Application for approval of a novel food

DHA-rich algal oil from *Schizochytrium* sp. T18

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Introduction

This submission concerns an oil from the micro-alga *Schizochytrium* sp. strain T18 which is rich in docosahexaenoic acid (DHA).

DHA-rich oil from the micro-alga *Schizochytrium* sp. T18 has previously received positive opinions on two occasions concerning its substantial equivalence to DHA-rich oil approved under the Novel Food Regulation (EC) No. 258/97[1]. On the first occasion, in 2012[2], the UK Advisory Committee on Novel Foods and Processes (ACNFP) concluded that it is substantially equivalent to the DHA-rich oil from an improved strain of the original wild type *Schizochytrium* sp. ATCC 20888 authorised by Commission Decisions 2003/427/EC[3] and 2009/778/EC[4]. An intention to place the oil on the EU market for the uses approved by those Decisions was notified to the Commission in accordance with Article 5 of Regulation (EC) No 258/97 in April 2012[5]. On the second occasion, in 2015[6], the Food Safety Authority of Ireland concluded that following a change in its production process from a solvent extraction to an enzymatic/aqueous extraction procedure, it remains substantially equivalent to the oil from the improved strain of *Schizochytrium* sp. ATCC 20888 in the approved uses now extended and consolidated by Commission Decision 2014/463/EU[7].

The present submission seeks approval for an extension of the use of DHA-rich oil from the micro-alga *Schizochytrium* sp. T18 to cooking fats, infant and follow-on formulae, and processed cereal-based foods and baby foods for infants and young children, as encompassed by the food uses and levels authorised for DHA-rich algal oil from *Schizochytrium* sp. strain ATCC PTA-9695 by Commission Decision (EU) 2015/545[8], extended by the further addition of use in fruit and vegetable purees.

Identification of information essential to the submission

The subject of this submission is manufactured by fermentation and extraction of the resultant oil fraction from the marine alga *Schizochytrium* sp. T18. It consists of a mixture of triglycerides. The product therefore falls into class 2.1 as defined by Commission Recommendation 97/618/EC of 29 July 1997[9] – complex foods from non-GM sources which have a history of food use in the European Community. In accordance with Table II, Annex I to the Commission Recommendation, the following elements of information are identified as necessary for the safety and nutritional evaluation of a novel food falling within Class 2.1:

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 NF or its source

XI. Nutritional information on the novel food

XII. Microbiological information on the novel food

XIII. Toxicological information on the novel food

These elements are presented in the following sections I through XIII.

I. Specification of the novel food

Structured Scheme I of Commission Recommendation 97/608/EC requires the following questions to be addressed:

- Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?
- Is the information representative of the novel food when produced on a commercial scale?
- Is there an appropriate specification (including species, taxon *etc.* for living organisms) to ensure that the novel food marketed is the same as that evaluated?

These questions are addressed in this section.

Composition of the novel food

The subject of this application is a yellow to orange coloured, semi-solid to liquid oil that is extracted from the wild-type heterotrophic micro-alga *Schizochytrium* sp. T18. It is comprised predominantly of a mixture of triglycerides, in which the principal fatty acid is docosahexaenoic acid (DHA).

The viscosity of the oil (its presentation as a semi-solid or liquid) is directly related to its content of myristic and palmitic acids and is managed through the fermentation conditions to meet the requirements of its application in foods. In dry blending processes, where ingredients in powdered form are mixed together to achieve a uniform blend of macro- and micronutrients, the algal oil must be encapsulated into a powdered form in order to protect it from auto-oxidation during blending or in the finished product. In such applications a semi-solid oil is more advantageous since its higher viscosity physically aids the encapsulation process. In wet blending processes where ingredients are blended together, homogenized and pasteurized prior to spray drying to produce a powdered product, a more liquid form is advantageous and algal oil with a lower concentration of saturated fatty acids is preferred.

The proximate analyses, fatty acid profiles and analyses of the sterol contents of three non-consecutive batches of oil of each type (liquid and semi-solid) are presented in Tables 1, 2 and 3 respectively. Batches 16039, 16040 and 16041 are of liquid oil and batches N-2-006C, N-2-008-C and N-2-010-C are of semi-solid oil. The relevant certificates of analysis and the analytical methods used are provided in Appendices 1 and 2. The reproducibility of the results between batches demonstrates the consistency of the production process.

Table 1 – Proximate analysis

Parameter	***Batch number***					
	16039	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**
Moisture (%)	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05
Ash (%)	<0.05	<0.05	<0.05	<0.1	<0.1	<0.1
Protein (%)	<0.10	<0.10	<0.10	<0.15	<0.15	<0.15
Fat (%)	100.24	99.17	99.86	101.44	100.31	100.49
Carbohydrate (%)	0	0	0	<0.1	<0.1	<0.1

The results of the proximate analyses (Table 1) are consistent across the batches and confirm that the product is essentially exclusively oil.

Table 2 – Fatty acid profile (% area)

Fatty acid	***Batch number***					
	16039	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**
12:0 Lauric	0.92	0.74	0.79	0.97	1.01	1.01
14:0 Myristic	12.30	9.0	9.5	13.12	13.63	13.65
14:1 Myristoleic	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
15:0 Pentadecanoic	0.68	0.45	0.56	0.42	0.51	0.52
16:0 Palmitic	22.67	21.46	21.76	27.87	29.45	29.39
16:1 Palmitoleic	6.16	3.63	4.21	2.1	2.2	2.23
17:0 Heptadecanoic	0.15	0.12	0.14	<0.10	<0.10	0.10
18:0 Stearic	0.77	0.83	0.78	0.84	0.85	0.85
18:1 Oleic + cis-vaccenic	7.49	8.06	7.26	2.17	1.81	1.85
18:2 Linoleic	0.34	0.78	0.56	<0.10	<0.10	<0.10
18:3 gamma-Linolenic	0.24	0.42	0.33	0.13	0.11	0.12
18:4 Octadecatetraenoic	0.24	0.32	0.30	0.23	0.20	0.21
20:0 Eicosanoic (arachidic)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
20:1 Eicosenoic	<0.10	<0.10	<0.10	<0.01	<0.01	<0.01
20:3 (n-6) Eicosatrienoic	<0.10	<0.10	<0.10	0.15	<0.10	<0.10
20:4 (n-6) Arachidonic	0.65	0.76	0.75	0.74	0.64	0.63
20:5 (n-3) Eicosapentaenoic	1.08	1.59	1.49	1.12	0.90	0.90
22:0 Docosanoic	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
22:5 (n-6) Docosapentaenoic	7.21	7.65	8.12	8.38	7.73	7.78
22:6 (n-3) DHA	37.1	42.47	41.98	40.54	39.64	39.60
24:0 Tetracosanoic	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10

The fatty acid profile (Table 2) is consistent across batches. All of the fatty acids detected are well-known components of the human diet and found in both animal and vegetable food sources. The major fatty acids comprise DHA, myristic acid, palmitic acid, and docosapentaenoic acid. Literature searches did not identify safety/toxicity concerns related to any individual fatty acid or their ratios in the proposed DHA algal oil.

