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; NDNS = National Diet and Nutrition Survey; UK = United Kingdom.

As the use and use levels of DHA from DHA-B are to be harmonised to those recently approved/in process of approval for DHA-O, the intake data summarized in the tables above are considered to be directly comparable to anticipated intakes of DHA-B.

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

DHA-B has been developed to provide an alternative source of DHA in conventional food, food supplement products, and infant and follow-on formulae.

Uses of DHA-B in infant formulae and follow-on formulae are intended to be an alternative or replacement of currently approved uses of DHASCO®. The concentrations of DHA are comparable between the two oils. As such, intakes of DHA from the proposed use of DHA-B in infants and young children are expected to be similar to current levels.

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

There are no proposed geographical restrictions.

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

As stated previously, DHA-B is intended to replace fish and other algal oils in food uses already approved in the EU.

# X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

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

a) “Is there information from previous direct, indirect, intended, or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?”

b) “Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?”

## X.A Is there information from previous direct, indirect, intended, or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?

A summary of the current approvals for DHA- and EPA-rich algal oils are presented in Table X.1-1. There are no proposed food uses (excluding supplements) of DHA-B that are outside of the currently approved/proposed uses of DHA- and EPA-rich algal oils. As such, the intakes assessments of DHA and EPA from DHA-O (presented in Section IX.B) are considered to be an accurate estimate of the anticipated human exposure to DHA from DHA-B from proposed food uses in the EU.

| Table X.1-1 Summary of Authorised/Proposed Uses of DHA- and EPA-rich Oil | | | | |
|---|---|---|---|---|
| **Food Category Use Group** | **Maximum Use Level of DHA (or DHA + EPA in the case of DHA-O)** | | | |
| | **DHASCO®** | **DHA-S** | **DHA-O** | **DHA-B** |
| Dairy products except milk-based drinks | Not limited | 200 mg/100 g or for cheese products 600 mg/100 g | 600 mg/100 g for cheese; 200 mg/100 g for milk products (including milk, fromage frais, and yogurt products) excluding drinks | 600 mg/100 g for cheese; 200 mg/100 g for milk products (including milk, fromage frais, and yogurt products) excluding drinks |
| Dairy analogues except drinks | Not limited | 200 mg/100 g or for analogues to cheese products 600 mg/100 g | 200 mg/100 g or for analogues to cheese products | 200 mg/100 g or for analogues to cheese products |
| Spreadable fat and dressings | Not limited | 600 mg/100 g | 600 mg/100 g | 600 mg/100 g |
| Cooking fats | Not limited | - | 360 mg/100 g | 360 mg/100 g |
| Breakfast cereals | Not limited | 500 mg/100 g | 500 mg/100 g | 500 mg/100 g |
| Food supplements | Not limited | 200 mg per daily dose as recommended by the manufacturer | 3,000 mg per daily dose as recommended by the manufacturer; 450 mg per daily dose for pregnant and lactating women | 3,000 mg per daily dose as recommended by the manufacturer; 450 mg per daily dose for pregnant and lactating women |
| Dietary foods for special medical purposes | Not limited | In accordance with the particular nutritional requirements of the persons for whom the products are intended | In accordance with the particular nutritional requirements of the persons for whom the products are intended | 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 | Not limited | 200 mg/meal replacement | 250 mg/meal replacement | 250 mg/meal replacement |
| Bakery products (Breads and rolls) | Not limited | 200 mg/100 g | 200 mg/100 g | 200 mg/100 g |
| Cereal and nutrition bars | Not limited | 500 mg/100 g | 500 mg/100 g | 500 mg/100 g |
| Non-alcoholic beverages (including milk based beverages) | Not limited | 60 mg/100 mL | 80 mg/100 mL | 80 mg/100 mL |
| Infant and follow-on formula | Used in accordance with Commission Directive 2006/141/EC[1] | - | - | Used in accordance with Commission Directive 2006/141/EC[1] |

[1] The DHA content shall not exceed that of n-6 long chain polyunsaturated fatty acids in accordance with Commission Directive 2006/141/EC (Commission of the European Communities, 2006a).

## X.B Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?

The toxicological and allergenicity aspects of DHA -rich oils were discussed in two previous Advisory Committee on Novel Foods and Processes (ACNFP) Opinions (ACNFP, 2002, 2008). The potential for allergenicity is discussed in further detail in Section XIII.

# XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on the Scientific Committee on Food (SCF) guidelines, the following questions must be addressed pertaining to the nutritional information available on the novel food (Commission of the European Communities, 1997):

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

This question has been addressed in the section which follows.

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

As shown in Section I.B.2.2, DHA-B is compositionally similar to other DHA-rich oils that have been approved for use in food in the EU. DHA-B will be used as an alternative source of DHA to existing ingredients on the market.

# XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be addressed pertaining to the microbiological information available for the novel food (Commission of the European Communities, 1997):

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

This point is addressed in the section that follows.

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

As discussed in Section II, both aqueous recovery and pasteurisation are employed in the manufacture of DHA-B, which is itself 100% lipid with very low water activity, and therefore, neither the source organism nor microbial contaminants will be able to survive in the final product. Furthermore, DHA-B is manufactured in accordance with cGMP and DSM continues to comply with the requirements of *Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs* (European Parliament and the Council of the European Union, 2004a,b)[10]. The results of microbiological analysis of DHA-B conducted at Eurofins Central Analytical Laboratories are presented below in Table XII.A-1 and Appendix 2. The results demonstrate the absence of the source organism and microbiological contaminants.

**Table XII.A-1 Microbiological Analysis Results for DHA-B**

| Test Method | Specification | DHA-B Lot No. | | |
|---|---|---|---|---|
| | | 08-6530 | 08-6586 | 08-6592 |
| Aerobic Plate Count | <10 CFU/g | <1 | <10 | <10 |
| Yeast | <10 CFU/g | <1 | <10 | <10 |
| Mould | <10 CFU/g | <1 | <10 | <10 |
| Total Coliforms | <10 CFU/g | <0.3 | <3 | <3 |
| *Escherichia coli* | <10 CFU/g | <0.3 | <3 | <3 |
| *Staphylococci Coagulase+* | <10 CFU/g | <10 | <10 | <10 |
| *Salmonella* | Negative /25g | Neg | Neg | Neg |

CFU = colony forming units; Neg = negative.

