Fermentalg

March 20, 2018

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Office of Food Additive Safety HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD, 20740

Dear Sir or Madam:

Accompanying this letter is a notice pursuant to regulations of the Food and Drug Administration found at 21 CFR Part 170 setting forth the basis for the conclusion reached by the submitter, Fermentalg, that DHA 350 from *Schizochytrium* sp. strain FCC-1324 is generally recognized as safe under the intended conditions of use described in the notice. The notice is contained in a binder. In addition, we include a CD that contains a complete copy of the notice. I hereby certify that the electronic files contained on the CD were scanned for viruses prior to submission, and thus certified as being virus-free using Symantec Endpoint Protection.

Sincerely,

(b) (6)

Hywel Griffiths
Chief Scientist
Email: hgriffiths@fermentalg.com

GRAS Notice for DHA Algal Oil from *Schizochytrium* sp. FCC-1324 for Use in Infant Formula

Prepared for:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Prepared by:
Fermentalg
4 Rue Rivière, 33500 Libourne
France

March 20, 2018

GRAS Notice for DHA Algal Oils from *Schizochytrium* sp. FCC-1324 for Use in Infant Formula

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GRAS Notice for DHA Algal Oil from *Schizochytrium* sp. FCC-1324 for Use in Infant Formula

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285 (U.S. FDA, 2017a), Fermentalg hereby informs the United States (U.S.) Food and Drug Administration (FDA) that docosahexaenoic acid (DHA) algal oil derived from *Schizochytrium* sp. FCC-1324 (referred to as DHA 350 herein), manufactured by Fermentalg, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Fermentalg's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below. In addition, as a responsible official of Fermentalg, Hywel Griffiths hereby certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Fermentalg and pertinent to the evaluation of the safety and GRAS status of DHA 350 as an ingredient for addition to food.

Signed,

(b) (6)

Hywel Griffiths

20TH MARCH 2018

Date

1.1 Name and Address of Notifier

Fermentalg
4 Rue Rivière, 33500 Libourne
France

1.2 Common Name of Notified Substance

DHA algal oil

1.3 Conditions of Use

Fermentalg's DHA 350 is intended for use as an ingredient in exempt (pre-term) and non-exempt (term) infant formula (ages from birth to 12 months) in accordance with current good manufacturing practices (cGMP) and in combination with a source of arachidonic acid (ARA). The ratio of DHA to ARA would range from 1:1 to 1:2. The intended use level is up to 0.5% (w/w) of fatty acids, similar to all other approved uses for incorporation of DHA in infant formula. DHA-rich oil shall not be used in combination with any other added oil that is a significant source of EPA or DHA.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2017a), DHA 350 manufactured by Fermentalg, has been concluded to have GRAS status for use as an ingredient for addition to infant formula as described in Section 1.3 on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification (GRN) will be made available to the U.S. FDA for review and copying upon request during business hours at the offices of:

Fermentalg
4 Rue Rivière, 33500 Libourne
France

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency's review of the notice, Fermentalg will supply these data and information.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Fermentalg's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Description

Fermentalg's DHA 350 oil is extracted and refined from the wild-type heterotrophic micro-algae *Schizochytrium* sp. FCC-1324. This oil is considered substantially equivalent in its source, composition, nutritional value, and metabolism to the GRAS-notified substance described in GRN 137 (Martek Biosciences Corporation, 2003) and contains DHA at a level of approximately 35% (by weight). The substantial equivalence of Fermentalg's oil is supported by the decision of the Food Safety Authority of Ireland (FSAI), which considered Fermentalg's DHA 350 to be substantially equivalent to the Martek Biosciences Corporation's oil in terms of composition, nutritional value, metabolism, intended use and level of undesirable substances as set out in Article 3.4 of the novel food Regulation EC No 258/97 (EC, 1997; FSAI, 2014).

Information about DHA, the major component of DHA 350, is provided below. Information characterizing the identity of the production organism is presented in Section 2.2.

2.1.1 Chemical Name

4,7,10,13,16,19-docosahexaenoic acid

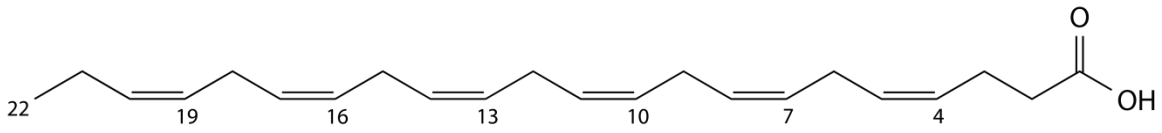
2.1.2 Molecular Formula

$C_{22}H_{32}O_2$

2.1.3 Chemical Abstract Service (CAS) Number

6217-54-5

2.1.4 Chemical Structure



2.2 Source Organism

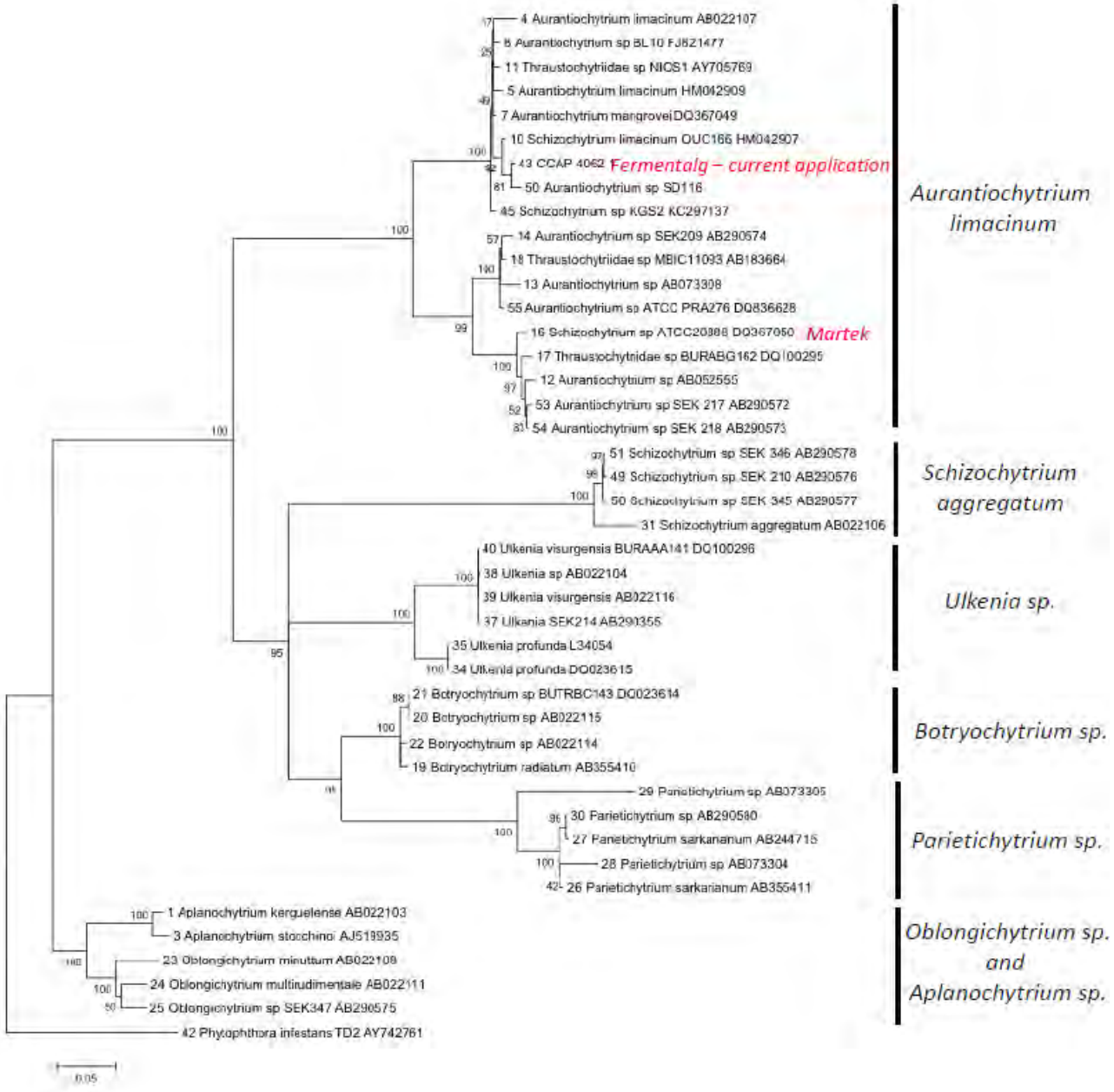
2.2.1 Phenotypic Identity

Fermentalg's DHA 350 is produced *via* fermentation using the single cell marine micro-algae, *Schizochytrium* strain FCC-1324. The taxonomic classification of this strains is as follows:

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

Figure 2.2.1-1 shows the phylogenic tree generated *via* the comparison of sequences of the small subunit of ribosomal DNA (18S SSU-rDNA) of strains in both the genus *Schizochytrium* and the genus *Ulkenia*. This figure demonstrates that Fermentalg's production strain [*i.e*., FCC-1324 (corresponding to CCAP 4062 1 in Figure 2.1-1)] is closely related to the production organism used to manufacture the DHA-rich oil that was the subject of GRN 137 (Martek Biosciences Corporation, 2003) (*i.e.,* Schizochytrium ATCC-20888 in Figure 2.1-1). An oil produced *via* fermentation using *Schizochytrium* ATCC PTA 9695 was the subject of GRN 553 (DSM Nutritional Products, 2014) while strain *Thraustochytrium sp* ONC T18 was used to produce the DHA oil described in GRN 677 (Mara Renewables Corporation, 2016).

Figure 2.2.1-1 Phylogeny of *Aurntiochytrium, Schizochytrium, Sicyiodochytrium* and *Traustochytrium* Genera, Collectively Referred to as *Schizochytrium*



The production organism can be grown to a high cell density using a carbon-based substrate. The components of the fermentation medium are listed in Table 2.2.1-1.

Table 2.2.1-1 Components of Fermentation Medium for FCC-1324

Fermentation Medium for FCC-1324		
	Compound	**CFR Citation**
Carbon + Salt	Glucose, 1 H_20 Sea salt	21 CFR §184.1857 (U.S. FDA, 2017a) 21 CFR §182.1 (U.S. FDA, 2017a)
Minerals	$MgSO_{4,}$ $7H_20$	21 CFR § 184.1443 (U.S. FDA, 2017a)
	K_2SO_4	184.1643 (U.S. FDA, 2017a)
	KH_2PO_4	21 CFR § 175.105 (U.S. FDA, 2017a)
	$MnCl_2$ 4 H_20	21 CFR § 184.1446 (U.S. FDA, 2017a)
	$ZnS0_{4,}$ 7 H_20	21 CFR § 182.8997 (U.S. FDA, 2017a)
	CoCl2, 6 H_20	--
	Na_2MoO_4, 2 H_20	--
	$CuSO_4$, 5 H_20	21 CFR § 184.1261 (U.S. FDA, 2017a)
	$NiSO_4$, 6 H_20	--
	$FeSO_4$, 7 H_2O	21 CFR § 184.1315 (U.S. FDA, 2017a)
Vitamins	Thiamine (B_1)	21 CFR § 182.8159 (U.S. FDA, 2017a)
	Cobalamin (B_{12})	21 CFR § 184.1945 (U.S. FDA, 2017a)
	Panthoenate (B_5	21 CFR § 184.1212 (U.S. FDA, 2017a)
Nitrogen	$(NH_4)_2SO_4$	21 CFR § 184.1143 (U.S. FDA, 2017a)
Chelator	Na_2EDTA	21 CFR § 172.135 (U.S. FDA, 2017a)
Anti-foam	BIOSPUMEX 153K	-