Table 3 – Sterol content (% total sterols)

Sterol	***Batch number***					
	16039	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**
Cholesterol	21.7	12.8	12.6	24.3	32.9	32.2
Brassicasterol	6.5	4.6	6.3	<0.1	<0.1	<0.1
24-methylene cholesterol	2.8	2.3	3.3	3.9	7.1	6.1
Campesterol	1.5	3.9	3.2	1.2	1.4	2.7
Campestanol	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Stigmasterol	22.5	23.1	21.7	<0.1	7.2	6.9
delta-7-campesterol	<0.1	<0.1	<0.1	3.4	7.0	6.7
delta-5,23-stigmastadienol	3.0	3.8	<0.1	6.9	6.2	7.7
Clerosterol	14.5	19.3	17.9	8.8	8.2	6.3
beta-sitosterol	14.8	11.4	13.7	13.4	9.4	11.5
Sitostanol	<0.1	0.5	<0.1	<0.1	<0.1	<0.1
delta-5-avenasterol	3.8	4.7	5.7	1.4	1.2	1.3
delta-5,24-stigmastadienol	4.1	6.8	6.2	7.0	3.9	6.1
delta-7-stigmastenol	<0.1	<0.1	<0.1	26.1	14.0	11.0
delta-7-avenasterol	5.0	5.1	9.1	3.6	1.5	1.4
Total Sterols (mg/kg fat)	900	1070	831	2310	1900	1990

The individual sterols and stanols identified (Table 3) are generally present in the diet from vegetable and animal sources such as edible oils. At the level detected of less than 0.25% total on the fat basis, under the intended conditions of use (Section IX), the total intake of sterols from the *Schizochytrium* sp. T18 oil will be minimal.

Identification of Impurities

a. Elemental analysis

The results of elemental analysis for six non-consecutive batches of oil are presented in Table 4. The results confirm that the oil is compliant with the maximum limit for lead imposed by the requirements of Commission Regulation (EC) No 1881/2006 for the presence of lead in oils intended for human consumption[10].

Table 4 – Elemental analysis

Analyte	***Batch number/result (mg/kg)***					
	16039	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**
Arsenic	<0.1	<0.1	<0.1	<0.01	<0.01	<0.01
Chromium	<0.05	<0.05	<0.05	<0.1	<0.1	<0.1
Copper	<0.1	<0.1	<0.1	0.08	0.02	0.03
Iron	0.5	<0.1	<0.1	<0.02	<0.02	<0.02
Lead	<0.05	<0.05	<0.05	<0.01	<0.01	<0.01
Manganese	<0.1	<0.1	<0.1	<0.01	<0.01	<0.01
Mercury	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01
Molybdenum	<0.1	<0.1	<0.1	<0.05	<0.05	<0.05
Nickel	<0.1	<0.1	<0.1	0.3	0.3	0.3
Phosphorus	<3	<3	<3	<2	<2	<2
Silicon	110	51	67	79	80	75
Sulphur	3	<2	<2	1.6	<1.0	<1.0

b. Microbiological analysis

The results of microbiological assay of six non-consecutive batches of oil are presented in Table 5. The results for *Salmonella*, *E. coli*, coagulase+ *Staphylococcus*, yeasts, moulds and total coliforms were all at, or below the relevant assay thresholds.

Table 5 – Microbiological analysis

Assay	***Batch number***					
	16039	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**
Salmonella	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g
Escherichia coli	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Staphylococci, coagulase+	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Yeast	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Mould	<10 CFU/ml	<10 CFU/ml	<10 CFU/ml	<10 CFU/g	<10 CFU/g	<10 CFU/g
Total Coliforms	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g

CFU = Colony Forming Units

Specification

The common or usual name of the food is DHA-rich algal oil. It complies with the specification presented in Table 6. The specification reflects the quality and characteristics of the material presented for market.

Table 6 – Specification for DHA-rich oil from *Schizochytrium sp*. T18

Parameter	***Specification***	***Test method***
Acid Value (KOH/g)	0.5 maximum	AOCS Cd 3d-63
Peroxide Value (meq/kg)	5.0 maximum	AOCS Cd 8-53
Moisture (%)	0.05 maximum	AOAC 930.15
Unsaponifiables (%)	3.5 maximum	AOCS Ca 6b-53
Trans-fatty acids (%)	2.0 maximum	AOCS 2a-94
DHA (% relative)	35 minimum	AOCS Ce 2-66/AOCS Ce 1b-89
Arsenic (mg/kg)	<0.1	AOAC vol. 90 (2007) 844-856 (Mod)
Copper (mg/kg)	<0.1	AOCS Ca 17-01
Iron (mg/kg)	<0.2	AOCS Ca 17-01
Mercury (mg/kg)	<0.1	J. AOAC vol. 90 (2007) 844-856 (Mod)
Lead (mg/kg)	<0.1	J. AOAC vol. 90 (2007) 844-856 (Mod)

Consistency of the production process

Results of the analysis of six non-consecutive batches of oil are provided in Table 7 and confirm that the above specification is consistently met.

Table 7 – Analysis of multiple lots of product

Parameter	***Batch number***					
	16039	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**
Acid Value (mg KOH/g)	0.05	0.06	0.05	0.06	0.06	0.06
Peroxide Value (meq/kg)	1.0	1.0	1.3	1.06	<0.1	<0.1
Moisture (%)	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05
Unsaponifiables (%)	0.3	0.4	0.3	2.97	2.43	2.50
Trans-Fatty Acids (%)	0.2	0.2	0.2	<0.05	<0.05	<0.05
DHA (% Relative)	37.1	42.5	42.0	40.5	39.6	39.6
Arsenic (mg/kg)	<0.1	<0.1	<0.1	<0.01	<0.01	<0.01
Copper (mg/kg)	<0.1	<0.1	<0.1	0.08	0.02	0.03
Iron (mg/kg)	0.15	<0.1	<0.1	<0.02	<0.02	<0.02
Mercury (mg/kg)	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01
Lead (mg/kg)	<0.05	<0.05	<0.05	<0.01	<0.01	<0.01

Shelf-life and stability

Oxidative stability was assessed by measurement of residual DHA and peroxide and anisidine values after storage for 7 and 12 months under frozen conditions. DHA algal oil is typically shipped and stored under a nitrogen blanket in a closed, light resistant container under frozen conditions (-25°C). The results presented in Table 8 support the stability of the frozen product for a period of one year. Proposed labelling will recommend product use (“best before date”) within 1 year of the date of manufacture.