[10] Corrigendum to Regulation (EC) No 852/2004 (- http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:226:0003:0021:EN:PDF)

# XIII TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

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

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

   or

c. “Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?”

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

These questions are addressed in the section which follows.

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

As mentioned above, DHA-B is compositionally similar to other DHA-rich oils that have been approved for use in food in the EU. DHA-B will be used as an alternative source of DHA to existing ingredients on the market.

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

The absence of algal toxins and other contaminants is presented in Sections III.D.1 and XII. No new toxicants or changes in the levels of existing toxicants have been identified compared to other DHA- and EPA-rich algal oils.

## XIII.C Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?

The safety of DHA-B is supported by several lines of evidence. Firstly, other DHA-rich oils have a history of safe use in the same food categories as those proposed for DHA-B. Both fish oil and DHASCO® have been widely used in infant and follow-on formulae since the mid 1990's. Furthermore, DHA-S (DHA-rich oil produced by a similar strain of *Schizochytrium*) and DHA-O (DHA- and EPA-rich oil produced by a similar strain of *Schizochytrium*) have been approved as novel foods for the same food uses as DHA-B (excluding infant and follow-on formula). A summary of the authorised uses of the DHA- and EPA-rich algal oils is provided in Table X.1-1.

Secondly, the safety of DHA, the predominant fatty acid constituent of DHA-B, is well established. EFSA has reviewed the safety of supplemental DHA and EPA intake and has concluded that intakes of up to 5 g/day of DHA and EPA were not anticipated to pose human health concerns in adults (EFSA, 2012). Additionally, health claims pertaining to DHA in conventional foods and infant and follow-on formula are authorised as *per* Commission Regulation (EU) No 432/2012 (European Commission, 2012) and Commission Directive 2006/141/EC (Commission of the European Communities, 2006a). These lend further support that intake of DHA from DHA-B is not anticipated to cause adverse health effects at the proposed levels of use.

Lastly, DSM has commissioned a genotoxicity suite and 90-day oral toxicity studies with an *in utero* phase in rats as confirmatory preclinical studies in support of the safety of DHA-B. The 90-day oral toxicity study with the *in utero* phase was of the same design as those described in the Cargill's approved (and uncontested) novel food application for refined arachidonic acid-rich oil (Novel Foods Unit, 2011). Thus, based on the review and subsequent approval of the arachidonic acid rich oil as a novel food ingredient in infant formula, this study design was considered to be appropriate to substantiate the safety of

DHA-B for its similar use in infant and follow-on formulae. The results of the 90-day oral toxicity study and genotoxicity studies conducted with DHA-B are described in the following sections.

## XIII.C.1 Confirmatory Pre-clinical Safety Studies Conducted on DHA-B

### *XIII.C.1.1 Repeat Exposure Studies*

90-day toxicity study in the rat

This study was conducted in accordance with Organization for Economic Cooperation and Development (OECD) Test Guideline No. 408 (OECD, 1998a) and U.S. Redbook Guideline IV.C.4.a (U.S. FDA, 2003). During the *in utero* phase, DHA-B was administered at dietary levels of 1 (low-dose), 3 (mid-dose), or 5 (high-dose) % to $F_0$ rats (13 males and 26 females/ group). Two control groups also were included in the study, one that received a standard low fat basal diet and one a basal diet supplemented with 5% tuna oil. Males were fed the test or control diets for 4 weeks before mating, and throughout the mating and gestation periods. Females were fed the test or control diets for 4 weeks before mating, and throughout the mating, gestation, and lactation periods. In the subsequent 90-day $F_1$ phase, the test diets were fed to randomly selected offspring from each litter (1 to 2 animals/sex/ litter) according to their original *in utero* groups. Twenty $F_1$ animals/sex/group were selected to proceed to the 90-day dietary phase. Parameters evaluated in the $F_0$ and $F_1$ generations included viability, signs of gross toxicity, behavioural changes, body weights, food consumption, and fertility, reproductive, and developmental indices. Gross necropsies were performed on $F_0$ dams on post-natal day (PND) 22, and the ovaries and uteri from all $F_0$ animals were removed and weighed. $F_0$ males also were sacrificed on the corresponding PND 22 of their respective females and were subjected to gross necropsy. All unselected pups were culled and macroscopically examined on PND 22. Parameters assessed in the $F_1$ generation included viability, signs of gross toxicity, behavioural changes, ophthalmology, body weights, food consumption, functional observation battery (FOB), motor activity, haematology, clinical chemistry, urinalysis, organ weights, and gross pathology. Histopathological examination was performed on selected organs and tissues from the both control and high-dose groups.

During the pre-mating phase of the study, parental intakes of DHA-B were equivalent to doses of 757.2, 2,293.9, or 3,860.4 mg/kg body weight/day in males; and 894.9, 2,613.3, and 4,319.8 mg/kg body weight/day in females. Intakes of the fish oil control were equivalent to 3,837.0 and 4,434.5 mg/kg body weight in males and females, respectively. During gestation, the mean overall daily intake of DHA-B for confirmed pregnant rats was 778.0, 2309.9, and 3728.5 mg/kg body weight/day. Intakes of the fish oil control were equivalent to 3824.9 mg/kg body weight.

No test article-related mortalities were observed in parental animals at any period, and no clinical signs of toxicity were observed. No significant differences in body weight, body weight gain, or food consumption were observed during the pre-mating, mating, or gestation

periods compared to basal diet controls. A statistically significant decrease in food consumption was observed in dams receiving DHA-B at all doses during lactation (days 0 to 22) compared to the basal diet; however, this was comparable to that of the tuna oil control group and did not have effects on pup's general signs or weight gain. Similarly, a statistically significant increase in food efficiency for pre-mating females (days 0 to 7) was observed compared to the basal diet control; however, this was comparable to that of the tuna oil control group and was not considered adverse by the authors. These effects on food consumption and food efficiency were considered to be the result of an increase in caloric load associated with the high-fat diet (9.2% of fat in the DHA-B and the tuna oil control diets *versus* 5% of fat in the basal diet).