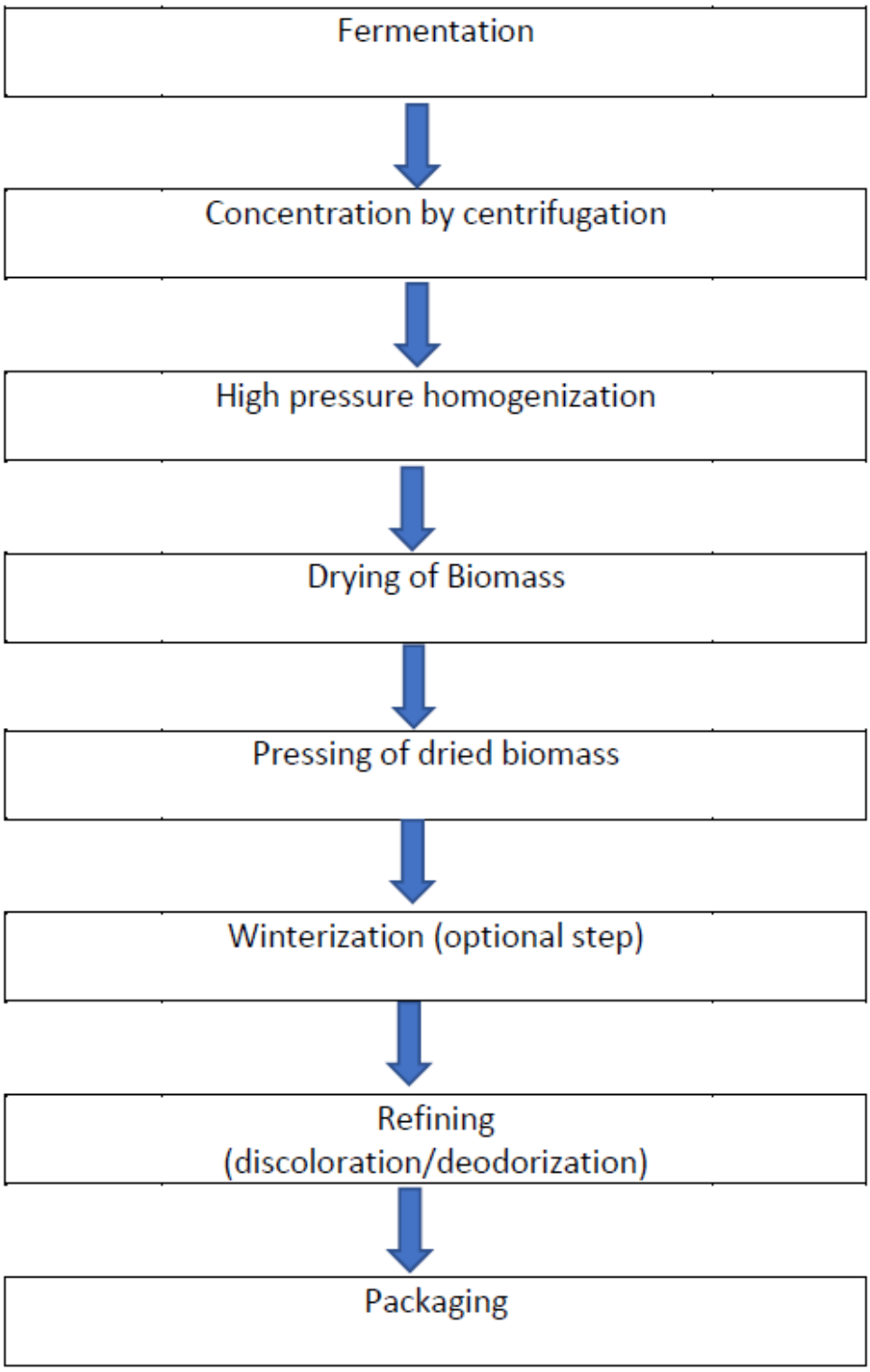
CFR = Code of Federal Regulations.

2.3 Manufacturing

Fermentalg's DHA 350 is produced in accordance with Hazard Analysis Critical Control Point (HACCP) and cGMP including quality control (QC) checks at every stage of the production process. Upstream (fermentation) processing includes the sterilization of growth media and all vessels/containers/fermenters. The fermentation is carried out in the absence of light under axenic conditions. All of these steps (from fermentation to refining) provide conditions that minimize the risk of contamination with foreign microorganisms. No solvents are used to obtain the crude-DHA rich oil.

The manufacturing flow process for DHA 350 is shown in Figure 2.3-1. Additional details follow.

Figure 2.3-1 Schematic of the Production Process of DHA 350



The production process for DHA 350 consists of 3 distinct stages (*i.e*., contained fermentation, oil extraction, and oil refining). DHA 350 is produced with a fermentation process using a single cell marine micro-alga, *Schizochytrium* sp. FCC-1324. This organism is grown to a high cell density using a carbon-based substrate. Operating parameters such as temperature, aeration, agitation, and pH are controlled throughout the process to ensure that results, in terms of cell growth and oil production, are reproducible. During the process, the fermentation is fed further with a solution of glucose, ammonium sulfate and potassium dihydrogen phosphate. The pH is controlled with either sodium or ammonium hydroxide. All ingredients used in the preparation of the culture medium are food-grade and are sterilized before use, except for sodium or ammonium hydroxide, which are considered auto-sterilizing.

To extract the oil, cells (biomass) from the liquid fermentation medium are (optionally) concentrated by centrifugation or filtration, and treated in a process involving food-grade, non-genetically modified organism (GMO) enzymes (*e.g.,* Alcalase from Novazyme) so that the cells are lysed and oil is liberated.

This process is carried out under an inert atmosphere in the presence of FDA-permitted antioxidants (*e.g*., mixed tocopherols, ascorbyl palmitate). Currently, a natural blend of tocopherols from sunflower is used at a level of around 3,000 ppm total tocopherols in the final refined oil, although these antioxidants may be replaced with other FDA-permitted antioxidants on client demand or if more effective at preventing oxidation under specific conditions. The separation of oil, water, and remaining cellular matter is carried out by centrifugation and an optional clarification by filtration is used to remove any remaining solid matter. All steps are carried out under an inert atmosphere. The crude oil is subsequently refined using processes and techniques common in the edible oil refining industry being degumming, neutralization, decoloration, and deodorization. After the deodorization step, further FDA-permitted antioxidants may be added to ensure stability. In keeping with standard industry practice, the algal oil is diluted with food-grade high-oleic sunflower oil to standardize DHA content across batches. Fermentalg's DHA-rich oil is then packaged in airtight and light-proof containers with low oxygen permeability.

2.4 2.4 Product Specifications and Batch Analyses

2.4.1 Proposed Product Specifications

The proposed product specifications for DHA 350 is provided in Table 2.4.1-1

Table 2.4.1-1 Chemical Specifications for DHA 350

Specification Parameter	Specification	Method
Color[a]	Report	Lovibond
Acid value	Max. 0.5 mg KOH/g	NF EN ISO 660
Peroxide value (PV)	Max. 5.0 meqO_2/kg	NF EN ISO 3960
Moisture and volatiles	Max. 0.05%	NF EN ISO 662
Unsaponifiables	Max. 3.5%	NF EN ISO 3596
Trans fatty acids	Max 1%	NF EN ISO 12966-2 and NF EN ISO 5508
DHA		
Area %	Min. 35%	NF EN ISO 12966-2 and NF EN ISO 5508
mg/g	Min. 350 mg/g	
Elemental Analysis		
Arsenic	< 0.1 mg/kg	Internal method
Copper	< 0.05 mg/kg	NF EN ISO 8294
Iron	< 0.2 mg/kg	NF EN ISO 8294

Table 2.4.1-1 Chemical Specifications for DHA 350

Specification Parameter	Specification	Method
Mercury	< 0.04 mg/kg	Internal method
Lead	< 0.01 mg/kg	NF EN ISO 12193
Cadmium	< 0.01 mg/kg	Internal method

DHA = docosahexaenoic acid; KOH = potassium hydroxide.
[a] DHA 350 has a light yellow to orange color, largely due to the presence of the naturally occurring carotenoids astaxanthin and beta-carotene but is not intended for use as a color additive.

2.4.2 Microbiological Specifications

Upstream (fermentation) processing includes the sterilization of growth media and all vessels/containers/fermenters used to grow the production organism and produce oil. Fermentation takes place in industrial fermenters. Extraction of the oil is carried out without utilization of any organic solvent. Both bleaching and deodorization use high temperatures under vacuum.

All of these steps (from fermentation to deodorization) provide conditions that minimize the risk of growth of foreign microorganisms. Microbiological testing is nevertheless a routine part of the final QC testing prior to release of the oil to ensure compliance with the limits shown in Table 2.4.2-1.

Table 2.4.2-1 Microbiological Specifications for DHA 350

Specification Parameter	Specification	Method
Aerobic microorganisms	< 1,000 CFU/g	NF EN ISO 4833-1
Yeasts	< 100 CFU/g	NF EN ISO V08-059
Molds	< 100 CFU/g	NF EN ISO V08-059
Coliforms	< 10 MPN/g	NF EN ISO V08-050
E. coli	Negative/g	NF EN ISO 16649-2
Coagulase positive *Staphylococci*	< 10 CFU/g	NF EN ISO V08-057-1

CFU = colony forming units; MPN = most probable number.

2.4.3 Batch Analyses

The results of 3 non-consecutive batches of DHA 350 show that the ingredient is manufactured consistent with the proposed chemical specifications (Table 2.4.3-1). Compliance with microbial specifications is shown in Table 2.4.3-2. Certificates of analysis are provided in Appendix 1.

Table 2.4.3-1 Summary of the Chemical Product Analysis for 3 Lots of DHA 350

Parameter	Specification	Manufacturing Lot		
		OLD-03-B4-1-2017	OLD-03-B7-2017	OLD-03-B9-2-2017
Color[a]	Report	Lovibond: 1.7 R, 16.0 Y Gardner 3.3	Gardner: 2.7	Lovibond: 1.5 R, 16.6 Y Gardner 3.3
Acid value	Max, 0.5 mg KOH/g	0.1	0.4	0.1
Peroxide value (PV)	Max. 5 meq/kg	0.4	1.1	0.3
Moisture and volatiles	Max 0.05 %	< 0.05%	< 0.05%	< 0.05%

Table 2.4.3-1 Summary of the Chemical Product Analysis for 3 Lots of DHA 350

Parameter	Specification	Manufacturing Lot		
		OLD-03-B4-1-2017	OLD-03-B7-2017	OLD-03-B9-2-2017
Unsaponifiables	Max. 3.5%	1.42% ± 0.30	1.86% ± 0.30	1.40% ± 0.30
Trans fatty acids	Max. 1%	0.2	None Detected	0.2
DHA				
Area %	Min. 35%	38.5	37.4	38.8
mg/g	Min. 350 mg/g	361	367	362
Elemental Analysis				
Arsenic	< 0.1 mg/kg	< 0.01 mg/kg	< 0.03 mg/kg	< 0.01 mg/kg
Copper	< 0.05 mg/kg	< 0.005 mg/kg	0.035 mg/kg	< 0.005 mg/kg
Iron	< 0.2 mg/kg	0.041 mg/kg	0.03 mg/kg	0.038 mg/kg
Mercury	< 0.04 mg/kg	< 0.005 mg/kg	< 0.005 mg/kg	< 0.005 mg/kg
Lead	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg
Cadmium	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg

DHA = docosahexaenoic acid; KOH = potassium hydroxide.
[a] DHA 350 has a light yellow to orange color, largely due to the presence of the naturally occurring carotenoids astaxanthin and beta-carotene but is not intended for use as a color additive.

Table 2.4.3-2 Summary of the Microbiological Product Analysis for 3 Lots of DHA 350

Specification Parameter		Manufacturing Lot		
		OLD-03-B4-1-2017	OLD-03-B7-2017	OLD-03-B9-2-2017
Aerobic microorganisms	< 1,000 CFU/g	< 1,000	< 1,000	< 1,000
Yeasts	< 100 CFU/g	< 10	< 10	< 10
Molds	< 100 CFU/g	< 10	40	< 10
Coliforms	< 10 MPN/g	< 1	< 1	< 1
Thermotolerant coliforms	< 10 CFU/g	< 1	< 1	< 1
E. coli	Negative/g	Absent	Absent	Absent
Coagulase positive *Staphylococci*	< 10 CFU/g	Absent	Absent	Absent

CFU = colony forming units.

2.4.4 Additional Analytical Information

The fatty acid profiles of Fermentalg's DHA 350, as well as that of Martek's oil as described in GRN 137 (Martek Biosciences Corporation, 2003), is shown in Table 2.4.4-1. These data demonstrated a good repeatability fermentation process, as well as a substantially equivalent composition to Martek's oil.

Table 2.4.4-1 Fatty Acid Profile of DHA 350

Fatty Acid	Martek Oil Analysis (Composition by Area %)	Manufacturing Lot			
		NF1	NF2	NF3	Mean
12:0	0.1	0.2	0.2	0.2	0.2
14:0	5.1	4.0	3.9	4.2	4.0

Table 2.4.4-1 Fatty Acid Profile of DHA 350

Fatty Acid	Martek Oil Analysis (Composition by Area %)	Manufacturing Lot			
		NF1	NF2	NF3	Mean
16:0	14.6	42.8	46.5	44.8	44.7
16:1n7	0.2	0.1	0.1	0.2	0.1
18:0	0.9	1.1	1.1	1.1	1.1
18:1n9	16.5	0.6	0.4	0.5	0.5
18:1n7	Sum with oleate	Sum with oleate	Sum with oleate	Sum with oleate	-
18:2n6	1.4	0.8	0.5	0.5	0.6
18:4n3	0.3	0.2	0.1	0.1	0.2
20:3n6	0.4	0.1	0.1	0.1	0.1
20:4n6	1.2	0.3	0.2	0.3	0.3
20:4n3	0.8	0.5	0.4	0.4	0.2
20:5n3	1.2	0.2	0.2	0.2	0.2
22:5n6	16.0	8.3	7.6	7.6	7.8
22:5n3	0.6	0.1	0.2	0.2	0.2
22:6n3	**38.3**	**39.2**	**37.3**	**38.0**	**38.2**
Other	2.5	1.5	1.2	1.5	1.4
Total saturated fatty acid	20.7	48.1	51.7	50.3	50.0
Total MUFA	16.7	0.7	0.5	0.7	0.6
Total PUFA	60.2	49.6	46.4	47.3	47.8

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid.