Table 8 – Results of stability study

Batch number	Test parameter	Specification	Result at time (months)		
			0	7	12
N-2-008-C; frozen	DHA (%)	minimum 35%	39.6	39.3	42.6
	Peroxide value (meq/kg)	< 5	<1.0	1.3	1.0
	Anisidine value	<15	NA	8.7	9.3

Two batches have also been tested after storage under accelerated stability conditions over a period of 8 weeks and found to be stable. The results of the accelerated storage tests are presented in Table 9.

Table 9 – Results of accelerated stability study

Batch number/test conditions	Test parameter	Specification	Result at time (weeks)		
			0	4	8
N-2-008-C; refrigerated at 5°C	DHA (%)	Minimum 35%	39.6	38.9	38.4
	Peroxide value (meq/kg)	<5	<0.1	2.2	1.5
N-2-010-C; stored at 25°C at 60% relative humidity	DHA (%)	Minimum 35%	39.6	38.6	38.3
	Peroxide Value (meq/kg)	<5	<0.1	2.8	1.4

II. Effect of the production process

Structured Scheme II of Commission Recommendation 97/608/EC requires the following questions to be addressed:

- Does the novel food undergo a production process?
- 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?
- Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?"
- Are the means identified for controlling the process to ensure that the novel food complies with its specification?
- Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?
- After processing is the novel food likely to contain micro-organisms of adverse public health significance?

These questions are addressed in this section.

Overview of production process

An oil rich in polyunsaturated fatty acids (PUFA), in particular DHA, is produced by a heterotrophic fermentation process with a single cell marine micro-alga of the genus *Schizochytrium*. The fermentation process uses a medium containing carbon and nitrogen sources, bulk and trace mineral nutrients, and vitamins. The oil is produced and accumulates intracellularly during fermentation. After the fermentation is complete, the crude algal oil is recovered following disruption of the algal cell walls and is refined by techniques commonly used in the commercial processing of edible oils. The oil is manufactured in accordance with Hazard Analysis Critical Control Point (HACCP) procedure and cGMP, including quality control (QC) checks at every stage of the production process. All the steps in the production process are conducted under conditions that minimize the risk of contamination with foreign materials.

- **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?**

  The fermentation, oil extraction and refinement procedures use processes commonly in use in the production of edible oils widely used as food ingredients.

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

  The fermentation process takes place under axenic conditions in accordance with HACCP-based principles to ensure that the growth of undesirable micro-organisms is excluded. The fatty acid profile of the refined oil (Table 2) comprises fatty acids typically present in common edible oils and fats and which may therefore be regarded as toxicologically and nutritionally equivalent to those found in conventional foods.

- **Are the means identified for controlling the process to ensure that the novel food complies with its specification?**

  The fermentation process takes place under axenic conditions in accordance with HACCP-based principles and the extraction and refinement procedures employ processes commonly used in the edible oil industry for which the operating parameters are well understood. The refined oil is routinely monitored to ensure that it consistently meets the specification set out in the previous section using the methods listed (Section I Specification of the Novel Food).

- **Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?**

  As described above, the fermentation process takes place under axenic conditions in accordance with HACCP-based principles and the extraction and refinement procedures employ processes commonly used in the edible oil industry for which the operating parameters are well understood. The refined oil and the biomass from which it is obtained have been screened for a range of algal toxins to confirm that they are not produced by the source organism under the process conditions (Section III and Appendix 1). The final product is routinely monitored to ensure that it consistently complies with the limits for the contaminants set out in the previous section (Section I Specification for the Novel Food).

- **After processing is the novel food likely to contain micro-organisms of adverse public health significance?**

  The fermentation process takes place under axenic conditions in accordance with HACCP-based principles to ensure that the growth of undesirable micro-organisms is excluded. The refined algal oil is routinely monitored to ensure that it consistently meets the microbiological criteria for *Salmonella*, *Escherichia coli*,

coagulase positive *Staphylococci*, yeast, mould and total coliforms set out in Table 5, Section I.

III. History of the organism used as the source of the novel food

Structured Scheme III of Commission Recommendation 97/608/EC requires the following questions to be addressed:

- Is the novel food obtained from a biological source, i.e., a plant, animal or micro-organism?
- Has the organism used as the source of the novel food been derived using GM?
- Is the source organism characterised?
- Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?

These questions are addressed in this section.

Identity of the source organism

The source of the DHA-rich algal oil that is the subject of this submission is a strain of marine micro-alga of the genus *Schizochytrium*, originally owned by Ocean Nutrition Canada and designated as ONC T18, now under the ownership of Mara Renewables Corp. and re-designated more simply as T18. It has not been subjected to any form of genetic modification.

Characterisation of the source organism

The micro-algal family *Thraustochytriaceae* has historically comprised seven genera, *Japanochytrium*, *Schizochytrium*, *Ulkenia*, *Althornia*, *Diplophrys*, *Aplanochytrium* and *Thraustochytrium*, all of which are referred to as thraustochytrids. Under this classificatory scheme, the source organism strain T18 has previously been assigned to the genus *Thraustochytrium* (Burja *et al*., 2006[11]). The genera *Schizochytrium*, *Ulkenia and Thraustochytrium* comprise marine protists commonly found in marine and estuarine environments. DHA-rich oils from two species (represented by three separate strains) of *Schizochytrium*[7, 8, 12] and one species of *Ulkenia*[13] have previously been the subject of European Commission authorisation decisions approving their use in foods.

In recent times the taxonomic structure of the family *Thraustochytriaceae* has been the subject of discussion and the redistribution of some of the component organisms into a broader suite of genera has been proposed, in particular in relation to members of the genus *Schizochytrium* (Yokoyama and Honda, 2007[14]) and the genus *Ulkenia* (Yokoyama, Salleh and Honda 2007[15]). In light of the on-going debate, Ocean Nutrition Canada, when it was the owner of T18, commissioned an expert review of the relationship between the thraustochytrid strain T18 and *Schizochytrium* sp. ATCC 20888, the parent wild-type strain which