Fertility and reproductive performance parameters of males and females were comparable between DHA-B groups and controls. No significant effects on mean gestation length, gestation index, number of implantation sites, number of corpora lutea, pre-implantation loss, post-implantation loss, stillbirth, live births, or viability indices were observed compared to controls.

No significant differences in litter loss, litter size, litter or pup weight, sex ratio, time and body weight to attainment of developmental indices and sexual maturity, or pup survival were noted compared to controls. A statistically significant lower body weight was observed in pups receiving the high-dose DHA-B and the tuna oil control groups compared to the basal diet control during post-natal day 14 through to weaning, and in all DHA-B groups and the tuna oil controls following weaning. These reductions were considered to be non-adverse because they were seen in the tuna oil control as well and all mean and individual body weights values were within an acceptable range for biological variation based on the age and strain of these animals. Pups in all groups continued to thrive and gain weight and exhibited appropriate developmental landmarks for their age.

No test article-related macroscopic abnormalities were observed in pups culled at PND 22. In the parental animals, no adverse test article-related changes in macroscopic findings or organ weights were observed. Although yellow-discoloured peritoneal fat was observed in 17% of the high-dose dams, this was considered non-adverse and expected from previously reported studies with LC-PUFAs (see discussion below). Taken together, there were no pre-mating, mating, reproductive, or early developmental effects attributed to DHA-B in the *in utero* phase of the study, and all indices remained within historical control values for age- and strain-matched rats.

In the 90-day dietary phase of the study, intakes of DHA-B were equivalent to doses of 644.9, 1,973.3, or 3,278.9 mg/kg body weight/day in males, and 754.2, 2,331.2, and 3,788.4 mg/kg body weight/day in females, respectively. Intakes of the tuna oil were equivalent to 3,236.8 and 3,760.5 mg/kg body weight/day in males and females, respectively.

No mortalities were observed and no clinical signs of toxicity were noted during the 90-day dietary phase. No test article-related ophthalmoscopic findings or test article-related

differences in the functional observational battery or motor activity were observed compared to controls. No test article-related adverse changes in haematology, clinical chemistry, coagulation, or urinalysis parameters were observed, and all differences in these parameters from the basal diet control were determined to be within historical control data or without histological correlates and thus were deemed to be incidental.

Compared to the basal diet control, a transient, statistically significant lower body weight were observed in animals receiving all doses of DHA-B and tuna oil beginning in the latter part of the lactation period; however, these were recovered by the middle of the 90-day period and were considered to be non-adverse by the authors. A significant decrease in food consumption accompanied by an increase in food efficiency were observed in animals receiving DHA-B or tuna oil compared to the basal diet control over the course of the 13 weeks; however, these were anticipated due to the high caloric content of the test diets, and considered to be compensatory, non-adverse and toxicologically insignificant by the study authors.

Two incidences of moderate granulomatous infiltration of retroperitoneal fat were observed in high-dose males. A similar incidence of minimal/moderate granulomatous infiltration was noted in the adipose issue of the mammary gland fat pad in 4 high-dose males and 2 high-dose females. These were considered to be possibly related to the test article. These findings were in line with several previous published LC-PUFA oil studies reports (Pollard and Sanders, 1993; Muggli, 1994; Ando *et al.*, 2000; Kroes *et al.*, 2003), which were explained by elevated demand for antioxidants with high loads of PUFA oils (Muggli, 1994). Therefore, the authors considered these effects to be non-adverse because they were not specifically ascribed to the test substance but rather attributable to exposure to the high levels of dietary LC-PUFA.

A slight to moderate cytoplasmic vacuolation of cortical cells in the zona fasciculate of the adrenal glands was observed in high-dose animals and the tuna oil control. This was attributed to the increased dietary fat content compared to that of the basal diet control and were not accompanied by changes in adrenal organ weight. Therefore, these findings were considered non-adverse by the authors. No other remarkable macroscopic or microscopic findings were reported.

Compared to the basal diet control, some changes in liver, heart, testes, kidney, and spleen weight were reported; however, these were without histological correlates, without a dose relationship, and thus were deemed to be toxicologically insignificant by the authors.

Based on the results of the study, the authors derived a no-observed-adverse-effect level (NOAEL) of 3,278.9 and 3,788.4 mg/kg body weight/day for male and female rats, the highest doses tested.

### *XIII.C.1.2 Short-term Tests for Mutagenicity/Genotoxicity*

#### XIII.C.1.2.1 Bacterial Reverse Mutation (Ames) Assay

A bacterial reverse mutation test (Ames test) was conducted to assess the potential mutagenicity of DHASCO®-B in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2*uvr*A (BSL Bioservice Study No. 105886 – BSL, 2011a). This study was conducted in compliance with the OECD Test Guideline No. 471 (OECD, 1997a), Commission Regulation (EC) No. 440/2008 (Commission of the European Communities, 2008), and the Environmental Protection Agency (EPA) Test Guideline OPPTS 870.5100 (U.S. EPA, 1998a). The solvent vehicle, dimethyl sulfoxide (DMSO), served as a negative control for all strains. One of the following compounds was employed as a positive control for assays conducted in the absence of S9 metabolic activation: sodium azide ($NaN_3$), 4-nitro-o-phenylene-diamine (4-NOPD), or methylmethanesulfonate (MMS). For assays conducted in the presence of metabolic activation, 2-aminoanthracene (2-AA) was employed as the positive control. Bacterial strains were treated with DHA-B at concentrations of 31.6, 100, 316, 1,000, 2,500, or 5,000 µg/plate. No biologically relevant increases in revertant colony numbers of any of the 5 tester strains were observed following treatment with DHA-B at any concentration level, neither in the presence or absence of metabolic activation. Positive control agents substantially induced the number of revertant colonies compared to the negative control, confirming the sensitivity of the assay. Based on these findings, the investigators concluded that DHA-B did not induce gene mutations by base-pair changes or frameshifts in the genomes of the tester strains used and therefore was non-mutagenic.