As shown in the table above, there are generally minor differences in the levels of the various fatty acids present in Martek's oil *vs*. Fermentalg's DHA 350. Exceptions include myristate (14:0), palmitate (16:0), and oleate (18:1n9); however, as these are all common dietary fatty acids, and at the intended use levels of Fermentalg's oil, these differences are not expected to make a difference with regard to safety, nutritional value, or metabolic impact.

Proximate analysis demonstrates that Fermentalg's DHA 350 is free from protein and carbohydrate (limit of detection of 0.1%). Although there are no reports of toxin production by any members of the *Thraustochytriaceae* family, member, Fermentalg has analyzed three samples of DHA 350 for the presence of algal toxins. As demonstrated in Table 2.4.4-2, no toxins were detected.

Table 2.4.4-2 Algal Toxin Screening for DHA 350

Toxin	Limit of Detection	Manufacturing Lot		
		NF1	NF2	NF3
Azaspiracids	5 µg/kg	< 5 µg/kg	< 5 µg/kg	< 5 µg/kg
Pectinotoxins	5 µg/kg	< 5 µg/kg	< 5 µg/kg	< 5 µg/kg
Yessotoxin	20 µg/kg	< 20 µg/kg	< 20 µg/kg	< 20 µg/kg
Okadaic Acid	5 µg/kg	< 5 µg/kg	< 5 µg/kg	< 5 µg/kg
Domoic Acid	1 mg/kg	< 1 mg/kg	< 1 mg/kg	< 1 mg/kg
Diarrhetic Shellfish Poison (DSP)	5 µg/kg	< 5 µg/kg	< 5 µg/kg	< 5 µg/kg
Paralytic Shellfish Poison (PSP)	20 µg/kg	< 20 µg/kg	< 20 µg/kg	< 20 µg/kg

The sterol composition of a representative batch of Fermentalg's DHA 350 is presented in Table 2.4.4-3. This table also provides comparisons to other *Schizochytrium* sp.-derived DHA oils already in the food supply. The sterol composition of Fermentalg's DHA 350 is identical to that of other DHA algal oil derived from *Schizochytrium* sp. which have attained GRAS status (GRN 553 and 677) (U.S. FDA, 2015a, 2017b). As shown in Table 2.4.4-3, Fermentalg's product does not contain new components, and the slight differences in the relative proportions of various sterols between Fermentalg's DHA350 and other DHA oil products are not expected to be affect safety.

Table 2.4.4-3 Comparative Sterol Profile

Sterols	Fermentalg's DHA 350 Manufacturing Lot: ITE_17_0182	DSM Nutritional Products GRN 553 Manufacturing Lot: 08-9530	Mara Renewables Corporation GRN 677 Manufacturing Lot: N-2-006-C
Cholesterol	33.8 %	13.3 %	24.3 %
Brassicasterol and/or Ergosterol[a]	9.5 %	1.3 %	< 0.1 %
24 methyl-cholesterol	0.3 %	1.3 %	3.9 %
Campesterol	0.4 %	0.1 %	1.2 %
Campestanol	< 0.1 %	2.0 %	< 0.1 %
Stigmasterol	1.9 %	64.2 %	< 0.1 %
Delta7-Campesterol	< 0.1 %	0.4 %	3.4 %
D5,23 Stigmastadienol	< 0.1 %	1.0 %	6.9 %
Clerosterol and/or fucosterol[a]	45.1 %	1.6 %	8.8 %
Beta-sitosterol	6.3 %	10.2 %	13.4 %
Sitostanol	0.1 %	0.5 %	< 0.1 %
Delta5-Avenasterol	0.2 %	1.7	1.4 %
Delta 5,24 Stigmastadienol	0.7 %	0.4	7.0 %
Delta7-Stigmasterol	1.4 %	1.7	26.1 %
Delta7-Avenasterol	0.3 %	0.3	3.6 %
Total sterol content	10,058 mg/kg of oil	0.56 wt%	2,310 mg/kg fat

[a] Two sterol compounds that have the same retention time.

2.5 Stability

The stability of DHA 350 is expected to be similar to other algal oils with a similar DHA content. Results of an stability study under accelerated storage conditions (*i.e.*, 40°C ± 2°C and 75% ± 5% relative humidity) on DHA 350 show that the fatty acid profile of DHA 350 remains unchanged over 8 weeks (Table 2.5-1). Furthermore, analysis of DHA 350 was conducted in parallel with a sample of oil from DSM Nutritional Products (Batch Number: VY00213006). Results confirm that the rate of accumulation of oxidation products, measured using peroxide value and para-anisidine values, is similar between the oils. No significant change in the DHA content was observed for either oil during the test. Real-time stability analysis of DHA 350 is ongoing.

Table 2.5-1 Stability of DHA 350 Under Accelerated Storage Conditions

Fatty Acid	Time 0	1 Week	4 Weeks	8 Weeks
12:0	0.16 %	0.17 %	0.15 %	0.14 %
14:0	4.49 %	4.16 %	4.07 %	3.90 %
14:1	0.26 %	0.15 %	0.13 %	0.23 %
15.0	0.14 %	0.10 %	0.10 %	< 0.05 %
16:0	48.39 %	46.99 %	47.44 %	46.37 %
16:1	0.15 %	0.14 %	0.12 %	0.16 %
16:2	< 0.05 %	< 0.05 %	< 0.05 %	< 0.05 %
16:3	< 0.05 %	< 0.05 %	< 0.05 %	< 0.05 %
16:4	< 0,05 %	< 0,05 %	< 0,05 %	< 0,05 %
17.0	0.06 %	0.06 %	0.08 %	< 0.05 %
17:1	0.12 %	0.10 %	0.11 %	0.10 %
18:0	1.41 %	1.38 %	1.41 %	1.42 %
18:1	1.36 %	1.33 %	1.28 %	1.31 %
18:2 (n-6)	0.83 %	0.86 %	0.82 %	0.84 %
18:3 (n-6)	< 0.05 %	0.07 %	0.10 %	< 0.05 %
18:3 (n-3)	2.29 %	2.33 %	2.30 %	2.28 %
18:4 (n-3)	0.19 %	0.20 %	0.20 %	0.21 %
20:0	0.09 %	0.08 %	0.08 %	0.08 %
20:1	< 0.05 %	< 0.05 %	< 0.05 %	< 0.05 %
20:2 (n-6)	< 0.05 %	< 0.05 %	< 0.05 %	< 0.05 %
20:3 (n-6)	0.11 %	0.08 %	0.08 %	0.09 %
20:4 (n-6)	0.10 %	0.08 %	0.06 %	0.06 %
20:3 (n-3)	< 0.05 %	< 0.05 %	< 0.05 %	< 0.05 %
20:4 (n-3)	0.39 %	0.36 %	0.35 %	0.38 %
20:5 (n-3)	0.28 %	0.29 %	0.27 %	0.29 %
22:0	0.09 %	0.10 %	0.10 %	0.09 %
22:1	0.61 %	0.62 %	0.59 %	0.62 %
22:2 (n-6)	< 0.05 %	< 0.05 %	< 0.05 %	< 0.05 %
22:4 (n-6)	< 0.05 %	< 0.05 %	< 0.05 %	0.06 %
21:5 (n-3)	< 0.05 %	0.05 %	< 0.05 %	< 0.05 %
22:4 (n-3)	< 0.05 %	< 0.05 %	< 0.05 %	< 0.05 %
22:5 (n-6)	5.54 %	5.83 %	5.92 %	6.04 %
22:5 (n-3)	0.11 %	0.13 %	< 0.05 %	0.14 %

Table 2.5-1 Stability of DHA 350 Under Accelerated Storage Conditions

Fatty Acid	Time 0	1 Week	4 Weeks	8 Weeks
22:6 (n-3)	32.56 %	34.11 %	34.08 %	34.92 %
24:0	0.08 %	0.08 %	0.08 %	0.08 %
24:1	0.06 %	0.06 %	< 0.05 %	0.05 %
Total saturated fatty acids	54.91 %	53.11 %	53.51 %	52.08 %
Total MUFA	2.55 %	2.40 %	2.23 %	2.48 %
Total PUFA	42.40 %	44.39 %	44.20 %	45.31 %
n-3	35.81 %	37.48 %	37.21 %	38.28 %
n-6	6.58 %	6.91 %	6.99 %	7.03 %
Total fatty acids	98.5 g/100g	98.0 g/100g	97.8 g/100g	95.2 g/100g
Total DHA	321 mg/g	334 mg/g	328 mg/g	332 mg/g
Other Parameters				
Para-anisidine index	7.2	9.0	15,8	23,9
Peroxide index	2.1 meqO_2/kg ± 1.0	7.7 meqO_2/kg ± 3.1	11.0 meqO_2/kg	16.3 meqO_2/kg

DHA = docosahexaenoic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid.

Part 3. §170.235 Dietary Exposure

3.1 History of Use in Food

DHA is primarily consumed through the ingestion of fatty fish, which contain high amounts of polyunsaturated fatty acids (PUFAs) with concentrations of w-3 fatty acids ranging from 0.1 to 5.3 g/100 g (Ascherio *et al.,* 1995; Sanders, 1989). The estimated consumption of DHA and EPA in the United States is approximately 100 mg/day (Kris-Etherton *et al.,* 2009).

DHA-rich oils from numerous sources are considered GRAS for use in foods and/or infant formula (GRN 41, 137, 138,319,384,469, 527, 553) (U.S. FDA, 2001, 2004a,b, 2010, 2012, 2013, 2015a,b). DHA algal oils from *Schizochytrium* strains related to Fermentalg's production organisms were described in GRN 137, GRN 553, and GRN 677 (U.S. FDA, 2004a, 2015a, 2017b). Two pending notices for DHA oil produced in *Schizochytrium* sp. (GRN 731 and 732) (U.S. FDA, 2017c,d) are listed in the inventory that were not yet available at the time of this dossier compilation. Other sources of the DHA-rich algal oils include related organisms (*i.e.*, *Ulkenia* sp., *Crypthecodinium cohnii,* SAM2179, *Chlorella protothecoides* strain S 106, and *Prototheca moriformis* strain S2532. In addition to algal oils, other sources of DHA such as tuna/fish oil are approved by the FDA for addition to human food and infant formula.

3.2 Estimated Consumption of DHA 350

3.2.1 Estimated Consumption from Intended Conditions of Use in Infant Formula

Fermentalg estimated intake from infant formula using the same rationale presented and discussed in previous GRAS submissions (GRN 553 and GRN 677) (U.S. FDA, 2015a, 2017b). It is assumed that infants consume about 100 to 120 kcal/kg body weight (bw)/day, of which fat constitutes approximately 50% of calories, or approximately 5.5 to 6.7 g fat/kg bw/day (1 g of fat is equivalent to 9 kcal). Assuming incorporation of the proposed DHA ingredient at a maximum use level of 0.5% of fatty acids, the intake of DHA would be 27 to 33 mg/kg bw/day. This DHA intake estimate is in agreement with current

recommendations for DHA consumption by pre-term and term infants of 18 to 60 mg/kg bw/day (Koletzko *et al.,* 2014).

DHA 350 is intended for use in infant formula in an identical manner as the currently approved oils Therefore, Fermentalg's oil will replace, rather than add to, intake from these oils.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with the use of DHA 350.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable

Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

Fermentalg's determination that its DHA oil are GRAS under the conditions of intended use in foods as described herein is based on scientific procedures. Much of the information related to the safety of algal DHA oils have been previously reviewed (see GRN 137, 553, 677) (U.S. FDA, 2004a, 2015a, 2017b). A summary of the main findings is provided in Section 6.3.

6.2 Literature Search

As noted previously, the published scientific literature has been reviewed in several previous GRAS Notices, most recently in May of 2017. An updated search of the published scientific literature was conducted through August 2017 using the search program ProQuest to identify published studies relevant to the safety of DHA from *Schizochytrium sp*. and other sources. The search was conducted on databases including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and Toxfile®. One additional publication, Falk *et al.* (2017); which included a 15-day developmental study and a reproductive study of DHA-rich oil from *Schizochytrium* in Wistar rats, was identified. Details of this study are provided in Section 6.3.

6.3 Toxicology Studies

As noted in Section 6.1, information related to the safety of other algal DHA oils have been previously reviewed (see GRN 137, 553, 677) (U.S. FDA, 2004a, 2015a, 2017b). A summary of safety studies on the source organism is provided in Table 6.3-1. Details of pivotal safety data on DHA-rich oil are included in Table 6.3-2.