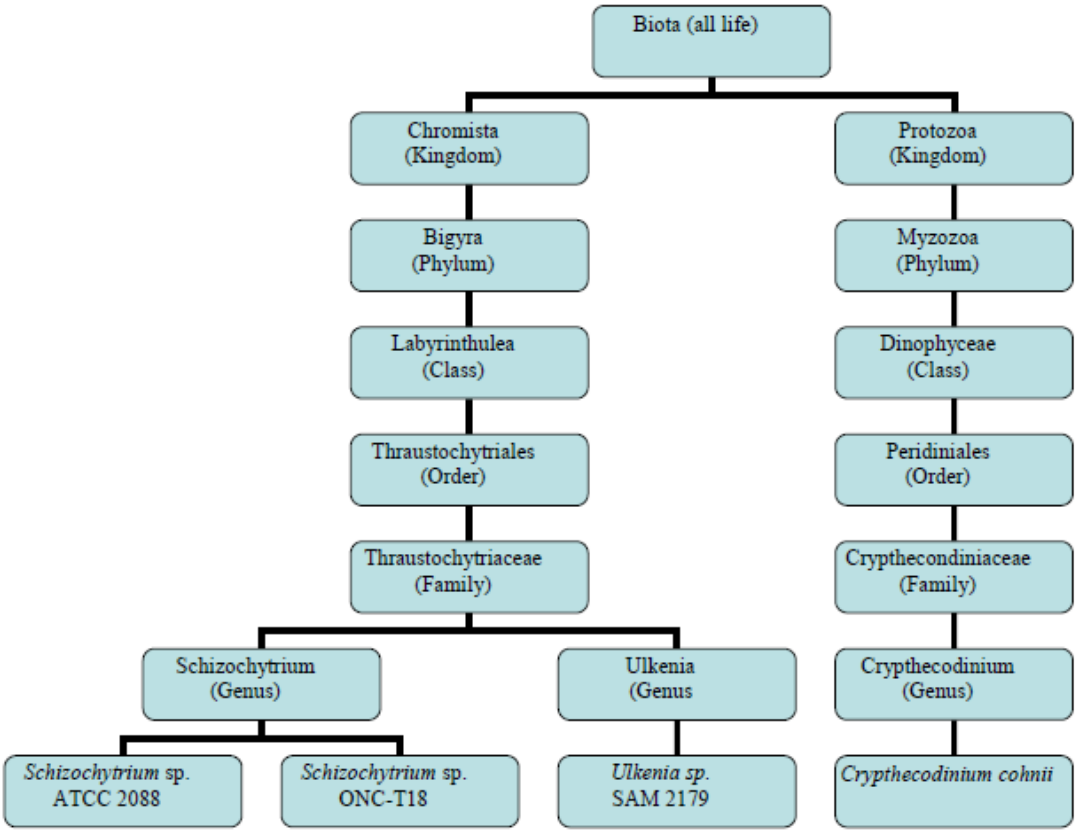
is the basis of Commission authorization decision 2014/463/EC[7]. The review concluded that, on the basis of their morphological characteristics, their pigment and fatty acid profiles and a comparison of small subunit ribosomal DNA (SSU-rDNA) sequences, and notwithstanding the on-going scientific debate about the taxonomy of the family *Thraustochytriaceae* as a whole, these two organisms are closely related and that strain T18 is more appropriately to be considered as falling within the genus *Schizochytrium sensu lato*. The report of this study is attached as Appendix 2. The conclusion has been supported by an additional independent expert review attached as Appendix 3.

The taxonomic classification of the source organism is therefore as follows[16]:

Kingdom: *Chromista*
Phylum: *Bigyra*
Class: *Labyrinthulea*
Order: *Thraustochytriida*
Family: *Thraustochytriaceae*
Genus: *Schizochytrium*

The taxonomic relationship between *Schizochytrium* sp. T18 and the source organisms of the micro-organism-derived DHA-rich oils currently in use in foods on the EU market can thus be represented as set out in Figure 3.

Figure 1 - Taxonomic relationship between DHA-rich oil producing micro-organisms



(Note: DHA-rich oil from the protozoan *Crypthecodinium cohnii* was in use in food in the EU before Regulation (EC) No 258/97 came into effect; it therefore did not require evaluation and explicit approval as a novel food)

Absence of toxins

Toxin production by *Schizochytrium sp*. T18 is unlikely since there are no reports of toxin production by any of the *Thraustochytriacea*, the family of which *Schizochytrium* is a member. To confirm the absence of toxins, samples of the refined algal oil and the biomass (freeze-dried) from which it is obtained have been screened for the following algal toxins:

Domoic Acid	Okadaic Acid
Gymnodimine	Dinophysistoxin-1
Desmethylspirolide C	Dinophysistoxin-2
Azaspiracid-1	Yessotoxin
Azaspiracid-2	Prymnesin-1
Azaspiracid-3	Prymnesin-2
Pectenotoxin-2	

None of the toxins were detected in extracts of the oil or the freeze-dried biomass from which the oil is obtained (report at Appendix 1).

IX. Anticipated intake/extent of use of the novel food

Structured Scheme IX of Commission Recommendation 97/608/EC requires the following questions to be addressed:

- Is there information on the anticipated uses of the novel food based on its properties?
- Is there information to show anticipated intakes for groups predicted to be at risk?
- Will introduction of the novel food be restricted geographically?
- Will the novel food replace other foods in the diet?
- Are any of the foods replaced significant nutritional sources?
- Does the probable level of substitution have a nutritional significance for any population groups?

These questions are addressed in this section.

Anticipated uses of DHA-rich algal oil from *Schizochytrium* sp. T18

Dietary recommendations from national and international bodies for intakes of n-3 long chain polyunsaturated fatty acids (mostly as EPA and DHA), as reviewed by the European Food Safety Authority[17], range from 200 to around 600 mg/day for adults, and from 40 to 250 mg/day for infants older than six months, children and adolescents. Algal oil from *Schizochytrium* sp.T18 is requested for use in food as an alternative source of DHA to those currently in use, for example marine oils from fish, krill and other algal sources. It is requested for use in the same foods and at the same levels as those currently authorised by Commission Implementing Decision (EU) 2015/545[8], with the additional application of use in fruit/vegetable purees. The list of foods and levels of use authorised by Decision (EU) 2015/545 is reproduced in Table 12 with food descriptions modified where relevant to take account of recent changes to the legislation concerning foods for particular nutritional uses and foods for specific groups[18], and with the addition of fruit/vegetable purees.

Table 10 – Proposed food uses and use levels for algal oil from *Schizochytrium* sp. T18

Food category	*Maximum level of use expressed as DHA*
Dairy products except milk-based drinks	200 mg/100 g; 600 mg/100 g in the case of cheese products
Dairy analogues except drinks	200 mg/100 g; 600 mg/100 g in the case of cheese analogues
Spreadable fat and dressings	600 mg/100 g
Breakfast cereals	500 mg/100 g
Food supplements	250 mg DHA per daily dose as recommended by the manufacturer for normal population 450 mg DHA per daily dose as recommended by the manufacturer for pregnant and lactating women
Foods intended for use in energy-restricted diets for weight reduction (including foods intended for use in total diet replacement for weight control as defined in Regulation (EU) No 609/2013)	250 mg per meal replacement equivalent
Foods intended to meet the expenditure of intense muscular effort	200 mg/100 g
Foods intended for people with gluten intolerance	200 mg/100 g
Foods for special medical purposes as defined in Regulation (EU) No 609/2013	Used in accordance with Regulation (EU) 2016/128 (50 mg/100 kcal in the case of foods intended for infants)
Bakery products (breads and rolls), sweet biscuits	200 mg/100 g
Cereal bars	500 mg/100 g
Cooking fats	360 mg/100 g
Non-alcoholic beverages (including dairy analogue and milk-based drinks)	80 mg/100 ml
Infant formula and follow-on formula	Used in accordance with Regulation (EU) 2016/127*
Processed cereal-based foods and baby foods for infants and young children, including those defined in Regulation (EU) No 609/2013	200 mg/100 g
Fruit/vegetable puree	100 mg/100 g

*DHA is currently permitted as a voluntary addition to infant and follow-on formulae under Directive 2006/141/EC. Under Regulation (EU) 2016/127, which comes fully into effect in February 2020, the addition of DHA will become mandatory in the range 20 mg/100 kcal minimum to 50 mg/100 kcal maximum.