#### XIII.C.1.2.2 *In-vitro* Mammalian Chromosome Aberration Test

An *in vitro* mammalian chromosome aberration test in human lymphocytes was conducted to further evaluate the genotoxic potential of DHA-B in the presence and absence of metabolic activation (BSL Bioservice Study NO. 105887 – BSL, 2011b). This study was conducted in accordance with OECD Test Guideline No. 473 (OECD, 1997b), Commission Regulation (EC) No. 440/2008 (Commission of the European Communities, 2008), EPA Test Guideline OPPTS 870.5375 (U.S. EPA, 1998b), and the International Conference on Harmonisation (ICH) Guideline S2(R1) (ICH, 2011). The culture medium served as the negative control. Ethylmethanesulfonate (EMS) and cyclophosphamide (CPA) were employed as the positive control in assays conducted in the absence and presence of metabolic activation, respectively. In the first experiment, cells were treated with DHA-B at concentrations of 250, 500, 1,000, 2,500, or 5,000 µg/mL for 4 hours in the presence or absence of metabolic activation (short-term assay) or for 24 hours without metabolic activation (continuous treatment assay). Additional experiments were conducted due to inconsistent results observed at some concentrations in the assay conducted with metabolic activation. In the second experiment, cells were treated with DHA-B at concentrations of 3,000, 4,000, or 5,000 µg/mL for 4 hours in the presence of metabolic activation.

Precipitation was observed in all experiments at concentrations of 1,000 µg/mL and greater. Therefore, a revised second experiment was conducted to evaluate the test article without

visible precipitate at lower concentrations of 400, 500, 750, or 1,000 µg/mL for 4 hours in the presence of metabolic activation. Cytotoxicity was only observed in the continuous treatment assay in which the mitotic index was decreased to 66% at the highest concentration (5,000 µg/mL). DHA-B did not induce chromosomal aberrations in human lymphocytes in all assays conducted in the absence of metabolic activation. In the first short-term assay conducted with metabolic activation, an increase in the frequency of chromosomal aberrations was noted at a concentration of 500 µg/mL compared to historical control data, and a borderline value (4% aberrant cells) was noted at a concentration of 5,000 µg/mL. In the revised second experiment, an increase in the chromosomal aberration frequency was observed at concentration of 500 µg/mL and greater; however, no dose-response relationship was observed. In both experiments, positive controls induced distinct and biologically relevant increases in the incidence of cells with structural chromosomal aberrations. No biologically relevant increase in the frequency of polyploidy cells was observed in any experiment. DHA-B did not induce structural chromosomal aberrations in human lymphocytes in the absence of metabolic activation, but induced an increase in the frequency of chromosomal aberrations in the presence of metabolic activation; however, given that the clastogenic effect was relatively moderate and a dose-response relationship was not observed, the study authors concluded that the results of the *in vitro* chromosomal aberration test were equivocal.

XIII.C.1.2.3 *In vivo* Mouse Micronucleus Test –

An *in vivo* micronucleus test was performed to investigate the genotoxic potential of DHA-B in mice (BSL Bioservice Study Report No. 105888 – BSL, 2011c). This study was conducted in accordance with OECD Guideline No. 474 (OECD, 1997c), Commission Regulation (EC) NO. 440/2008 (Commission of the European Communities, 2008), and EPA Test Guideline OPPTS 870.5395 (U.S. EPA, 1998c). In a preliminary dose-range finding study, NMRI mice (1/sex) were administered the test article at a single dose of 2,000 mg/kg body weight *via* intraperitoneal (i.p.) injection with no signs of toxicity observed. Therefore, this dose was selected as the maximum tolerable dose in the main micronucleus test. In the main micronucleus test, NMRI mice (5/sex) were administered DHA-B at a single dose of 2,000 mg/kg body weight *via* i.p. injection. The negative and positive control group was administered cottonseed oil and 40 mg/kg body weight of CPA, respectively. No toxicity was observed in animals administered the test article. DHA-B did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse. The incidence of micronuclei in the negative control group was reported to be within the range of historical laboratory control data. A significant increase in micronuclei was observed in the positive control group, thus confirming the validity of the assay. Therefore, DHA-B obtained *via* aqueous extraction was considered to be non-genotoxic as assessed in the *in vivo* mammalian micronucleus test.

## XIII.C.2 Supporting Pre-clinical Safety Studies Conducted on an Alcoholic Extraction Preparation of DHA-B

Additional studies conducted on DHA-B prepared *via* an alternative method of extraction by isopropyl alcohol (85 to 100%) are available. DHA-B produced by alcoholic extraction meets the same specifications of DHA-B produced by aqueous extraction and is compositionally similar. Batch analyses of DHA-B produced by alcoholic extraction compared to aqueous extraction is presented in Table XIII.C.2-1. Due to the similarities between the two substances, studies conducted on the alcoholic preparation provide supporting evidence of the safety of DHA-B (aqueous extraction) and are discussed in the following sections.