Studies have been conducted to determine the safety of *Schizochytrium* sp. algae and algal oil derived from *Schizochytrium* sp. algae. *Schizochytrium* sp. algae is not mutagenic in the *Salmonella typhimurium*, Chinese hamster ovary cells, human peripheral blood lymphocytes, and murine bone marrow (Hammond *et al*., 2002). No treatment-related effects were observed in rats in a 13-week dietary study (Hammond *et al*., 2001a). A no-observed-adverse-effect-level (NOAEL) of 22,000 mg/kg bw was determined by Hammond *et al*. (2001b) for maternal and developmental toxicity in rats. Lower no-observed-effect-levels (NOELs) of 600 mg/kg bw and 18,000 mg/kg bw were established for maternal and developmental toxicity in rabbits, respectively (Hammond *et al*., 2001b).

Algal oil derived from *Schizochytrium* sp. algae was found to be not mutagenic in Ames, chromosome aberration, and *in vivo* micronucleus assays (Fedorova-Dahms *et al*., 2011a; Schmitt *et al*., 2012a; Lewis *et al*., 2016). The acute oral median lethal dose (LD_{50}) of DHA algal oil is greater than 2,000 mg/kg bw/day, the highest dose tested (Schmitt *et al*., 2012a; Lewis *et al.,* 2016). In subchronic toxicity studies, no toxicologically significant adverse effects have been seen following gavage administration of DHA oil to rats at levels of up to 5,000 mg/kg/day or administration in the diet at levels up to 5% in rats and piglets (Schmitt *et al*., 2012a; Fedorova-Dahms *et al.*, 2014; Lewis *et al.*, 2016). Likewise, DHA oil was without developmental toxicity (Schmitt *et al.,* 2012b). A NOAEL of 5% DHA-rich algal oil was also established from a study exposing rats in utero for 28 days and as F1 rats for 90 days (Fedorova-Dahms *et al*., 2011b). In a second such study with the same exposure duration, the NOAEL for F_0 male and female and F_1 male systemic toxicity was considered to be 50,000 ppm (highest concentration administered) and 25,000 ppm for F_1 female systemic toxicity (higher mean body weight, body weight gain, and food consumption). No adverse effects on reproduction or development were seen (Schmitt *et al.*, 2012b). Furthermore, the FDA has reviewed numerous GRNs for substantially equivalent or similar products, including three for DHA algal oils from closely related *Schizochytrium* strains (GRN 137, 553, and 677), and has issued “no questions” letters to these notifications (U.S. FDA, 2004a, 2015a, 2017b).

Table 6.3-1 Safety Data for *Schizochytrium* sp. algae

Reference	Study Type	Test System	Exposure	Findings/Comments
Hammond *et al*. (2001a)	13-week Dietary	Rat Sprague-Dawley	0, 400, 1,500, 4,000 mg/kg bw	No treatment-related adverse effects observed.
Hammond *et al*. (2001b)	Developmental Dietary	Rat Sprague-Dawley	0.6, 6, 30%	NOAEL = 22,000 mg/kg bw for maternal and developmental toxicity.
Hammond *et al*. (2001b)	Developmental Gavage	Rabbit New Zealand White (SPF)	180, 600, 1,800 mg/kg bw	NOEL = 600 mg/kg bw/day for maternal toxicity. NOEL = 1,800 mg/kg bw/day for developmental toxicity.
Hammond *et al*. (2001c)	One-generation reproductive dietary	Rat Sprague-Dawley	0, 0.6, 6, 30%	No effects observed on estrus cycle or reproductive performance of F_0. Litter size, sex ratio, offspring viability, and physical development of F_1.
Hammond *et al*. (2002)	Ames +/- S9	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537	0, 5, 15, 50, 150, 500 µg/plate	Not mutagenic.
Hammond *et al*. (2002)	CHO AS52/XPRT gene mutation	Chinese hamster ovary AS52 cells	-S9: 200, 500, 1,000, 2,000, 5,000 µg/mL +S9: 200, 700, 850, 900, 1,000 µg/mL	Not mutagenic.
Hammond *et al*. (2002)	Chromosome aberration	Human peripheral blood lymphocytes	125, 250, 500, 750 µg/mL	Not clastogenic.
Hammond *et al*. (2002)	Micronucleus	Male CD-1 Mice	500, 1,000, 2,000 mg/kg	No chromosomal effects.

bw = body weight; NOAEL = no-observed-adverse-effect-level; NOEL = no-observed-effect-level.

Table 6.3-2 Safety Data for DHA-rich oil from *Schizochytrium*

Reference	Study Type	Test System	Exposure	Findings/Comments
Fedorova-Dahms *et al*. (2011a)	Ames +/- S9	*Salmonella typhimurium:* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2 *uvrA*	Up to 5,000 µg/plate	No biologically relevant increases in revertant colonies.
Fedorova-Dahms *et al*. (2011a)	Chromosome aberration +/- S9	Human lymphocytes	Up to 5 µL/mL Exp 1: 4 hr +/- S9 Exp 2: 4 hr with +S9 24 with -S9	No toxic effects or biologically relevant increases in chromosomal aberration.
Fedorova-Dahms *et al*. (2011a)	*In vivo* Micronucleus	Mouse	Maximum 2,000 mg/kg of oil	No biologically relevant increases in micronuclei.
Fedorova-Dahms *et al*. (2011a)	90-day	Rat Sprague-Dawley Male and Female	0.5% (312 mg/kg bw/day), 1.5% (965 mg/kg bw/day), 5% (3,246 mg/kg bw/day)	NOAEL of 5% Males: 3,149 mg/kg bw/day Females: 3,343 mg/kg bw/day Based on the body surface area, the human equivalent dose is about 30 g oil/day for a 60 kg adult.
Fedorova-Dahms *et al*. (2011b)	*In utero* (28-day), 90-day exposure, 30-day recovery	Rat Sprague-Dawley	0.5% (5,000 ppm), 1.5% (15,000 ppm), 5% (50,000 ppm)	NOAEL of 5% dietary DHA-rich oil for juvenile male and female rats over a 90-day post-natal period following pre-natal parental exposure and during maternal lactation. Resulting in 4,122 and 4,399 mg/kg bw/day for male and female rats respectively, averaging to 4,260 mg/kg bw/day. Authors suggested an average daily intake of 19 to 51 mg/kg bw/day for infants and 255 g/day for a 60 kg adult.
Fedorova-Dahms *et al*. (2014)	21-day Subacute Toxicity Oral (diet)	Pre-weaning farm piglets Domestic Yorkshire Crossbred Swine Male and female	0.32% (dose volume of 500 mL/kg/day)	No test article-related effects on growth, development, hematology, clinical chemistry, coagulation and urinalysis measures. No adverse effects based on macro- and microscopic pathology evaluations at necropsy.

Table 6.3-2 Safety Data for DHA-rich oil from *Schizochytrium*

Reference	Study Type	Test System	Exposure	Findings/Comments
Schmitt *et al.* (2012a)	Acute Toxicity	Female Sprague-Dawley rats	5,000 mg/kg bw	Acute oral LD_{50} was greater than 5,000 mg/kg of body weight.
Schmitt *et al.* (2012a)	Subchronic Toxicity	Sprague-Dawley rats	TOX: Basal diet, tuna oil control (50,000 ppm), or 10,000, 25,000 ppm, or 50,000 ppm DHA-rich oil in the diet REC: Vehicle control or 5,000 mg/kg bw/day for 90-days, 28-day recovery period	DHA-rich algal oil was well-tolerated at these dietary levels as evidenced by the absence of major treatment-related changes in the general condition and appearance of the rats, neurobehavioral endpoints, growth, feed and water intake, ophthalmoscopic examinations, routine hematology and clinical chemistry parameters, urinalysis, or necropsy findings. The no observed adverse effect level (NOAEL), the highest level fed, was determined to be 50,000 ppm, the highest dose tested, and equivalent to at least 3,305 and 3,679 mg/kg bw/day, for male and female rats, respectively.
Schmitt *et al.* (2012a)	Ames +/- S9	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537; E. coli WP2uvrA.	313, 625, 1,250, 2,500, and 5,000 µg/plate	Not mutagenic.
Schmitt *et al.* (2012a)	Chromosome aberration +/- S9	Human peripheral blood lymphocytes	*Initial Assay* -S9: 235, 336, and 480 µg/mL +S9: 480, 686, and 980 µg/mL *Confirmatory assay* -S9: 500, 750, and 1,000 µg/mL +S9: 11,000, 1,250, and 1,500 µg/mL	Not clastogenic.
Schmitt *et al.* (2012a)	*In vivo* Micronucleus Test	Sprague-Dawley rats	500, 1,000, and 2,000 mg/kg	Not clastogenic.
Schmitt *et al.* (2012b)	Prenatal Developmental Toxicity Study	Sprague-Dawley rats	400, 1,000, and 2,000 mg/kg/day by gavage on Gestation Days 6 to 19	No test article-related clinical findings. Based on the absence of maternal or developmental toxicity at any dosage level, a dosage level of 2,000 mg/kg/day was considered to be the NOAEL for maternal toxicity and embryo/fetal development.

Table 6.3-2 Safety Data for DHA-rich oil from *Schizochytrium*

Reference	Study Type	Test System	Exposure	Findings/Comments
Schmitt *et al*. (2012b)	*In utero* (28-day), 90-day exposure	Rat Sprague-Dawley Male and Female	0, 50,000 ppm DHA fish oil, 10,000, 25,000 or 50,000 ppm algal oil for the F0 and F1 generations.	The NOAEL for F_0 male and female and F_1 male systemic toxicity was considered to be 50,000 ppm (highest concentration administered) and 25,000 ppm for F_1 female systemic toxicity (higher mean body weight, body weight gain, and food consumption). F_0 reproductive performance values, estrous cycle length, gestation length, or the process of parturition, and the numbers of former implantation sites and unaccounted-for sites were unaffected by algal oil exposure. Postnatal survival and developmental parameters in the F_1 generation were unaffected by algal oil exposure at all dietary concentrations. There were no neurotoxic effects noted at any algal oil exposure level.
Lewis *et al.* (2016)	Acute Toxicity	Female Wistar rats	5,000 mg/kg	Acute oral LD_{50} was greater than 5,000 mg/kg of body weight
Lewis *et al.* (2016)	28-day Subacute Toxicity	Wistar rats	0 (vehicle control) 1,000 mg/kg bw, 2,500 mg/kg bw, or 5,000 mg/kg bw of DHA-rich oil by gavage for 28 days.	No mortality was observed at any dose level throughout the treatment period and there were no differences in body weight or feed consumption among any of the groups. No treatment-related clinical signs or symptoms were observed in any of the animals. No changes were seen upon ophthalmological examinations. Likewise, no significant differences were seen in hematology, serum biochemistry, or urinalysis. The NOAEL was thus considered to be 5,000 mg/kg/day
Lewis *et al*. (2016)	90-day Subchronic Toxicity		TOX: Basal diet, vehicle control, 1,000, 2,500, or 5,000 mg/kg bw/day by gavage for 90 days. REC: Vehicle control or 5,000 mg/kg bw/day for 90-days, 28-day recovery period	DHA-rich oil did not produce any toxicologically significant changes in physical, physiological, biochemical, hematological, and histopathological parameters. The NOAEL value was thus considered to be 5,000 mg/kg bw/day, the highest dose tested.
Lewis *et al*. (2016)	Ames +/- S9	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537; E. coli WP2uvrA.	0.062, 0.185, 0.556, 1.667, 2.5, 3.75, and 5 mg/plate	Not mutagenic.

Table 6.3-2 Safety Data for DHA-rich oil from *Schizochytrium*

Reference	Study Type	Test System	Exposure	Findings/Comments
Lewis *et al*. (2016)	Chromosome aberration +/- S9	Human peripheral blood lymphocytes	Phase I (4-hour exposure) :0.00 (negative control), 0.00 (vehicle control), 1.25, 2.5, and 5.0 mg DHA-rich oil/mL Phase 2 (24-hour exposure) 1.25, 2.5 and 5.0 mg DHA-rich oil/mL culture	Not clastogenic.
Lewis *et al.* (2016)	*In vivo* Micronucleus Test	Wistar rats	1,000, 2,500, or 5,000 mg/kg bw/day	Not clastogenic.