Anticipated intakes by groups predicted to be at risk

Intakes of DHA from algal oil from *Schizochytrium* sp. T18 when used in accordance with the applications set out in Table 11 were estimated using food consumption data from the EFSA Comprehensive European Food Consumption Database[19]. For this purpose, the food categories proposed for use (Table 12) were converted into the EFSA FoodEx2[20] coding system, which is used to record values in the EFSA Comprehensive European Food Consumption Database. DHA concentration values used as inputs to the dietary exposure model were derived from the use levels in Table 12 and are provided in Appendix 4.

For foods intended for use in energy-restricted diets for weight reduction purposes it was assumed that a typical meal size was 250g (based on food retailers' information – see for example Boots UK[21]) giving a DHA concentration of 1000 mg/kg. For infant and follow on formulae (and foods intended for special medical purposes) a standard energy content of not less than 60 kcal (250 kJ)

and not more than 70 kcal (295 kJ) per 100 ml, as required by Regulation (EU) 2016/127[22], was taken. Applying a maximum DHA use level of 50 mg/100 kcal gives a maximum DHA level in reconstituted formulae of 35 mg/100g. Using a standard re-constitution ratio gives 238 mg/100g in dried formula powder.

It was not possible to convert daily doses of DHA from food supplement use into use levels suitable for exposure modelling with the EFSA Comprehensive database and so intakes from this source are addressed separately.

These values were entered into the Comprehensive European Dietary Exposure Model (CEDEM)[23] which is based on the methodology from the EFSA Food Additive Intake Model (FAIM)[24] and uses data taken from the published version of the EFSA Comprehensive European Food Consumption database[19].
The EFSA Comprehensive European Food Consumption Database comprises data from 19 European countries sub-divided, where the availability of data permits, into 6 age-bands to include potential at-risk sub-populations:

1. Infants: up to and including 11 months
2. Toddlers: from 12 up to and including 35 months of age
3. Other children: from 36 months up to and including 9 years of age
4. Adolescents: from 10 up to and including 17 years of age
5. Adults: from 18 up to and including 64 years of age
6. Elderly: from 65 up to and including 74 years of age
7. Very elderly: from 75 years of age and older

Each survey relating to one of the age ranges defined above has been extracted into the CEDEM dietary exposure model. In the CEDEM model the population average and 95th percentile consumer intakes from each food category is calculated. High level intake for each population group is estimated by adding the highest 95th percentile value from any group to the sum of the population averages for all other foods.

Because the Comprehensive database is drawn from many different sources, the duration of each survey and the quality of the data can vary. This means that not all food categories have been reported at all levels of definition in the FoodEx categorisation system. It was judged that working at level three (L3) in the system was most appropriate for matching with food additive authorisation categories but this has meant that in some cases data were included at L2 where more precise data were unavailable. This would lead to conservatism in those estimates.

Some of the surveys included relatively small numbers of subjects which resulted in very few measures of consumption for certain foods. EFSA warns that where the number of observations is lower than 60, the 95th and higher percentiles may not be statistically robust. In order to remove this uncertainty, the database has been constructed without P95 values where the number of observations was less than 60. In some country/age-group combinations the total number of subjects

was very small and in some cases the total was less than 60 individuals. This mainly affects some infant and elderly populations and in these cases the average intake is reported but not the high level. This is indicated by 'insufficient consumers' in the reporting tables.

The output of the dietary exposure modelling using CEDEM is summarised in Table 13, where the lowest and highest country-reported values for the average and high level potential intakes by each age group are presented. Average potential intakes of DHA from use as a novel food ingredient ranged from 7.8 mg/kg bw/day for adults in Spain up to 59.3 mg/kg bw/day for toddlers in Belgium. High level potential intakes ranged from 10.2 mg/kg bw/day for the elderly in Sweden up to 190.8 mg/kg bw/day for infants in Bulgaria. The main sources of high level intakes were fermented milk products (including yogurts), porridge and flavoured milk. Detailed results are provided in Appendix 4.

Table 11 - Dietary exposure modelling results

		Population Average Intake	Total High Level Intake
INFANTS			
	Min*	17.77	43.15
	Max*	45.70	190.82
TODDLERS			
	Min*	28.91	42.36
	Max*	59.34	110.14
OTHER CHILDREN			
	Min*	16.75	28.71
	Max*	51.11	101.02
ADOLESCENTS			
	Min*	8.44	13.31
	Max*	26.00	47.81
ADULTS			
	Min*	7.75	14.33
	Max*	17.88	39.55
ELDERLY			
	Min*	8.52	11.22
	Max*	18.14	39.94
VERY ELDERLY			
	Min*	8.81	10.19
	Max*	14.07	29.97

* lowest/highest estimated national intake (mg/kg bw/day)

Intakes of DHA for supplement users, given the daily dose recommendations of 250 or 450 mg/day, would range from 4.2 mg/kg bw/day for 60 kg adults in the normal population up to 7.5 mg/kg bw/day for pregnant and lactating women. Supplement use would therefore make a relatively low contribution to total intake for the majority of consumers.

From about the age of 4 – 6 months onwards infants will be starting to take a mixed diet, including infant formula/follow-on formula together with cereal-based baby foods and moving on to dairy products and other non-specialised foods. These will be reported in the EFSA Comprehensive database by the CEFC database and are reflected in the CEDEM analysis in Table 12. For bottle-fed infants up to the age of 6 months for whom the sole source of nutrition is formula (or breast milk) and who may be assumed to be fed in accordance with the manufacturers' instructions, the maximal feeding rates for infants of 4 months age (around 7 kg weight) equate to an energy intake of around 750 – 800 kcals/day. Infants of this age fed solely on formula might therefore expect to take in up to 400 mg DHA per day at a use rate of 50 mg/100 kcal, which would represent a DHA intake of around 57 mg/kg bw/day. This value lies between average and high level intakes for infants obtaining DHA from all sources described by the CEDEM model.