**Table XIII.C.2-1 Summary of Batch Analyses Data of DHA-B Produced by Aqueous and Alcoholic Extraction**

| Tests | Spec. | DHA-B (Aqueous)[1] | DHA-B (Alcoholic) Batch No [2] |
|---|---|---|---|
| Specifications | | | |
| Free fatty acids (%) | Not more than 0.4 | 0.063 | 0.113 |
| Unsaponifiables (%) | Not more than 3.5 | 0.903 | 0.92 |
| DHA (%) | Not less than 35 | 42.74 | 43.76 |
| Fatty acid profile | | | |
| 14:0 Myristic | N/A | 1.163 | 1.997 |
| 15:0 Pentadecanoic | N/A | 0.237 | 0.487 |
| 16:0 Palmitic | N/A | 13.48 | 20.357 |
| 17.0 Heptadecanoic (Margaric) | N/A | ND | 0.130 |
| 18:0 Stearic | N/A | 1.68 | 1.437 |
| 18:1(n-9)* Oleic | N/A | 26.32 | 16.76 |
| 18:1(n-7)* cis-vaccenic | N/A | 0.257 | 0.133 |
| 18:2 Linoleic | N/A | 2.07 | 1.253 |
| 20:0 Eicosanoic (Arachidic) | N/A | 0.323 | 0.373 |
| 20.1 Eicosenoic acid | N/A | 0.140 | ND |
| 20:4(n-6) Arachidonic | N/A | 0.667 | 0.850 |
| 20:5(n-3) Eicosapentaenoic | N/A | 6.033 | 7.140 |
| 22:0 Docosanoic (Behenic) | N/A | 0.363 | 0.257 |
| 22:5(n-6) Docosapentaenoic | N/A | 2.513 | 2.700 |
| 22:5(n-3) Docosapentaenoic | N/A | 0.710 | 0.573 |
| 24:0 Tetracosanoic (Lignoceric) | N/A | 0.137 | 0.110 |
| 22:6(n-3) Docosahexaneoic | N/A | 42.74 | 43.76 |

[1] Average of batches R223, 08-6586, and 08-6592
[2] Averages of batches 98-5570, 98-5572, and 98-5999
ND = not detected; N/A = not applicable.

### *XIII.C.2.1 Repeat Exposure Studies*

DHA-B*90-Day Repeated Dose Study with in utero Phase in Rats*

The potential toxicity of DHA-B obtained *via* alcoholic extraction was investigated in an additional 90-day dietary toxicity study, preceded by an *in utero* phase, in Sprague-Dawley rats (Fedorova-Dahms *et al.,* 2011a). This study was conducted in accordance with OECD Test Guideline No. 408 (OECD, 1998a) and U.S. Redbook Guideline IV.C.4.a (U.S. FDA, 2003). During the *in utero* phase, DHA-B was administered at dietary levels of 0.5 (low-dose), 1.5 (mid-dose), or 5 (high-dose) % to $F_0$ rats (13 males and 26 females/group). Two control groups also were included in the study, one that received a standard low-fat basal diet and one a basal diet supplemented with 5% fish oil. Males were fed the test or control diets for 4 weeks before mating, and throughout the mating and gestation periods. Females were fed the test or control diets for 4 weeks before mating, and throughout the mating, gestation, and lactation periods. In the subsequent 90-day $F_1$ phase, the test diets were fed to randomly selected offspring from each litter (1 to 2 animals/sex/litter) according to their original *in utero* groups. Twenty $F_1$ animals/sex/group were selected to proceed to the 90-day dietary phase and half of them (10 animals/sex/group) were included in a 30-day recovery period. Parameters evaluated in the $F_0$ and $F_1$ generations included viability, signs of gross toxicity, behavioural changes, body weights, food consumption, and fertility, reproductive, and developmental indices. Gross necropsies were performed on all post-partum $F_0$ dams, and the ovaries and uteri from all $F_0$ animals were removed and weighed. All unselected pups were macroscopically examined on PND 22. Parameters assessed in the $F_1$ generation included viability, signs of gross toxicity, behavioural changes, ophthalmology, body weights, food consumption, FOB, motor activity, haematology, clinical chemistry, urinalysis, organ weights, and gross pathology. Histopathological examination was performed on selected organs and tissues from the both control and high-dose groups.

There were no test-article related effects on mortality, clinical observations, body weight, body weight gain, food consumption, or food efficiency observed in the $F_0$ generation. Dietary administration of DHA-B was not reported to adversely affect the fertility or reproductive performance of the $F_0$ rats. There were no significant differences in litter size, stillbirth index, sex ratio, gestation index, pup viability, survival or mean pup weights reported among groups during the lactation period. The normal attainment of developmental landmarks (*i.e.,* hair growth, pinna detachment, incisor eruption, and eye opening) was reported to be comparable among the control and test groups. There were no test article-related macroscopic findings observed in any of the post-natal dams or unselected pups. Significantly lower absolute ovarian and uterine weights were observed in the fish oil control and the high-dose dams, and significantly lower relative ovarian and uterine weights were noted in high-dose dams compared to those given the basal diet control; however, the investigators did not consider these findings to be toxicologically significant as the changes were small in magnitude and within historical control values.

In the subsequent $F_1$ 90-day phase, no mortality, clinical observations, or ophthalmological abnormalities related to administration of DHA-B were noted in any group. There were no

test article-related changes in motor activity or behaviour assessed in the FOB battery observed in any test group. There were no significant differences in mean body weights observed in males administered DHA-B compared to their respective basal diet controls. Mid- and high-dose females exhibited higher body weights during the latter portion of the administration period until the end of the recovery phase compared to the basal diet controls (statistical significance not reported). There were no statistically significant differences in food consumption noted in the test groups compared to the basal diet control group.

Statistically significant changes in haematology parameters were observed in high-dose males compared to their respective basal diet controls, including decreased mean erythrocyte counts and decreased haematocrit levels. Although the reduction in haematocrit levels was still statistically significant at the end of the recovery period, the investigators noted that resolution was evident. Moreover, the investigators did not consider these findings to be clinically significant due to the low magnitude of change and their reversibility. In addition, similar findings have been observed in male rats in other studies conducted on long-chain polyunsaturated fatty acids (LC-PUFA) (Burns *et al.*, 1999; Lina *et al.*, 2006; Casterton *et al.*, 2009; Fedorova-Dahms *et al.*, 2011b).