[a] Untreated control group was for the prenatal developmental study only.
[b] Males were dosed for the duration of one spermatogenic cycle (84 days) and females were dosed for 2 estrous cycles (14 days), during pregnancy (22 days) and during nursing/lactation (21 days). In addition, both sexes were dosed during mating

6.4 Updated Discussion of Safety

The literature search discussed in Section 6.2 identified one publication, Falk *et al.* (2017), which included a 15-day developmental study and a reproductive study of DHA-rich oil from *Schizochytrium* in Wistar rats. In the developmental toxicity study, pregnant Wistar rats (24 rats/group) were untreated (control) or received vehicle control (corn oil) or 1,000, 2,500, or 5,000 mg/kg bw/day of DHA-rich oil *via* gavage from gestation Days 6 through 20. No mortality or clinical signs indicative of toxicity occurred during the course of the study in any of the dose groups. No treatment-related changes in food consumption or body weight were observed. Gross observations of dams revealed no treatment-related lesions, and there were no significant differences in the weight of the reproductive organs, implantation, and cornea lutea of the right and left cornu, and pre-and post-implantation loss of fetuses between DHA-rich oil and control and vehicle control treated groups. Likewise, there were no significant differences between groups with respect to the incidence of fetal viability and sex ratio, or fetal weight changes. There were no significant or dose dependent differences compared to control for the external observations (*i.e*., fetal size, generalized arrested development, kinked tail, bent tail, bulged eyelid, microphthalmia, subcutaneous hemorrhage, or malformed head). The NOAEL for maternal toxicity, embryo/fetal development, and parental reproductive toxicity for DHA-rich oil by gavage was 5,000 mg/kg bw/day, the highest dose tested.

In the reproductive toxicity study, male and female Wistar rats were administered vehicle control (corn oil), or 1,000, 2,500, or 5,000 mg/kg bw/day of DHA- rich oil *via* gavage throughout the mating period, pregnancy, and the nursing and lactation period. No treatment-related mortality was observed in the parental (F0) or pup generation (F1) during the course of the study. There was no dose response relationship in pup mortality or treatment-related clinical signs. No significant differences in the mean body weight were observed for the F0 generation. A slight increase in the body weight gain of male rats was observed from Day 1 to Day 64 (30% and 37%) for the mid- and high-dose groups. Higher food consumption compared to control was observed in males in the low-dose group for Weeks 5, 9, and 10 and on Days 4 and 6 of gestation in females of all DHA dose groups. In the F1 group, no differences in between control and all treatment groups was observed or body weight or body weight gain.

There were no significant differences between any DHA-rich oil dose group and the control group for mean litter size, sex ratio, live birth index, weaning index, number of implantation sites, corpora lutea, and pre- and post-implantation loss. There were no differences in female fertility index, gestation index, fecundity index, estrus cycle length, or gestation period. No treatment-related gross or microscopic changes were seen in the F1 generation, and there were no significant differences in absolute and relative organ weights. The NOAEL paternal or maternal treatment-related reproductive toxicity for the DHA-rich oil was 5,000 mg/kg bw/day.

6.5 Clinical Safety

Numerous clinical trials have been conducted on DHA-containing fish and marine-based oils. The trials have included adults, children, and infants. In a recent 106-day clinical study, healthy term infants were fed a milk-based formula with 17 mg/100 kcal DHA derived either from *Crypthecodinium cohnii* (DHASCO®) or DHA derived from *Schizochytrium* sp. algae (DHASCO®-B) to evaluate effects on growth and tolerance. Results are provided in Table 6.5-1. No significant differences in growth rates by gender were seen through 120 days of age, and *Schizochytrium* oil was equivalent with respect to DHA as measured by total red blood cell(RBC) DHA levels. The adverse events reported were not statistically different between groups or were concluded by the study physicians to be not related to the infant formulas. The most commonly reported adverse events were gastroesophageal reflux and respiratory infection. Overall, the published scientific literature continues to support the safety of DHA-algal oils from *Schizochytrium* in infant formula.

Table 6.5-1 Clinical Safety of DHA-Algal Oil in Infants (Yeiser *et al.*, 2016)

Study Population	Study Duration Study Design/Type of Study	Intervention/ Dose	Study Objective	Safety-Related Endpoints Measured/ Monitored	Safety-Related Results	Additional Outcomes with Possible Safety Implications	Reference
Healthy infants (202 M and 158 F) Age: 10 to 14 days ni = 360 nf = 267[a]	106-day study (Day 14 to 120) Multicenter, DB, R, C, P, prospective study	Test: cow milk-based formula with 17 mg/100kcal DHASCO®-B (DHA derived from *Schizochytrium* sp. algae) Control: cow milk-based formula with 17 mg/100kcal DHASCO® (DHA derived from *Crypthecodinium cohnii*) All formulas included a 1:1 ratio of PDX and GOS prebiotics (4 g/L)	To evaluate the effect of a milk-based liquid formula with DHASCO®-B DHA, ARASCO® ARA, and a prebiotic blend of polydextrose (PDX) and galactooligosaccharides (GOS) on the growth of and tolerance in healthy term infants.	• AEs[b] • Tolerance (gas, fussiness, stool characteristics as indicators)	• 9 serious AEs reported that were individually evaluated by physicians and determined to be unrelated to study formulas[c] • NSD between groups in AEs reported (most common: gastroesophageal reflux and upper respiratory infection) • NSD between test and controls among 27 subjects (8%) who discontinued study due to formula intolerance, as determined by investigators	• NSD in growth rates	Yeiser *et al.* (2016)

AE = adverse event; ARA = arachidonic acid; C = controlled; DB = double blind; DHA = docosahexaenoic acid; F = females; M = males; ni = initial study sample size; nf = final sample size after drop-outs and exclusions from analyses; NSD = no statistically significant difference; P = parallel; R = randomized.

[a] There were no statistically significant differences between groups for study discontinuation or discontinuation related to study formula.

[b] Including in the body as a whole, the skin, eyes, ears, nose and throat, as well as gastrointestinal, respiratory, cardiovascular, endocrine, musculoskeletal, nervous system, and urogenital systems, as well metabolic and nutrition related effects.

[c] Serious AEs included those that resulted in death, was life-threatening, required hospitalization or prolonged such stay, resulted in persistent or significant disability or incapacity, or was a congenital anomaly/birth defect.

6.6 Expert Panel Evaluation

Fermentalg has concluded that its DHA 350, manufactured consistent with cGMP and meeting food-grade specifications, is GRAS for use as in select food categories as described in Part 1.3, on the basis of scientific procedures. Fermentalg's conclusion on the GRAS status of DHA 350 under the conditions of its intended use is based on its substantial equivalence in its source, composition, nutritional value, and metabolism to the GRAS-notified substance described in GRN 137 (Martek Biosciences Corporation, 2003). Furthermore, the safety of the production organism and DHA algal oils under the intended conditions of use have been demonstrated in a series of preclinical toxicology studies and clinical safety studies.

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients unanimously concluded on the GRAS status of the DHA 350 under conditions of its intended use. The Expert Panel consisted of the following qualified scientific experts: Dr. John Thomas (Adjunct Professor, Indiana University School of Medicine), Dr. Michael Pariza (Professor Emeritus, Food Science, Director Emeritus, Food Research Institute, University of Wisconsin-Madison) and Dr. David Bechtel (President, Bechtel Consulting Inc.).

The Expert Panel, convened by Fermentalg, independently and critically evaluated all data and information presented herein and concluded that DHA 350, meeting appropriate food-grade specifications and manufactured consistent with cGMP, is safe and suitable for use as an ingredient in select food categories as described in Part 1.3, and is GRAS based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of the DHA 350 is presented in Appendix 2.

6.7 Conclusions

Based on data and information presented herein Fermentalg has concluded that DHA 350 can be determined to be Generally Recognized as Safe (GRAS) on the basis of scientific procedures.

The GRAS status of DHA 350 is further supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended use of DHA 350, as described herein, is GRAS.

Therefore, the intended use of DHA 350 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

Part 7. §170.255 List of Supporting Data and Information

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Part	Section §	Section Title
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
	170.203	Definitions
	170.205	Opportunity to submit a GRAS notice
	170.210	How to send your GRAS notice to FDA
	170.215	Incorporation into a GRAS notice
	170.220	General requirements applicable to a GRAS notice
	170.225	Part 1 of a GRAS notice: Signed statements and certification
	170.230	Part 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or...
	170.235	Part 3 of a GRAS notice: Dietary exposure
	170.240	Part 4 of a GRAS notice: Self-limiting levels of use
	170.245	Part 5 of a GRAS notice: Experience based on common use in food before 1958
	170.250	Part 6 of a GRAS notice: Narrative
	170.255	Part 7 of a GRAS notice: List of supporting data and information in your GRAS notice
	170.260	Steps you may take before FDA responds to your GRAS notice
	170.265	What FDA will do with a GRAS notice
	170.270	Procedures that apply when the intended conditions of use of a notified substance include use in...
	170.275	Public disclosure of a GRAS notice
	170.280	Submission of a supplement
	170.285	Disposition of pending GRAS affirmation petitions
172—Food additives permitted for direct addition to food for human consumption	172.135	Disodium EDTA
175—Indirect food additives: adhesives and components of coatings	175.105	Adhesives
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe
	182.8159	Biotin
	182.8997	Zinc sulfate
184—Direct food substances affirmed as generally recognized as safe	184.1143	Ammonium sulfate
	184.1212	Calcium pantothenate
	184.1261	Copper sulfate
	184.1315	Ferrous sulfate
	184.1443	Magnesium sulfate
	184.1446	Manganese chloride
	184.1472	Menhaden oil
	184.1643	Potassium sulfate
	184.1857	Corn sugar
	184.1945	Vitamin B 12

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APPENDIX 1
Certificates of Analysis

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Pessac, le 18/03/2014

v/réf. :
n/réf. : EIMA – D2A02 – Procedure of equivalency deposit

ANALYSIS CERTIFICATE

Contract followed by: JOFFRE Florent

METHODS :

DETERMINATION OF ACID VALUE AND ACIDITY (NF EN ISO 660) (*)

DETEMRINATION OF PEROXIDE VALUE (NF EN ISO 3960) (*)

DETERMINATION OF WATER AND VOLATIL CONTENT (NF EN ISO 662) (*)

DETERMINATION OF PARA-ANISIDNE VALUE (NF EN ISO 6885) (*)

DETERMINATION OF TOCOPHEROLS AND TOCOTRIENOLS IN OILS AND FATS BY HIGH PERFORMANCE LIQUID PERFORMANCE (NF EN ISO 9936) (*)

PREPARATION AND ANALYSIS of FATTY ACID METHYL ESTERS BY GAS PHASE CHROMATOGRAPHY (NF EN ISO 12966-2 et NF EN ISO 5508) (*)

DETERMINATIONOF UNSAPONIFIABLE CONTENT (method using diethylic oxide (NF EN ISO 3596)) (*)

DETERMINATION OF ARSENIC BY ATOMIC ABSORBTION SPECTROMETRY (method ITERG) - LQ = 0,05 mg/kg

DETERMINATION OF LEAD BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 12193) (*) - LQ = 0,01 mg/kg

DETERMINATION OF IRON BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

DETERMINATION OF COPPER BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

DETERMINATION O 4 PAH (Polycyclic Aromatic Hydrocarbons) BY HIGH PERFORMANCE LIQUID PERFORMANCE – REVERSE PHASE (ITERG method

DETERMINATION OF COLOR USING THE LOVIBOND METHOD-automatic method (NF ISO 27608)

MICROBIOLOGICAL DETERMINATIONS (subcontracted analysis)

SAMPLES

Reception date	:	27/01/2013
Nature	:	**REFINED AND ANTI-OXIDANT SUPPLEMENTED ALGAE OIL NF1**
Remark	:	ITERG code E14-032

RESULTS

FATTY ACIDS: COMPOSITION AND CONTENT (REFINED AND ANTI-OXIDANT SUPPLEMENTED ALGAE OIL NF3)

FATTY ACIDS	Oil NF1
12 : 0	0,2
14 : 0	4.0
14 : 1	<0,1
15 : 0	0,1
16 : 0	42.8
16 : 1	0,1
16 : 2	<0,1
16 : 3	<0,1
16 : 4	<0,1
17 : 0	0,1
17 : 1	<0,1
18 : 0	1,1
18 : 1	0,6
18 : 2 (n-6)	0,8
18 : 3 (n-6)	0,1
18 : 3 (n-3)	0,1
18 : 4 (n-3)	0,2
20 : 0	0,1
20 : 1	<0,1
20 : 2 (n-6)	<0,1
20 : 3 (n-6)	0,1
20 : 3 (n-3)	<0,1
20 : 4 (n-6)	0,3
20 : 4 (n-3)	0,5
20 : 5 (n-3)	0,2
22 : 0	0,1
22 : 1	<0,1
22 : 4 (n-6)	<0,1
22 : 5 (n-6)	8,3
22 : 5 (n-3)	0,1
22 : 6 (n-3)	39,2
24 : 0	<0,1
24 : 1	<0,1
unidentified	0,9
Total FA in the sample (g/100g)	**93,8**
DHA content (g/100g)	**36.77**