Estimates of population average intakes of DHA from novel food use range from about 8 mg/kg bw/day (in the case of the elderly) up to around 60 mg/kg bw/day (in the case of toddlers) depending on age range and country of residence. High level intakes have the potential to range up to 190 mg/kg bw/day but for this level of intake to be sustained in the longer term it would be necessary for an infant always to consume products containing the maximum concentrations of DHA and this scenario is likely to be overly conservative.

Will introduction of the novel food be restricted geographically?

It is not the intention to restrict the introduction of DHA-rich algal oil from *Schizochytrium* sp. T18 as a novel food geographically.

Will the novel food replace other foods in the diet?

DHA-rich algal oil from *Schizochytrium* sp. T18 is intended to provide an alternative source of DHA to other sources currently on the market.

Are any of the foods replaced significant nutritional sources?

DHA-rich algal oil from *Schizochytrium* sp. T18 will be nutritionally equivalent to the sources of DHA it replaces

Does the probable level of substitution have a nutritional significance for any population groups?

When used in the food categories and at the use levels listed in Table 11, DHA-rich algal oil from *Schizochytrium* sp. T18 will provide a nutritionally significant source of dietary DHA for consumer groups across the whole age range.

X. Information from previous human exposure to the novel food or its source

Structured Scheme X of Commission Recommendation 97/608/EC requires the following questions to be addressed:

- 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?

- Is there information to demonstrate that exposure to the novel food is unlikely to give rise to nutritional, microbiological, toxicological and/or allergenicity problems?

Information on previous exposure to the novel food and its source, and related sources, is presented in this section. Information concerning nutritional, microbiological and toxicological and/or allergenicity aspects of the novel food is presented in Sections XI, XII and XIII respectively.

Previous use of DHA-rich oil from *Schizochytrium* sp. T18

DHA-rich oil from *Schizochytrium* sp. T18 has previously been judged by the UK ACNFP[2] to be substantially equivalent to the oil from *Schizochytrium* sp. authorised by Commission Decisions 2003/427/EC and 2009/778/EC, and an intention to place the oil on the EU market for the food uses listed in the Annexes to those Decisions was notified to the European Commission in April 2012 under Article 5 of Regulation (EC) No 258/97[5]. In accordance with that Article and with Commission Decision 2014/463/EU[7], the oil has been accepted in the EU since that date for the food uses listed in Table 14.

Table 12 – Food uses authorised by Commission Decisions 2003/427/EC and 2009/778/EC

Food category	***Maximum level of use expressed as DHA***
Dairy products except milk-based drinks	200 mg/100 g; 600 mg/100 g in the case of cheese products
Dairy analogues except drinks	200 mg/100 g; 600 mg/100 g in the case of cheese analogues
Spreadable fat and dressings	600 mg/100 g
Breakfast cereals	500 mg/100 g
Food supplements	200 mg DHA 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 per meal replacement
Bakery products (breads and rolls)	200 mg/100 g
Cereal bars	500 mg/100 g
Non-alcoholic beverages (including dairy analogue and milk-based drinks)	60 mg/100 ml

These applications are subsumed within those now requested (Table 11) and will contribute an intake within that estimated in Section IX.

DHA-rich oils from micro-algae related to *Schizochytrium* sp. T18

DHA-rich oils from taxonomically related strains of thraustochytrid micro-algae (see Figure 3 for relationships) have been authorised for use in food in the EU since 2003 in the case of oils produced from a derived strain of *Schizochytrium* sp. strain ATCC 2088[3], and from *Ulkenia* sp.strain SAM2179[25]. Extensions to the range of foods in which these two oils may be used have been authorised over the intervening period[7,13] and, more recently, in 2015, DHA-rich and DHA and EPA-rich oils from two further strains of *Schizochytrium* sp. have been authorised for use in food in the EU[8,12].

DHA-rich algal oils from strains of *Schizochytrium* sp. and *Ulkenia* sp. are considered Generally Recognized as Safe (GRAS) for use in a range of foods, including infant formula, in the USA.[26,27,28]

XI. Nutritional information on the novel food

Structured Scheme XI of Commission Recommendation 97/608/EC requires the following question to be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

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

This question is addressed as follows:

Nutritional equivalence of DHA-rich algal oils

The proximate analyses and fatty acid profiles of six non-consecutive batches of DHA-rich algal oil from *Schizochytrium* sp. T18 are presented in Tables 1 and 2 respectively in Section I. The fatty acids identified are all well-known components of the human diet and are commonly found in edible oils and fats, including DHA-rich oils of fish and algal origin which the oil from *Schizochytrium* sp. T18 is intended to replace. At the intended levels of use (50 – 600 mg DHA-equivalent/100g of food, see Section IX), any small differences in the fatty acid profiles of the oils will have no significance for their relative nutritional values or metabolic impacts and the oil from *Schizochytrium* sp. T18 may be considered nutritionally equivalent to the oils it will replace.

XII. Microbiological information on the novel food

Structured Scheme XII of Commission Recommendation 97/608/EC requires the following question to be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- Is there information to show that the novel food is unlikely to contain micro-organisms and/or their metabolites of adverse public health significance?

This question is addressed as follows:

Conditions of the production process

Prior to production, the fermentation vessel all pipelines, and fermentation media are subjected to a timed, and controlled sterilisation process. The fermentation is carried out under axenic conditions and operating parameters, such as temperature, pH, aeration, and agitation, are controlled throughout the process. The vessel is operated under positive pressure to prevent any ingress by foreign organisms. Once fermentation is complete, the oil is released from the source organism by enzymatic disruption of the algal cell walls and the enzyme is deactivated by subjecting the broth to a temperature of greater than 85˚C for a period of at least 15 minutes. The oil is then separated from the hydrolysed biomass by centrifugation, refined and stored in sealed containers at a temperature no greater than 4°C. These process conditions ensure that no undesirable micro-organisms or their metabolites enter the production stream and no intact cells of the source organism are present in the finished oil. The oil itself is essentially 100% lipid, with very low water activity, and is inherently microbiologically stable.

Absence of microbiological contamination is confirmed by assays for *Salmonella*, *E. coli*, coagulase+ *Staphylococcus*, yeasts, moulds and total coliforms, all of which results are routinely found to be at, or below the relevant assay thresholds (see Section I, Table 5).

XIII. Toxicological information on the novel food

Structured Scheme XIII of Commission Recommendation 97/608/EC requires the following questions to be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?
- Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?

or

- 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?
- Is there information which suggests that the novel food might pose an allergenic risk to humans?

These questions are addressed in this section.