Males and females in the fish oil control and high-dose groups exhibited significantly higher mean ALP activity and lower serum cholesterol levels compared to their respective basal diet controls. Significantly higher triglyceride levels also were observed in males and females in the fish oil control group, and high-dose females compared to the basal diet control, and this effect was observed throughout the recovery phase. The investigators noted that the increase in ALP activity observed in the high-dose and fish oil control groups was consistent with the findings of other studies that evaluated the safety of LC-PUFA oils (Arterburn *et al.*, 2000; Lina *et al.*, 2006; Blum *et al.*, 2007; Casterton *et al.*, 2009; Fedorova-Dahms *et al.*, 2011b). Given that an increase in mean ALP activity also was observed in the fish oil control group, the investigators attributed this finding to the dietary administration of high levels of LC-PUFA. The investigators further noted that the reductions in cholesterol levels observed in the high-dose and fish oil control groups were expected, and that this effect was due to the lipid-lowering action of LC-PUFA (Harris *et al.*, 1988; Hempenius *et al.*, 2000; Ryan *et al.*, 2009).

At necropsy, mottled discoloration was noted in several livers of mid-dose females, high-dose males and females, and fish oil control males and females; however, these were not accompanied by histopathological findings and were not observed at the end of the recovery. Therefore, the investigators did not consider these findings to be test article-related.

A number of statistically significant changes in organ weights were noted in the test groups and the fish oil control group compared to the basal diet control. These included increased absolute and relative (to body) liver weights in high-dose and fish oil control females; increased relative liver (to brain) weights in high-dose and fish oil control males; increased absolute and relative kidney and spleen weights in high-dose females and fish oil control males and females; increased absolute and relative adrenal weights in high-dose and fish oil

control females; increased absolute heart weight in high-dose females; and increased absolute ovarian weight in high-dose females. However, the investigators did not consider any of the changes in organ weights to be test article-related as they were sex-specific, were not observed at the end of the recovery period, or were also observed in the fish oil control group. Instead, the investigators attributed the changes in organ weights observed in the high-dose group to the administration of a high LC-PUFA diet, and further noted that similar findings have been observed in dietary and gavage LC-PUFA studies (Burns *et al.*, 1999; Hempenius *et al.*, 2000; Lina *et al.*, 2006; Blum *et al.*, 2007; Casterton *et al.*, 2009).

Histopathological examination revealed an increased incidence of cytoplasmic vaculoation of cortical cells in the zona fasciculate of the adrenal glands in males and females in the fish oil control and high-dose groups. Minimal to slight splenic haematopoiesis was noted in the basal diet, fish oil control, and high-dose groups. Given that the histopathological findings observed in the high-dose group also were observed in the fish oil control group, the investigators did not consider these changes to be test article-related, but instead suggested that they were physiological adaptations to accommodate the large LC-PUFA load in the diet.

Based on the results of the study, the authors determined the NOAEL to be 50,000 mg/kg, the highest dose tested, for male and female rats over a 90-day post-natal period following pre-natal parental exposure and during maternal lactation. This dose level corresponds to 4,122 and 4,399 mg/kg body weight/day for male and female rats, respectively, or an average of 4,260 mg/kg body/day for both sexes.

*XIII.C.2.2 Short-term Tests for Mutagenicity/Genotoxicity*

XIII.C.2.2.1 Bacterial Reverse Mutation (Ames) Assay

The potential mutagenicity of DHA-B (obtained *via* alcoholic extraction) was investigated in the bacterial reverse mutation assay (Ames test) using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2 uvrA in the presence and absence of metabolic activation using the plate incorporation test (Fedorova-Dahms *et al.,* 2011a). This study was conducted in accordance with OECD Guideline No. 471 (OECD, 1997a). The solvent vehicle (ethanol) served as a negative control for all strains. One of the following positive controls was used in the assays conducted in the absence of metabolic activation: sodium azide ($NaN_3$), 4-nitro-o-phenylene-diamine (4-NOPD), and MMS. For the assays conducted in the presence of metabolic activation, 2-aminoanthracene (2-AA) was used as the positive control. Bacterial strains were treated with DHA-B at concentrations of 50, 158, 500, 1,580, 4,000, or 5,000 µg/plate. No precipitation or inhibition of bacterial growth was observed for any of the strains treated with DHA-B in the presence or absence of metabolic activation compared to the negative control. There were no biologically relevant increases (*i.e.,* more than a 2-fold or dose-related increase) in revertant colony numbers of any of the 5 tester strains observed following treatment with DHA-B at any concentration tested, either in the presence or absence of metabolic activation. Positive control agents increased the number of revertant colonies compared to the negative control, thus

confirming the sensitivity of the assay. Based on these findings, DHA-B was determined to be non-mutagenic as assessed in the Ames test.

XIII.C.2.2.2 *In vitro* Mammalian Chromosome Aberration Test in Human Lymphocytes (Fedorova-Dahms *et al.,* 2011a)

The genotoxic potential of DHA-B (obtained *via* alcoholic extraction) was further evaluated in a chromosomal aberration assay in human lymphocytes with and without metabolic activation (Fedorova-Dahms *et al.,* 2011a). This study was conducted in accordance with OECD Guideline No 473 (OECD, 1997b) and in compliance with OECD Principles of Good Laboratory Practices (GLP) (OECD, 1998b). The culture medium served as the negative control. Ethylmethanesulfonate (in the absence of metabolic activation) and cyclophosphamide (CP) (in the presence of metabolic activation) were employed as positive controls. In the short-term exposure (4-hour) experiment conducted in the presence and absence of metabolic activation, human lymphocytes were treated with the test article at concentrations of 1, 2.5, or 5.0 µL/mL. In the continuous (24-hour) experiment conducted in the absence of metabolic activation, cells were treated with the test article at concentrations of 1, 2.5, or 5.0 µL/mL. An additional short-term experiment was conducted in which cells were treated with the test article at concentrations of 3, 4, or 5 µL/mL in the presence of metabolic activation.

Precipitation was observed at concentrations of 0.125 µL/mL and above; however, no cytotoxicity was observed at any concentration. In order to evaluate the genotoxicity at higher doses, the test article was evaluated as a suspension by treating the test solution (containing the test article and cell culture media) with ultrasound for 20 to 30 minutes to produce a homogenous suspension. In all experiments, there was no induction of structural chromosomal aberrations observed in any of the doses tested. The frequency of chromosomal aberrations in the negative control group was within the range of historical control data. The positive controls increased the incidence of cells with structural chromosomal aberrations, thus confirming the validity of the assay. Based on the results of this study, the investigators concluded that DHA-B did not induce structural chromosomal aberrations in human lymphocyte cells.