DETERMINATION	Oil NF1
OLEIC ACIDITY (NF EN ISO 660)	0.06 ± 0.05 % (m/m)
PEROXIDE VALUE (NF EN ISO 3960)	0.7 ± 1.0 méqO_2/kg
COLOR (NF ISO 27608)	Lovibond 5″1/4 : 1.5R, 23.0Y
WATER AND VOLATIL CONTENT (NF EN ISO 662)	< 0,05 %
ANISIDINE VALUE (NF EN ISO 6885)	12,6
TOCOPHEROL CONTENT (NF EN ISO 9936)	3965 mg/kg ± 595
UNSAPONIFIABLE CONTENT (NF EN ISO 6885)	1,28 % ± 0,30
Arsenic (method ITERG)	< 0,01 mg/kg
Lead (NF EN ISO 12193)	< 0,01 mg/kg
Iron (NF EN ISO 8294)	0,011 mg/kg
Copper (NF EN ISO 8294)	< 0,005 mg/kg
4 HAP* content (method ITERG) among B(a)P	0,5 µg/kg <0,2 µg/kg

* B(a)anthracène, chrysène, B(b)fluoranthène, B(a)pyrène

DETERMINATION	OIL NF1
Research for Aerobic microorganisms 30°C (NF EN ISO 4833-1)	<1/g
Research for Yeast (NF V08-059)	<1/g
Research for moulds (NF V08-059)	<1/g
Research for coliforms suspected 30°C (NF V08-050)	<1/g
Research for thermotolerant coliforms 30°C (NF V08-060)	<1/g
Research for Escherichia coli (NF ISO 16649-2)	<1/g
Research for coagulase-positive staphylococci (NF V08-057-1)	<10/g

The coordinating technician

BLE Fabienne

Head of project

JOFFRE Florent

DETERMINATION OF FATTY ACID COMPOSITION
EXPERIMENTAL UNCERTAINTY ACCORDING TO NF EN ISO 12966-2 and NF EN ISO 5508 *

Fatty Acid (%)	Extended Uncertainty (%)
≤ 0,2	0,2
0,3	0,3
0,4 à 5,0	0,4
5,1 à 10,0	0,5
10,1 à 20,0	0,8
20,1 à 30,0	1,0
30,1 à 50,0	1,4
50,1 à 60	1,7
> 60	2,1

*** extension factor k=2**

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ANALYSIS CERTIFICATE

Contract followed by: JOFFRE Florent

METHODS :

DETERMINATION OF ACID VALUE AND ACIDITY (NF EN ISO 660) (*)

DETEMRINATION OF PEROXIDE VALUE (NF EN ISO 3960) (*)

DETERMINATION OF WATER AND VOLATIL CONTENT (NF EN ISO 662) (*)

DETERMINATION OF PARA-ANISIDNE VALUE (NF EN ISO 6885) (*)

DETERMINATION OF TOCOPHEROLS AND TOCOTRIENOLS IN OILS AND FATS BY HIGH PERFORMANCE LIQUID PERFORMANCE (NF EN ISO 9936) (*)

PREPARATION AND ANALYSIS of FATTY ACID METHYL ESTERS BY GAS PHASE CHROMATOGRAPHY (NF EN ISO 12966-2 et NF EN ISO 5508) (*)

DETERMINATIONOF UNSAPONIFIABLE CONTENT (method using diethylic oxide (NF EN ISO 3596)) (*)

DETERMINATION OF ARSENIC BY ATOMIC ABSORBTION SPECTROMETRY (method ITERG) - LQ = 0,05 mg/kg

DETERMINATION OF LEAD BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 12193) (*) - LQ = 0,01 mg/kg

DETERMINATION OF IRON BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

DETERMINATION OF COPPER BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

DETERMINATION O 4 PAH (Polycyclic Aromatic Hydrocarbons) BY HIGH PERFORMANCE LIQUID PERFORMANCE – REVERSE PHASE (ITERG method

DETERMINATION OF COLOR USING THE LOVIBOND METHOD-automatic method (NF ISO 27608)

MICROBIOLOGICAL DETERMINATIONS (subcontracted analysis)

SAMPLES

Reception date	:	27/01/2013
Nature	:	**REFINED AND ANTI-OXIDANT SUPPLEMENTED ALGAE OIL NF2**
Remark	:	ITERG code E14-189

RESULTS

FATTY ACIDS: COMPOSITION AND CONTENT (REFINED AND ANTI-OXIDANT SUPPLEMENTED ALGAE OIL NF2)

FATTY ACIDS	Oil NF2
12 : 0	0,2
14 : 0	3,9
14 : 1	0,2
15 : 0	0,1
16 : 0	46,5
16 : 1	0,1
16 : 2	<0,1
16 : 3	<0,1
16 : 4	<0,1
17 : 0	<0,1
17 : 1	0,2
18 : 0	1,1
18 : 1	0,4
18 : 2 (n-6)	0,5
18 : 3 (n-6)	0,1
18 : 3 (n-3)	0,1
18 : 4 (n-3)	0,1
20 : 0	0,1
20 : 1	<0,1
20 : 2 (n-6)	<0,1
20 : 3 (n-6)	0,1
20 : 3 (n-3)	<0,1
20 : 4 (n-6)	0,2
20 : 4 (n-3)	0,4
20 : 5 (n-3)	0,2
22 : 0	<0,1
22 : 1	<0,1
22 : 4 (n-6)	<0,1
22 : 5 (n-6)	7,6
22 : 5 (n-3)	0,2
22 : 6 (n-3)	37,3
24 : 0	0,1
24 : 1	<0,1
unidentified	0,3
Total FA in the sample (g/100g)	**96,1**
DHA content (g/100g)	**35.85**

DETERMINATION	OIL NF2
OLEIC ACIDITY (NF EN ISO 660)	0.05 ± 0.05 % (m/m)
PEROXIDE VALUE (NF EN ISO 3960)	1.7 ± 1.0 méqO_2/kg
COLOR (NF ISO 27608)	Lovibond 5″1/4 : 1.0R, 16.0Y
WATER AND VOLATIL CONTENT (NF EN ISO 662)	< 0,05 %
ANISIDINE VALUE (NF EN ISO 6885)	12,8
TOCOPHEROL CONTENT (NF EN ISO 9936)	4025 mg/kg ± 604
UNSAPONIFIABLE CONTENT (NF EN ISO 6885)	1,16 % ± 0,30
Arsenic (method ITERG)	< 0,01 mg/kg
Lead (NF EN ISO 12193)	< 0,01 mg/kg
Iron (NF EN ISO 8294)	0,024 mg/kg
Copper (NF EN ISO 8294)	< 0,005 mg/kg
4 HAP* content (method ITERG) among B(a)P	0,5 µg/kg <0,2 µg/kg

* B(a)anthracène, chrysène, B(b)fluoranthène, B(a)pyrène

DETERMINATION	Oil NF2
Research for Aerobic microorganisms 30°C (NF EN ISO 4833-1)	<1/g
Research for Yeast (NF V08-059)	<1/g
Research for moulds (NF V08-059)	<1/g
Research for coliforms suspected 30°C (NF V08-050)	<1/g
Research for thermotolerant coliforms 30°C (NF V08-060)	<1/g
Research for Escherichia coli (NF ISO 16649-2)	<1/g
Research for coagulase-positive staphylococci (NF V08-057-1)	<10/g

The coordinating technician

(b) (6)

BLE Fabienne

Head of project

(b) (6)

JOFFRE Florent

DETERMINATION OF FATTY ACID COMPOSITION
EXPERIMENTAL UNCERTAINTY ACCORDING TO NF EN ISO 12966-2 and NF EN ISO 5508 *

Fatty Acid (%)	Extended Uncertainty (%)
≤ 0,2	0,2
0,3	0,3
0,4 à 5,0	0,4
5,1 à 10,0	0,5
10,1 à 20,0	0,8
20,1 à 30,0	1,0
30,1 à 50,0	1,4
50,1 à 60	1,7
> 60	2,1

*** extension factor k=2**

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ANALYSIS CERTIFICATE

Contract followed by: JOFFRE Florent

METHODS :

DETERMINATION OF ACID VALUE AND ACIDITY (NF EN ISO 660) (*)

DETEMRINATION OF PEROXIDE VALUE (NF EN ISO 3960) (*)

DETERMINATION OF WATER AND VOLATIL CONTENT (NF EN ISO 662) (*)

DETERMINATION OF PARA-ANISIDNE VALUE (NF EN ISO 6885) (*)

DETERMINATION OF TOCOPHEROLS AND TOCOTRIENOLS IN OILS AND FATS BY HIGH PERFORMANCE LIQUID PERFORMANCE (NF EN ISO 9936) (*)

PREPARATION AND ANALYSIS of FATTY ACID METHYL ESTERS BY GAS PHASE CHROMATOGRAPHY (NF EN ISO 12966-2 et NF EN ISO 5508) (*)

DETERMINATIONOF UNSAPONIFIABLE CONTENT (method using diethylic oxide (NF EN ISO 3596)) (*)

DETERMINATION OF ARSENIC BY ATOMIC ABSORBTION SPECTROMETRY (method ITERG) - LQ = 0,05 mg/kg

DETERMINATION OF LEAD BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 12193) (*) - LQ = 0,01 mg/kg

DETERMINATION OF IRON BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

DETERMINATION OF COPPER BY ATOMIC ABSORBTION SPECTROMETRY (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

DETERMINATION O 4 PAH (Polycyclic Aromatic Hydrocarbons) BY HIGH PERFORMANCE LIQUID PERFORMANCE – REVERSE PHASE (ITERG method

DETERMINATION OF COLOR USING THE LOVIBOND METHOD-automatic method (NF ISO 27608)

MICROBIOLOGICAL DETERMINATIONS (subcontracted analysis)

SAMPLES

Reception date	:	27/01/2013
Nature	:	**REFINED AND ANTI-OXIDANT SUPPLEMENTED ALGAE OIL NF3**
Remark	:	ITERG code E14-507

RESULTS

FATTY ACIDS: COMPOSITION AND CONTENT (REFINED AND ANTI-OXIDANT SUPPLEMENTED ALGAE OIL NF3)

FATTY ACIDS	Oil NF3
12 : 0	0,2
14 : 0	4.2
14 : 1	<0,1
15 : 0	0,1
16 : 0	44,8
16 : 1	0,2
16 : 2	<0,1
16 : 3	<0,1
16 : 4	<0,1
17 : 0	<0,1
17 : 1	0,2
18 : 0	1,1
18 : 1	0,5
18 : 2 (n-6)	0,5
18 : 3 (n-6)	0,1
18 : 3 (n-3)	0,1
18 : 4 (n-3)	0,2
20 : 0	0,1
20 : 1	<0,1
20 : 2 (n-6)	<0,1
20 : 3 (n-6)	0,1
20 : 3 (n-3)	<0,1
20 : 4 (n-6)	0,3
20 : 4 (n-3)	0,4
20 : 5 (n-3)	0,2
22 : 0	<0,1
22 : 1	<0,1
22 : 4 (n-6)	<0,1
22 : 5 (n-6)	7,6
22 : 5 (n-3)	0,2
22 : 6 (n-3)	38,0
24 : 0	<0,1
24 : 1	<0,1
unidentified	0,9
Total FA in the sample (g/100g)	**95,9**
DHA content (g/100g)	**36.44**

DETERMINATION	Oil NF3
OLEIC ACIDITY (NF EN ISO 660)	0.05 ± 0.05 % (m/m)
PEROXIDE VALUE (NF EN ISO 3960)	0.7 ± 1.0 méqO_2/kg
COLOR (NF ISO 27608)	Lovibond 5″1/4 : 1.5R, 20.0Y
WATER AND VOLATIL CONTENT (NF EN ISO 662)	< 0,05 %
ANISIDINE VALUE (NF EN ISO 6885)	13,3
TOCOPHEROL CONTENT (NF EN ISO 9936)	4250 mg/kg ± 637
UNSAPONIFIABLE CONTENT (NF EN ISO 6885)	1,06 % ± 0,30
Arsenic (method ITERG)	< 0,01 mg/kg
Lead (NF EN ISO 12193)	< 0,01 mg/kg
Iron (NF EN ISO 8294)	0,018 mg/kg
Copper (NF EN ISO 8294)	< 0,005 mg/kg
4 HAP* content (method ITERG) among B(a)P	0,6 µg/kg <0,2 µg/kg

* B(a)anthracène, chrysène, B(b)fluoranthène, B(a)pyrène

DETERMINATION	Oil NF3
Research for Aerobic microorganisms 30°C (NF EN ISO 4833-1)	<1/g
Research for Yeast (NF V08-059)	<1/g
Research for moulds (NF V08-059)	<1/g
Research for coliforms suspected 30°C (NF V08-050)	<1/g
Research for thermotolerant coliforms 30°C (NF V08-060)	<1/g
Research for Escherichia coli (NF ISO 16649-2)	<1/g
Research for coagulase-positive staphylococci (NF V08-057-1)	<10/g

The coordinating technician

(b) (6)

BLE Fabienne

Head of project

(b) (6)

JOFFRE Florent

DETERMINATION OF FATTY ACID COMPOSITION
EXPERIMENTAL UNCERTAINTY ACCORDING TO NF EN ISO 12966-2 and NF EN ISO 5508 *

Fatty Acid (%)	Extended Uncertainty (%)
≤ 0,2	0,2
0,3	0,3
0,4 à 5,0	0,4
5,1 à 10,0	0,5
10,1 à 20,0	0,8
20,1 à 30,0	1,0
30,1 à 50,0	1,4
50,1 à 60	1,7
> 60	2,1

*** extension factor k=2**

APPENDIX 2
Expert Panel Consensus Statement

Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of DHA 350 for Use in Food and Infant Formula

February 12, 2018

INTRODUCTION

At the request of Fermentalg, an Expert Panel ("the Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a source of docosahexaenoic acid (DHA) in traditional foods and infant formula, DHA 350 would be "Generally Recognized as Safe" (GRAS), based on scientific procedures.