Existence of a traditional counterpart as a comparator

The European Food Safety Authority has previously evaluated the scientific basis for tolerable upper intake levels (ULs) for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA)[17]. While concluding that the available data are insufficient to establish ULs for them individually or combined, the Authority has concluded that supplemental intakes of up to 5 g/day for DHA and EPA combined for adults, and up to 1 g/day for DHA in isolation for the general population do not raise safety concerns. A wide range of fish oils and oils from marine organisms and micro-organisms, including micro-algae, have traditionally been used as food ingredients to supplement DHA in the diet. This history of use provides general support for the safety in use of DHA-rich oils as food ingredients.

Information on the absence of algal toxins and from toxicological studies further support the safety specifically of DHA-rich oil from *Schizochytrium* sp. T18 in the food uses requested and is presented below.

Absence of algal toxins

The absence of toxin production by the source organism and confirmation of their absence by analysis is discussed in relation to the taxonomical characterisation *Schizochytrium* sp. T18 in Section III. The report of the analytical study confirming absence is presented at Appendix 1.

Toxicological studies

Genotoxicity

A battery of *in vitro* and *in vivo* genotoxicity tests (microbial reverse mutation assay, *in vivo* rat bone marrow micronucleus assay, and chromosomal aberration assay in cultured human peripheral blood lymphocytes) has been conducted with DHA-rich algal oil from *Schizochytrium* sp. T18.

Microbial reverse mutation assay

DHA-rich algal oil from *Schizochytrium* sp. T18 was subjected to a microbial reverse mutation assay based on OECD Guideline 471 using *Salmonella typhimurum* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2*uvrA* (328) at levels of 313, 625, 1250, 2500 and 5000 µg/plate with and without S9 activation, and with appropriate vehicle and positive controls and monitoring for cytotoxicity. The DHA-rich algal oil did not cause an increase in the number of revertants in the presence or absence of S9 activation under the conditions of the assay (Schmitt et al., 2012a[29]).

In vivo, rat bone marrow micronucleus assay

DHA-rich algal oil from *Schizochytrium* sp. T18 was subjected to an *in vivo* rat bone marrow micronucleus assay based on OECD Guideline 474. Single doses of the test article were administered by gavage at 500, 1000 and 2000 mg/kg bw to 5 male Hsd:Sprague-Dawley rats per dose group, with appropriate negative and positive controls and monitoring for cytotoxicity. The DHA-rich algal oil did not induce a statistically significant increase in micronuclei in polychromatic erythrocytes (PCEs) at any of the doses tested and the oil was considered to be negative in the rat bone marrow micronucleus test under the conditions of the assay (Schmitt et al., 2012a[29]).

In vitro chromosomal aberration assay in cultured human peripheral blood lymphocytes

DHA-rich algal oil from *Schizochytrium* sp. T18 was subjected to a chromosomal aberration assay in cultured human peripheral blood lymphocytes. The assay was based on OECD Guideline 473. The test article was administered to cultures at 500, 750 and 1000 mg/ml in the absence of S9 activation and at 750, 1000 and 1500 mg/ml in the presence of S9 activation, with appropriate vehicle and positive controls. No significant increases in cells with chromosomal aberrations, polyploidy or endoreduplication were observed at any of the dose levels investigated. The DHA-rich oil was considered negative for the induction of

chromosomal aberrations, with or without S9 activation, under the conditions of the assay (Schmitt et al., 2012a[29]).

Acute toxicity

DHA-rich algal oil from *Schizochytrium* sp. T18 was administered to 3 female Sprague-Dawley rats in an acute toxicity study based on OECD Guideline 425. A single dose of 5000 mg/kg bw was administered by oral intubation and rats were observed over a 14-day period before necropsy on day 15. No deaths occurred and all rats continued to gain bodyweight over the study period. No gross abnormalities were noted at necropsy. Under the conditions of the assay, the acute oral LD_{50} of the DHA-rich oil was greater than 5000 mg/kg of body weight in Sprague-Dawley rats (Schmitt et al., 2012a[29]).

Sub-chronic toxicity

DHA-rich algal oil was administered at concentrations of 0, 10,000, 25,000 and 50,000 ppm in the diet to 10 male and 10 female Hsd:Sprague-Dawley rats per dose group in a 90-day repeat oral toxicity study carried out in accordance with OECD Guideline 408 without any significant toxicological manifestations (Schmitt et al., 2012a[29]). The algal oil was well-tolerated and there was an absence of major treatment-related effects on the general condition and appearance of the rats, neurobehavioral endpoints, growth, feed and water intake, ophthalmoscopic examinations, routine haematology and clinical chemistry parameters, urinalysis, and necropsy findings. The no observed adverse effect level (NOAEL) was the highest dietary concentration level of 50,000 ppm, equivalent to 3,305 and 3,679 mg/kg bw/day for male and female rats, respectively.

Reproductive/developmental toxicity

A developmental toxicity study and a 3-month dietary toxicity study with an *in utero* exposure phase were conducted on oil from *Schizochytrium* sp. T18 in Sprague-Dawley Crl:CD(SD) rats (Schmitt et al., 2012b[30]).

In the developmental toxicity study, which was conducted in accordance with OECD Guideline 414, the test material was administered orally by gavage to 3 groups of 25 pregnant female rats once daily from days 6 to 19 of gestation at dose levels of 400, 1000 and 2000 mg/kg bw/day at a dosage volume of 5 ml/kg bw in corn oil as a carrier. A concurrent control group of 25 pregnant female rats received corn oil alone on a comparable regimen. Animals were observed for mortality and moribundity twice daily, and clinical observations, body weights and food consumption were recorded at appropriate intervals. Necropsy was performed on day 20 of gestation and uteri, placentae and ovaries were examined and the number of foetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded and net body weights and body weight changes were calculated. The foetuses were weighed,

sexed and examined for external, visceral and skeletal malformations and developmental variations. Based on the absence of maternal and developmental toxicity at any dose level tested, the NOAEL for maternal toxicity and embryo/foetal development was considered to be 2000 mg/kg bw/day (the highest dose level tested).

The 3-month dietary toxicity study with *in utero* phase was conducted in general accordance with ECD Guidelines 415 and 408. Algal oil was administered at 0, 10,000, 25,000 and 50,000 ppm in the diet to four groups of male and female Sprague-Dawley Crl:CD(SD) rats (30/sex/group). Test diets were formulated by adding the algal oil at the allocated dosage in a DHA fish oil carrier (tuna) at the appropriate rate to basal diet to provide a constant volume of 50,000 ppm oil/test diet. An additional control group of rats (30/sex) received basal diet alone. F_0 males and females received basal or test diets for at least 70 and 14 days respectively prior to mating. F_0 males continued to receive the requisite diets through mating to the end of the study period. F_0 females continued to receive the diets through mating, gestation and lactation to the end of the study period. Offspring of the pairing of the F_0 animals were selected at postnatal day 21 (20/sex/dose group) to constitute the F_1 generation. The F_1 generation received basal or test diets from postnatal day 21 through to the end of the study period. F_0 males and females were exposed for 89-91 and 75-77 consecutive days respectively, and F_1 males and females were exposed for 106-107 and 110-111 consecutive days respectively.