XIII.C.2.2.3 *In vivo* Mouse Micronucleus Test

An *in vivo* micronucleus test was performed to investigate the genotoxic potential of DHA-B obtained *via* solvent extraction (Fedorova-Dahms *et al.,* 2011a). This study was conducted in accordance with OECD Guideline No. 474 (OECD, 1997c), and in compliance with OECD Principles of GLP. In a preliminary dose-range finding study, NMRI mice (3/sex/group) were orally administered the test article at a single dose of 2,000 mg/kg body weight with no signs of toxicity observed. Therefore, this dose was selected as the maximum tolerable dose in the main micronucleus test. In the main micronucleus test, NMRI mice (5/sex) were orally administered DHA-B at a single dose of 2,000 mg/kg body weight. The negative and positive control groups were administered corn oil and 40 mg/kg body weight of CP, respectively. No toxicity was observed in animals administered the test article. There was

no statistically significant increase in the number of cells with micronuclei observed in mice administered DHA-B. The incidence of micronuclei in the negative control group was reported to be within the range of historical laboratory control data. A significant increase in micronuclei was observed in the positive control group, thus confirming the validity of the assay. Therefore, DHA-B is considered to be non-genotoxic as assessed in the *in vivo* mammalian micronucleus test.

### XIII.C.3 Summary

DSM has commission a 90-day oral toxicity study with an *in utero* phase and a genotoxicity suite to support the safety of DHA-B. Based on the results of the 90-day study, the NOAEL for DHA-B is 3,278.9 and 3,788.4 mg/kg body weight/day for males and females, the highest dose tested. The safety of DHA-B is further supported by the results of a second 90-day oral toxicity study with an *in utero* phase conducted with an alternative method of extraction with isopropyl alcohol. The NOAEL derived from the study with DHA-B produced by alcoholic extraction is 4,122 and 4,399 mg/kg body weight/day for males and females, the highest dose tested. The results of these studies provide confirmatory evidence that intakes of DHA-B are not expected to pose adverse health effects at its intended use levels in food.

With regards to the potential mutagenicity and genotoxicity of DHA-B, negative findings were obtained in the Ames test and the *in vivo* mouse micronucleus test, while equivocal results were noted in the *in vitro* mammalian chromosome aberration test conducted on DHA-B obtained *via* aqueous extraction. Although equivocal results were obtained in the *in vitro* mammalian chromosome aberration assay, it should be noted that *in vitro* genotoxicity assays may produce both false negative and false positive results and that a positive result in any assay does not necessarily mean that the test compound poses a genotoxic hazard to humans (ICH, 2011). The ICH (2011) also indicated that "*although positive in vitro data may indicate intrinsic genotoxic properties of a compound, appropriate in vivo data determine the biological significance of these in vitro signals in most cases."* Therefore, given that negative findings were noted in a bacterial reverse mutation assay and the *in vivo* mouse micronucleus test, and negative results were obtained in the same test battery of mutagenicity/genotoxicity studies conducted on DHA-B obtained *via* isopropyl alcohol extraction (which is compositionally identical to DHA-B), it is highly unlikely that the DHA-B would have genotoxic potential despite the equivocal results obtained in the *in vitro* mammalian chromosome aberration test. Furthermore, several investigators have concluded that algal oils and algal biomass have no genotoxic potential (Arterburn *et al.,* 2000; Hammond *et al.,* 2002; Kroes *et al.*, 2003; Blum *et al.,* 2007; Fedorova-Dahms *et al.*, 2011b). A summary of the *in vitro* and *in vivo* mutagenicity and genotoxicity studies conducted on DHA-B is presented in Table XIII.C.3-1.

**Table XIII.C.3-1 Summary of *In vitro* and *In vivo* Mutagenicity and Genotoxicity Studies on DHA-B**

| Test System | Type | Results | Concentration | Reference |
|---|---|---|---|---|
| **DHA-B (Aqueous Extraction)** | | | | |
| *In vitro Studies* | | | | |
| *Escherichia coli* WP2uvrA | Mut (+/-S9) | Negative | 31.6, 100, 316, 1,000, 2,500, or 5,000 µg/plate | BSL Bioservice Study No. 105886 – BSL, 2011a |
| *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 | Mut (+/-S9) | Negative | 31.6, 100, 316, 1,000, 2,500, or 5,000 µg/plate | BSL Bioservice Study No. 105886 – BSL, 2011a |
| Human lymphocytes | CA (+/-S9) | Equivocal[a] | 250, 500, 1,000, 2,500, or 5,000 µg/mL (4-hr; +/-S9)<br><br>250, 500, 1,000, 2,500, or 5,000 µg/mL (24-hr; -S9)<br><br>3,000, 4,000, or 5,000 µg/mL (4-hr; +/-S9)<br><br>400, 500, 750, or 1,000 µg/mL (4-hr; +/-S9) | BSL Bioservice Study No. 105887 – BSL, 2011b |
| *In vivo* Studies | | | | |
| NMRI mice | MN | Negative | 2,000 mg/kg bw (i.p. injection) | BSL Bioservice Study No. 105888 – BSL, 2011c |
| **DHA-B (Alcoholic Extraction)** | | | | |
| *In vitro* Studies | | | | |
| *E. coli* WP2uvrA | Mut (+/-S9) | Negative | 50, 158, 500, 1,580, 4,000, or 5,000 µg/plate | Fedorova-Dahms *et al.,* 2011a |
| *S. typhimurium* TA98, TA100, TA1535, TA1537 | Mut (+/-S9) | Negative | 50, 158, 500, 1,580, 4,000, or 5,000 µg/plate | Fedorova-Dahms *et al.,* 2011a |
| Human lymphocytes | CA (+/-S9) | Negative | 1,0, 2.5, or 5.0 µL/mL (4-hr; +/-S9)<br><br>1,0, 2.5, or 5.0 µL/mL (24-hr; -S9)<br><br>3, 4, or 5 µL/mL (4-hr; +S9) | Fedorova-Dahms *et al.,* 2011a |
| *In vivo* Studies | | | | |
| NMRI mice | MN | Negative | 2,000 mg/kg bw (oral) | Fedorova-Dahms *et al.,* 2011a |

bw = body weight; CA = chromosomal aberration; i.p. = intraperitoneal; MN = micronucleus; Mut = mutation; S9 = metabolic activation

[a] Increased frequency of chromosomal aberrations was observed at concentrations of 500 µg/mL and greater in the absence of S9; however, no dose-response relationship was observed.