The Panel consisted of the below-signed qualified scientific experts: Michael W. Pariza, Ph.D. (University of Wisconsin), John A. Thomas, Ph.D. (Tom-Tox, LLC), and David Bechtel, Ph.D., D.A.B.T. (Bechtel Consulting). The Panel was selected and convened in accordance with the U.S. Food and Drug Administration (FDA)'s guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S. FDA, 2017a). Fermentalg ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety and toxicology. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Panel; no such conflicts of interest or "appearance issues" were identified. The Panel received a reasonable honorarium as compensation for their time; the honoraria provided to the Panel were not contingent upon the outcome of their deliberations.

The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources based on searches of the published scientific literature conducted through January 2018. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Fermentalg. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety of DHA 350 and its individual components.

Following their independent and collaborative critical evaluation of the data and information, the Panel convened *via* teleconference on February 12, 2018. The Panel reviewed their findings and, following discussion, unanimously concluded that the intended uses described herein of DHA 350 meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practices (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

COMPOSITION, MANUFACTURING, AND SPECIFICATIONS

Fermentalg's DHA 350 oil is extracted and refined from the wild-type heterotrophic micro-algae *Schizochytrium sp*. FCC-1324. This oil is considered substantially equivalent in its source, composition, nutritional value, and metabolism to the GRAS-notified substance described in GRN 137 (Martek Biosciences Corporation, 2003) and contains DHA at a level of approximately 35% (by weight).

Fermentalg's DHA-rich oil is produced in accordance with Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practices, including quality control (QC) checks at every stage of the production process. Upstream (fermentation) processing includes the sterilization of growth media and all vessels/containers/fermenters. The fermentation is carried out in the absence of light under axenic conditions. All of these steps (from fermentation to refining) provide conditions that minimize the risk of contamination with foreign microorganisms. No solvents are used to obtain the crude-DHA rich oil.

The *Schizochytrium* strain used in production of DHA 350 is closely related to the production organism used to manufacture other GRAS-notified DHA-rich oils (Martek Biosciences Corporation, 2003; DSM Nutritional Products, 2014; Mara Renewables Corporation, 2017). Analysis of 3 non-consecutive lots each of DHA 350 demonstrated that this process produces oils that reproducibly meet appropriate food-grade specifications. Fermentalg has demonstrated the absence of algal toxins in DHA 350.

In addition to DHA, Fermentalg's oils contain other fatty acids, as well as sterols. There are generally minor differences in the levels of the various fatty acids present in Martek's oil *vs*. Fermentalg's DHA 350. Exceptions include myristate (14:0), palmitate (16:0), and oleate (18:1n9); however, as these are all common dietary fatty acids, and at the intended use levels of Fermentalg's oils, these differences are not expected to make a difference with regard to safety, nutritional value, or metabolic impact.

Similarly, Fermentalg's product does not contain new sterol components, and the slight differences in the relative proportions of various sterols between Fermentalg's DHA 350 and other DHA oil products are not expected to affect safety. Proximate analysis demonstrates that Fermentalg's DHA 350 is free from protein and carbohydrates (limit of detection of 0.1%).

The stability of DHA 350 is expected to be similar to other algal oils with a similar DHA content. Results of a stability study under accelerated storage conditions (*i.e*., 40°C ± 2°C/relative humidity=75% ± 5%) on DHA 350 show that the fatty acid profile of DHA 350 remains unchanged over 8 weeks. Furthermore, analysis of DHA 350 was conducted in parallel with a sample of oil from DSM Nutritional Products (Batch Number: VY00213006). Results confirm that the rate of accumulation of oxidation products, measured using peroxide value and para-anisidine values, is similar between the oils. No significant change in the DHA content was observed for either oil during the test. Real-time stability analysis of DHA 350 is ongoing.

INTENDED USE AND ESTIMATED EXPOSURE

The oil is intended for use as a direct food ingredient in the food categories listed in 21 CFR §184.1472(a)(3) and summarized in Table 1. Use levels will be adjusted to account for the higher DHA content of Fermentalg's oil (35%) compared to menhaden oil (20% DHA + eicosapentaenoic acid [EPA]). DHA 350 will be used at roughly 50% of the levels listed in 21 CFR §184.1472(a)(3) (U.S. FDA, 2017b). Fermentalg's oils are not intended to be combined with any other added oil that is a significant source of EPA or DHA.

Table 1 Intended Uses and Use Levels

Category of Food	Maximum Level of Use in Food (as served)	
	Menhaden (21 CFR 184.1472(a)(3)	DHA 350
Baked goods, baking mixes, § 170.3[a](n)(1) of this chapter	5.0 percent	2.5 percent
Cereals, §170.3(n)(4) of this chapter	4.0 percent	2.0 percent
Cheese products, §170.3(n)(5) of this chapter	5.0 percent	2.5 percent
Chewing gum, §170.3(n)(6) of this chapter	3.0 percent	1.5 percent
Condiments, §170.3(n)(8) of this chapter	5.0 percent	2.5 percent
Confections, frostings, §170.3(n)(9) of this chapter	5.0 percent	2.5 percent
Dairy product analogs, §170.3(n)(10) of this chapter	5.0 percent	2.5 percent
Egg products, §170.3(n)(11) of this chapter	5.0 percent	2.5 percent
Fats, oils, §170.3(n)(12) of this chapter, but not in infant formula	12.0 percent	6.0 percent
Fish products, §170.3(n)(13) of this chapter	5.0 percent	2.5 percent
Frozen dairy desserts, §170.3(n)(20) of this chapter	5.0 percent	2.5 percent
Gelatins, puddings, §170.3(n)(22) of this chapter	1.0 percent	0.5 percent
Gravies, sauces, §170.3(n)(24) of this chapter	5.0 percent	2.5 percent
Hard candy, §170.3(n)(25) of this chapter	10.0 percent	5.0 percent
Jams, jellies, §170.3(n)(28) of this chapter	7.0 percent	3.5 percent
Meat products, §170.3(n)(29) of this chapter	5.0 percent	2.5 percent
Milk products, §170.3(n)(31) of this chapter	5.0 percent	2.5 percent
Nonalcoholic beverages, §170.3(n)(3) of this chapter	0.5 percent	0.25 percent
Nut products, §170.3(n)(32) of this chapter	5.0 percent	2.5 percent
Pastas, §170.3(n)(23) of this chapter	2.0 percent	1.0 percent
Plant protein products, §170.3(n)(33) of this chapter	5.0 percent	2.5 percent
Poultry products, §170.3(n)(34) of this chapter	3.0 percent	1.5 percent
Processed fruit juices, §170.3(n)(35) of this chapter	1.0 percent	0.5 percent
Processed vegetable juices, §170.3(n)(36) of this chapter	1.0 percent	0.5 percent
Snack foods, §170.3(n)(37) of this chapter	5.0 percent	2.5 percent
Soft candy, §170.3(n)(38) of this chapter	4.0 percent	2.0 percent
Soup mixes, §170.3(n)(40) of this chapter	3.0 percent	1.5 percent
Sugar substitutes, §170.3(n)(42) of this chapter	10.0 percent	5.0 percent
Sweet sauces, toppings, syrups, §170.3(n)(43) of this chapter	5.0 percent	2.5 percent
White granulated sugar, §170.3(n)(41) of this chapter	4.0 percent	2.0 percent

[a] U.S. FDA (2017c)

The proposed conditions of use in Table 1 will ensure that total intake of EPA or DHA does not exceed 3 g/person/day.

Fermentalg's DHA 350 is intended for use as an ingredient in exempt (pre-term) and non-exempt (term) infant formula (ages from birth to 12 months) in accordance with current good manufacturing practices and in combination with a source of arachidonic acid (ARA). The ratio of DHA to ARA would range from 1:1 to 1:2. The intended use level is similar to all other approved uses for incorporation of DHA in infant formula.

Fermentalg estimated intake from infant formula using the same rationale presented and discussed in previous GRAS submissions (GRN 553 and GRN 677). It is assumed that infants consume about 100 to 120 kcal/kg body weight (bw)/day, of which fat constitutes approximately 50% of calories, or approximately 5.5 to 6.7 g fat/kg bw/day (1 g of fat is equivalent to 9 kcal). Assuming incorporation of the proposed DHA ingredient at a maximum use level of 0.5% of fatty acids, the intake of DHA would be 27 to 33 mg/kg bw/day. This DHA intake estimate is in agreement with current recommendations for DHA consumption by pre-term and term infants of 18 to 60 mg/kg bw/day (Koletzko *et al.,* 2014).

Fermentalg's oils are intended for use in an identical manner in infant formulas as the currently marketed oil. Therefore, they will replace, rather than add to, intake from the currently marketed oils.

DATA PERTAINING TO SAFETY

The safety of DHA 350 under the conditions of intended use in foods as described herein is based on scientific procedures. Much of the information related to the safety of other algal DHA oils has been previously reviewed (see GRAS notices GRN No. 137, 553, 677). Studies were conducted to determine the safety of *Schizochytrium sp.* algae and algal oil derived from *Schizochytrium sp.* algae. *Schizochytrium sp.* algae is not mutagenic in the *Salmonella typhimurium*, Chinese hamster ovary cells, human peripheral blood lymphocytes, and murine bone marrow (Hammond *et al*., 2002). No treatment-related effects were observed in rats in a 13-week dietary study (Hammond *et al*., 2001a). A no-observed-adverse-effect level (NOAEL) of 22,000 mg/kg body weight (bw) was determined by Hammond *et al*. (2001b) for maternal and developmental toxicity in rats. Lower no-observed-effect-levels (NOELs) of 600 mg/kg bw and 18,000 mg/kg bw were established for maternal and developmental toxicity in rabbits, respectively (Hammond *et al*., 2001b).

Algal oil derived from *Schizochytrium sp.* algae was found to be not mutagenic in Ames, chromosome aberration, and *in vivo* micronucleus assays (Fedorova-Dhams *et al*., 2011a; Schmitt *et al*., 2012a; Lewis *et al*., 2016). The acute oral LD_{50} of DHA algal oil is greater than 2000 mg/kg bw/day, the highest dose tested (Schmitt *et al*., 2012a; Lewis *et al.,* 2012b). In subchronic toxicity studies, no toxicologically significant adverse effects have been seen following gavage administration of DHA oil to rats at levels of up to 5,000 mg/kg/day or administration in the diet at levels up to 5% in rats and piglets (Schmitt *et al.*, 2012a; Fedorova-Dahms *et al.*, 2014; Lewis *et al.*, 2016). Likewise, DHA oil was without developmental toxicity (Schmitt *et al.,* 2012b). A NOAEL of 5% DHA-rich algal oil was also established from a study exposing rats in utero for 28 days and as F1 rats for 90-days (Fedorova-Dahms *et al*., 2011b). In a second such study with the same exposure duration, the NOAEL for F_0 male and female and F_1 male systemic toxicity was considered to be 50,000 ppm (highest concentration administered) and 25,000 ppm for F_1 female systemic toxicity (higher mean body weight, body weight gain, and food consumption). No adverse effects on reproduction or development were seen (Schmitt *et al.*, 2012b). Furthermore, FDA has reviewed numerous GRAS Notifications for substantially equivalent or similar products, including three for DHA algal oils from closely related *Schizochytrium* strains (GRN 137, 553, and 677), and has issues "no questions" letters to these notifications (U.S. FDA, 2004, 2015 a,b, 2017d).

An updated search of the published scientific literature was conducted through August 2017 using the search program Proquest to identify published studies relevant to the safety of DHA from *Schizochytrium sp*. and other sources. The search was conducted on databases including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and Toxfile®. One additional publication,

Falk *et al.* (2017), which included a 15-day developmental study and a reproductive study of DHA-rich oil from *Schizochytrium* in Wistar rats, was identified. Details of this study are provided below.