There was no test article-related mortality in the F_0 and F_1 generations at any dose level studied. There were no adverse algal oil-related effects on mean F_0 male, F_0 female and F_1 male body weights, body weight gains, food consumption, and food efficiency at any exposure level. Higher mean body weight, body weight gain, and food consumption were noted for the F_1 females in the 50,000 ppm algal oil group throughout the generation and were considered to be a result of algal oil exposure. No adverse algal oil-related effects on macroscopic and microscopic examination and organ weights were noted for the F_0 and F_1 males and females and clinical pathology parameters for the F_1 males and females at any exposure level. For systemic toxicity, in F_0 males, F_0 females and F_1 males the NOAEL was considered to be 50,000 ppm in the diet, while in F_1 females the NOAEL was considered to be 25,000 ppm in the diet, based on higher mean body weight, body weight gain, and food consumption. The 50,000 ppm exposure level was equivalent to 3421 and 2339 mg/kg bw/day for F_0 males during pre-mating and after mating, respectively; 3558, 3117, and 7464 mg/kg bw/day for F_0 females during pre-mating, gestation, and lactation, respectively; and 3526 and 4138 mg/kg bw/day for F_1 males and females, respectively.

F_0 reproductive performance values, oestrous cycle length, gestation length, process of parturition, and the numbers of former implantation sites and unaccounted-for sites were unaffected by algal oil exposure at all dietary concentrations tested, as were F_1 generation postnatal survival and

developmental parameters. There were no neurotoxic effects noted at any algal oil exposure level. Based on these results, the NOAELs for reproductive toxicity, neonatal toxicity, developmental toxicity and neurotoxicity were considered to be the highest dose tested, 50,000 ppm in the diet.

The above toxicological studies are summarised in Table 15. The results support the safety of DHA-rich algal oil T18 for its proposed use in food.

Table 13 - Summary of toxicological studies on DHA-rich oil from Schizochytrium sp. T18

Study type/author	Results/observations
Genotoxicity/Mutagenicity	
Microbial reverse mutation assay; Schmitt et al., 2012a[29]	Negative
Rat bone marrow micronucleus assay; Schmitt et al., 2012a[29]	Negative
Chromosomal aberration assay in human peripheral blood lymphocytes; Schmitt et al., 2012a[29]	Negative
Acute Toxicity	
Oral LD_{50} in female rats; Schmitt et al., 2012a[29]	>5 g/kg bw
Subchronic Toxicity	
90-Day repeat dose oral toxicity study in rats; Schmitt et al., 2012a[29]	NOAEL: 50,000 ppm in the diet
Reproductive/Developmental Toxicity	
Developmental/maternal toxicity in rats (maternal exposure by gavage, once daily from days 6 – 19 of gestation); Schmitt et al., 2012b[30]	NOAEL for maternal toxicity and embryo/foetal development: 2000 mg/kg bw/day
3-Month dietary toxicity study with an *in utero* exposure phase in rats; Schmitt et al., 2012b[30]	NOAEL for systemic toxicity: 50,000 ppm in the diet for F_0 ♂/♀ and F_1 ♂; 25,000 ppm in the diet for F_1 ♀ (based on higher body weight gain and food consumption). NOAEL for repro., neonatal, developmental and neuro-toxicities: 50,000 ppm in the diet.

Allergenic potential

The product is a highly refined oil and the absence of protein as revealed by proximate analysis down to a limit of detection of 0.15% makes it highly unlikely that any allergens will be present.

Evaluation and conclusion by the applicant

DHA-rich oil is produced by heterotrophic fermentation of the single-celled micro-alga *Schizochytrium* sp. T18 under controlled, axenic conditions and is refined to a high degree of purity. The oil recovery and refining process ensure that no cells of the source organism remain in the oil and the process conditions ensure that no foreign micro-organisms enter the production stream. The refined oil consistently meets a specification appropriate to its use as a food ingredient, with a minimum DHA content of 35%.

The source organism is well characterised and taxonomically closely related to other micro-algae from which oils are approved for use in food in the EU.

The oil is intended to be used in a range of foods in which the use of other DHA-rich algal oils is already approved in the EU. The oil will provide an alternative to those other oils currently in use and it is compositionally and nutritionally equivalent to them.

Taxonomic considerations suggest that production of toxins by the source organism is unlikely and the absence of algal toxins from both the oil and the fermentation biomass from which it is obtained has been confirmed by analysis. The oil has not shown evidence of mutagenicity or genotoxicity in a standard battery of *in vitro* and *in vivo* assays. In a 90-day repeat feeding study in rats the NOAEL was 5% in the diet, equivalent to 3305 and 3679 mg/kg bw/day in males and females respectively (the highest dose levels administered). It did not produce any toxic manifestations in a reproductive toxicity study and a developmental/maternal toxicity study in rats. The absence of protein from the oil make it unlikely that any allergens are present.

The DHA-rich oil from *Schizochytrium* sp. T18 is therefore safe and suitable for the food uses requested.

REFERENCES

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients, Official Journal of the European Communities L 43, 14.2.1997, p. 1

[2] Advisory Committee on Novel Foods and Processes Opinion on substantial equivalence of a DHA rich oil from micro-algae considered under article 3(4) of the Novel Food Regulation (EC) 258/97, March 2012

[3] Commission Decision 2003/427/EC of 5 June 2003 authorising the placing on the market of oil rich in DHA (docosahexaenoic acid) 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, Official Journal of the European Union L 144, 12.6.2003, p. 13

[4] Commission Decision 2009/778/EC 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, Official Journal of the European Union L 278, 23.10.2009, p. 56

[5] Notifications pursuant to Article 5 of Regulation (EC) No 258/97 of the European Parliament and of the Council, entry No 184, 20 April 2012. European Commission website (consulted 24.05.2016) http://ec.europa.eu/food/safety/docs/novel-food_notifications_en.pdf

[6] Substantial equivalence of DHA-rich algal oil from *Schizochytrium sp*. (T18), Food Safety Authority of Ireland, December 21st, 2015

[7] Commission Implementing Decision 2014/463/EU of 14 July 2014 on authorising the placing on the market of 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 and repealing Decisions 2003/427/EC and 2009/778/EC, Official Journal of the European Union L 209, 16.7.2014, p. 55

[8] Commission Implementing Decision (EU) 2015/545 of 31 March 2015 authorising the placing on the market of oil from the micro-algae *Schizochytrium* sp. (ATCC PTA-9695) as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council, Official Journal of the European Union L 90, 2.4.2015, p. 7

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