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

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

There is no indication to suggest that DHA-B should elicit allergenic responses.

## XIII.E Additional Safety-Related Considerations

Whilst very high doses of fish oils have been suggested as increasing bleeding time, patients taking anticoagulation therapy are advised by their healthcare professionals not to consume large doses of fish oil. However this should be of no concern in relation to the consumption of products fortified with DHA-B. In 1997 and again in 2005, the United States Food and Drug Administration (FDA) stated that there is not a significant bleeding risk at intake levels of DHA+EPA at levels up to 3 g/day. Additionally, results from both short and intermediate-length clinical trials indicate that:

- "..the experience has been virtually unanimous: omega-3 fatty acid supplements do not increase the risk for clinically significant bleeding, even in patients also being treated with anti-platelet or antithrombotic medications." (Harris, 2007)
- There are no proposed geographical restrictions. DHA-B is an environmentally sustainable vegetarian alternative to fish. This should be a highly desirable prospect for all Member States at a time of stretched fishing stocks and the increasing amount of evidence supporting the importance of DHA to their citizens' health.
- As stated earlier, DHA-B is a simple replacement for fish and other algal oils in the European diet. It is proposed at "like-for like" uses and levels of addition, with the same nutritional value.

The safety of long-chain omega-3 fatty acids was further addressed in a recent Scientific Opinion on the Upper Tolerable Intake Level of EPA, DHA, and DPA published by EFSA (EFSA, 2012). In the Opinion, the Expert Panel concluded that current intake levels of long-chain polyunsaturated fatty acids were not associated with adverse effects in healthy children or adults, and supplemental intakes of DHA at a dose of 1 g/day did not raise safety

concerns for the adult population. In addition, the Expert Panel noted that intakes of EPA and DHA from food and food supplements in European populations were generally below 5 g/day and did not pose a safety concern with respect to bleeding complications at that intake level, even in subjects at high risk of bleeding (*e.g.*, taking acetylsalicylic acid or anti-coagulants). No tolerable upper intake level for DHA, EPA, or DHA (either individually or combined) was established due to the lack of sufficient data.

## OVERALL CONCLUSIONS

Martek has previously gained approval for a DHA-rich oil produced from *Schizochytrium* sp. (DHA-S) and a DHA and EPA-rich oil produced from another *Schizochytrium* microalgae (DHA-O), for general use as a nutritional ingredient in foods.

DSM has developed another strain of the *Schizochytrium* species (*i.e.*, the same species of *Schizochytrium* sp. from which the production strain for DHA-O was derived) to produce a new oil which is rich in DHA and is primarily being targeted as a replacement/alternative to DHASCO® in infant and follow-up formula but also as an alternative to DHA-S and DHA-O in conventional foods, PARNUTS, and food supplements. This new DHA-rich microalgal oil is given the abbreviation “DHA-B”.

The fatty acid and sterol profiles of DHA-B contain no new fatty acids that are not already consumed in either fish or vegetable oils. Extensive analysis shows the absence of significant levels of impurities or contaminants.

DHA-B is intended to be used as a direct replacement for existing sources of DHA in infant formula. It also is intended to be used as a direct food ingredient at the same uses and use-levels as currently approved for DHA-S and DHA-O in the EU. Intakes of DHA-B are anticipated to be similar to that of DHA-S, DHA-O, DHASCO®, and fish oils.

In addition to the extensive safety database already available on *Schizochytrium* sp. algal biomass and on DHA-S, Martek has conducted supporting confirmatory pre-clinical studies on DHA-B, including a 90-day rat study and a suite of mutagenicity studies. These studies follow the protocols that have been accepted previously for the approval of novel arachidonic acid rich oil for use in infant and follow-on formula. Additional supporting data also is available for an alternate form of DHA-B produced by isopropyl alcohol extraction. All of these show no significant adverse effects at the maximum dose/concentration tested. The NOAEL for DHA-B is 3,278.9 and 3,788.4 mg/kg body weight/day in males and females, respectively, based on the results of a 90-day oral toxicity study preceded by an *in utero* phase in rats, which included a fish oil control (fish oil is currently used in infant formula as a source of DHA). The absence of significant levels of protein and extensive history of safe consumption of DHA-S indicate there is no significant risk for allergenicity. DHA-B is therefore proposed as a safe and suitable vegetarian and sustainably produced alternative to fish oil and other approved algal oils for use in foods (including infant and follow-on formula) as a source of the important LC-PUFA DHA.

## GLOSSARY

| | |
|---|---|
| CFU | colony forming units |
| cGMP | current Good Manufacturing Practice |
| DHA | docosahexaenoic acid |
| DHA-B | DHA-rich microalgal oil from *Schizochytrium* sp |
| DHA-S | DHA-rich oil produced from *Schizochytrium* sp. |
| DHA-O | DHA and EPA-rich oil from *Schizochytrium* sp |
| *E. coli* | *Escherichia coli* |
| EFSA | European Food Safety Authority |
| EPA | eicosapentaenoic acid |
| EU | European Union |
| FDA | United States Food and Drug Administration |
| FOB | functional observational battery |
| LC-PUFA | long chain polyunsaturated fatty acids |
| NOAEL | no-observed-adverse-effect level |
| OECD | Organization for Economic Cooperation and Development |
| PARNUTS | Foods for Particular Nutritional Use |
| RM | reference material |
| *S. typhimurium* | *Salmonella typhimurium* |
| SCF | Scientific Committee on Food |
| U.S. | United States |

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