In the developmental toxicity study, pregnant Wistar rats (24 rats/group) were untreated (control) or received vehicle control (corn oil) or 1000, 2500, or 5000 mg/kg bw/day of DHA-rich oil via gavage from Gestation Days 6 through 20. No mortality or clinical signs indicative of toxicity occurred during the course of the study in any of the dose groups. No treatment-related changes in food consumption or body weight were observed. Gross observations of dams revealed no treatment-related lesions, and there were no significant differences in the weight of the reproductive organs, implantation, and cornea lutea of the right and left cornu, and pre-and post-implantation loss of fetuses between DHA-rich oil and control and vehicle control treated groups. Likewise, there were no significant differences between groups with respect to the incidence of fetal viability and sex ratio, or fetal weight changes. There were no significant or dose dependent differences compared to control for the external observations (*i.e*., fetal size, generalized arrested development, kinked tail, bent tail, bulged eyelid, microphthalmia, subcutaneous hemorrhage, or malformed head). The NOAEL for maternal toxicity, embryo/fetal development, and parental reproductive toxicity for DHA-rich oil by gavage was 5,000 mg/kg bw/day, the highest dose tested.

In the reproductive toxicity study, male and female Wistar rats were administered vehicle control (corn oil) or 1000, 2500, or 5000 mg/kg bw/day of DHA- rich oil via gavage throughout the mating period, pregnancy, and the nursing and lactation period. No treatment-related mortality was observed in the parental (F0) or pup generation (F1) during the course of the study. There was no dose response relationship in pup mortality or treatment-related clinical signs. No significant differences in the mean body weight were observed for the F0 generation. A slight increase in the body weight gain of male rats was observed from Day 1 to Day 64 (30 and 37%) for the mid- and high-dose groups. Higher food consumption compared to control was observed in males in the low-dose group for Weeks 5, 9, and 10 and on Days 4 and 6 of gestation in females of all DHA dose groups. In the F1 group, no differences in between control and all treatment groups was observed or body weight or body weight gain.

There were no significant differences between any DHA-rich oil dose group and the control group for mean litter size, sex ratio, live birth index, weaning index, number of implantation sites, corpora lutea, and pre- and post-implantation loss. There were no differences in female fertility index, gestation index, fecundity index, estrus cycle length, or gestation period. No treatment-related gross or microscopic changes were seen in the F1 generation, and there were no significant differences in absolute and relative organ weights. The NOAEL for paternal or maternal treatment-related reproductive toxicity for the DHA-rich oil was 5000 mg/kg bw/day.

Numerous clinical trials have been conducted on DHA-containing fish and marine-based oils. The trials have included adults, children, and infants. Overall, the published scientific literature continues to support the safety EPA/DHA intakes of up to 3 g/day from use in foods, and the clinical safety of DHA-algal oils from *Schizochytrium* in infant formula.

CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that DHA 350, meeting appropriate food grade specifications and produced in according with current good manufacturing practice, is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in foods specified herein. It is our professional opinion that other qualified experts would also concur in this conclusion.

(b) (6)

February 28, 2018

Michael W. Pariza, Ph. D.
Professor Emeritus, Food Science
Director Emeritus, Food Research Institute
University of Wisconsin-Madison

Date

(b) (6)

March 1, 2018

John A. Thomas, Ph.D.
Adjunct Professor
Department of Pharmacology & Toxicology
Indiana University School of Medicine Indianapolis, IN

Date

(b) (6)

March 3, 2018

David H. Bechtel, Ph.D., DABT
President
Bechtel Consulting, Inc.
Monroe, NJ

Date

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From:	Erica Cermak Intertek
To:	West-Barnette, Shayla; Morissette, Rachel; Hywel Griffiths
Subject:	RE: follow-up to phone call for GRNs 000776 and 000777
Date:	Tuesday, September 04, 2018 3:44:42 PM
Attachments:	image001.png image014.png image020.png image021.png image027.png image003.png GRN 000776 and GRN 000777 sterol supplement September 4 2018.docx

Dr. Morissette,

Please find attached the remaining responses to the questions received by email on June 17, 2018.

Regards,

Erica Cermak
Manager, Regulatory and Toxicology - Food & Nutrition
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From: West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Sent: Friday, August 31, 2018 1:57 PM
To: Erica Cermak Intertek <erica.cermak@intertek.com>; Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; Hywel Griffiths <hgriffiths@fermentalg.com>
Subject: RE: follow-up to phone call for GRNs 000776 and 000777

Thank you, Ms. Cermak. We look forward to receiving your responses on Tuesday.

Regards,

Shayla West-Barnette, Ph.D.
Supervisory Consumer Safety Officer

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From: Erica Cermak Intertek <erica.cermak@intertek.com>
Sent: Friday, August 31, 2018 1:48 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: RE: follow-up to phone call for GRNs 000776 and 000777

Dr. Morissette,

Fermentalg received the sterol analysis this evening local time. We will prepare the response to the remaining questions related to sterols and anticipate providing these to you by email on Tuesday, September 4th.

Regards,

Erica Cermak
Manager, Regulatory and Toxicology - Food & Nutrition
Health, Environmental & Regulatory Services (HERS)

Direct +1 908-290-7201
Skype erica.cermak.intertek
www.intertek.com

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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, August 24, 2018 11:45 AM
To: Erica Cermak Intertek <erica.cermak@intertek.com>; Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: RE: follow-up to phone call for GRNs 000776 and 000777

Thank you! I will forward this information to our review team.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Erica Cermak Intertek [mailto:erica.cermak@intertek.com]
Sent: Friday, August 24, 2018 11:40 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: RE: follow-up to phone call for GRNs 000776 and 000777

Dr. Morissette,

On behalf of Fermentalg, we respectfully submit this additional information in support of GRAS Notifications 000776 and 000777 in response to your questions received by email on June 17, 2018. It is our belief that this additional information provided as part of this notification adequately addresses the majority of your questions. As noted in your email below, we anticipate receipt of the sterol analysis by close of business next Friday, August 31st, and will provide the remaining responses upon receipt of this data.
My contact information is provided below. Please feel free to again contact me by phone or e-mail if you have any questions regarding this information.

Thank you,

Erica Cermak
Manager, Regulatory and Toxicology - Food & Nutrition
Health, Environmental & Regulatory Services (HERS)

Direct +1 908-290-7201
Skype erica.cermak.intertek
www.intertek.com

Intertek, New Jersey, USA

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Friday, August 24, 2018 10:15 AM
To: Hywel Griffiths <hgriffiths@fermentalg.com>
Cc: Erica Cermak Intertek <erica.cermak@intertek.com>; West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov>
Subject: follow-up to phone call for GRNs 000776 and 000777

Dear Dr. Griffiths,

Thank you for your phone call today to discuss the status of the responses to our questions for GRNs 000776 and 000777. You mentioned that the reason for the delay in responding to our questions is because the laboratory that you hired to test the sterols failed to provide those results in a timely manner; therefore, you have contracted with a separate company to perform those analyses. You mentioned that you can send the responses to the other questions now, excluding the sterol analyses, but that you anticipate having the sterol results by close of business (EST) next Friday, September 31. I agreed that sending what you have now would be best, with the expectation that we will receive the sterol response next week. If something changes, I requested that you contact Dr. Shayla West-Barnette, as I will be away next week. She will alert the review team and advise you on the next steps. You also mentioned that you would be amenable to withdrawing these notices should that become necessary. Please let me or Shayla know if you have any questions. We appreciate your keeping us apprised of the situation as it unfolds. I will look for your initial responses today. Please cc Dr. West-Barnette on that email as well.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Friday, August 24, 2018 9:51 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>

Subject: Re: response requested for GRNs 776 and 777

Dear Dr Morissette,

Do you have time for a quick 5 minute call? If so is there a number on which I could reach you?

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

Fermentalg

Tel. +335 57 250 252 | Mobile +337 61 33 37 96 | www.fermentalg.com [fermentalg.com] | Fermentalg – 4 Rue Rivière – 33500 Libourne |

On 24 Aug 2018, at 2:11 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dr. Griffiths,

I am following up on our conversation from last week. I have not received the responses to our questions for GRNs 776 and 777. Are you still planning to submit those responses by COB today? Withdrawing your notices and resubmitting them as I outlined below is still an option. If I do not hear back from you, we will need to assume that you are not planning to respond and will proceed with drafting no basis letters for these GRAS notices. Please let me know your intentions as soon as possible. I will be out of the office all next week. Dr. Shayla West-Barnette will be handling this matter while I'm away. Please cc her on all correspondence starting at 3 pm today EST. Email email address is Shayla.westbarnette@fda.hhs.gov. I hope to hear from you today about your intentions for these GRAS notices so that we can meet the 180-day mark.

Regards,

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Morissette, Rachel
Sent: Thursday, August 16, 2018 11:06 AM
To: 'Hywel Griffiths' <hgriffiths@fermentalg.com>
Subject: RE: questions for GRNs 000776 and 000777 (DHA algal oil)

Hi Hywel,

Thanks for your reply. Since we are already four weeks out from receipt of the questions, and typically 10 business days is the allowable time frame for responses from notifiers, early next week is preferable. If you don't think you'll be able to meet that timeframe, we'll have to discuss other options at this point, including withdrawing the notices and resubmitting revised versions that incorporate the questions that were raised in these notices, if necessary. I'll look out for your email next week.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Thursday, August 16, 2018 10:57 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: Re: questions for GRNs 000776 and 000777 (DHA algal oil)

Hi Rachel,

Thanks for your email. As you will have gathered from my out of office, I was away on vacation until today. The time it will take to review the response prepared by Intertek and check that we've collated all the data requested means I'm targeting next week for the reply. I hope this is acceptable.

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

<image007.png>

Tel. +335 57 250 252 | Mobile +337 61 33 37 96 | www.fermentalg.com [fermentalg.com] | Fermentalg – 4 Rue Rivière – 33500 Libourne |

On 14 Aug 2018, at 8:48 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Hi Dr. Griffiths,

I just wanted to check in and see when you anticipate sending your responses to our questions for GRNs 000776 and 000777?

Thanks,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Wednesday, July 18, 2018 11:31 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Erica Cermak Intertek <erica.cermak@intertek.com>
Subject: Re: questions for GRNs 000776 and 000777 (DHA algal oil)

Dear Ms Morissette,

Thank you for the letter. We will attempt to answer all questions within 10 business days, although with it already being holiday season in France we may have to ask for an extension for some of the questions requiring detailed technical responses.
In copy of this email is Erica Cermak of Intertek who was involved in the construction of the notifications and who may communicate on our behalf.

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

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On 17 Jul 2018, at 9:34 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dr. Griffiths,

Please see attached a letter with questions to be addressed for GRNs 000776 and 000777 (DHA algal oil). We request a response within 10 business days. Please let me know if you have any questions at this time.

Best regards,

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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<7-17-18 GRN776_777 Questions for Notifier.pdf>

5) Fermentalg provides a comparison of the sterol content of its GRN 000776 DHA algal oil with DSM Nutritional Product's (GRN 000553) oil and Mara Renewables Corporation's (GRN 000677) oil. For GRN 000776, there is a statement in the notice on p. 14 as follows:

"…the slight differences in the relative proportions of various sterols between Fermentalg's DHA350 and other DHA oil products are not expected to be [sic] affect safety."
Table 2.4.4-3 shows that (1) the level of total sterols in Fermentalg's DHA algal oil is higher than the total sterols in the GRN 000553 oil (0.56% w/w) and the GRN 000677 oil (0.23% w/w), and (2) the major sterols are not the same for GRNs 000776, 000553, and 000677. Please provide additional discussion and references to support the conclusion that these differences are not a safety concern for the intended use of Fermentalg's DHA algal oil in infant formulas for term and pre-term infants. Further, only a single batch analysis for sterols was reported for GRN 000776, with the comment that it is a representative batch. Please provide the results of a minimum of three non-consecutive batch analyses for sterols in order to characterize the sterol content of Fermentalg's DHA algal oil and to show typical levels of individual and total sterols.

The sterol profile of 3 non-consecutive batches of DHA 350 is shown in Table 3

Table 3 Sterol Profile of DHA 350

Sterol	Lot #0403012-A	Lot #0403014-A	Lot #0303010	Mara Renewables GRN 000677* (Range)	DSM Nutritional Products GRN 000553* (Range)
Cholesterol	44.1%	50.4%	54.7%	12.6-32.9%	9.8-14.4%
Brassicasterol and/or Ergosterol[1]	7.8%	8.1%	8.1%	<0.1-6.5%	0.9-1.7%
Campesterol	<0.1%	<0.1%	<0.1%	1.2-3.9%	1.5-2.2%
Campestanol	<0.1%	<0.1%	<0.1%	<0.1%	0.1
Stigmasterol	1.7%	3.0%	1.9%	<0.1-23.1%	60.6-65.3%
Delta 7-Campesterol	<0.1%	<0.1%	<0.1%	<0.1-7.0%	0.4-0.6%
D5,23 Stigmastadienol	<0.1%	<0.1%	<0.1%	<0.1-7.7%	0.8-1.0%
Chlerosterol and/or fucosterol[1]	29.4%	25.9%	26.8%	6.3-19.3%	1.6%
Beta-sitosterol	4.4%	3.9%	3.4%	9.4-14.8%	9.7-14.6%
Sitostanol	<0.1%	<0.1%	<0.1%	<0.1-0.5%	0.5-0.6%
Delta5-Avenasterol	0.9%	0.4%	0.2%	1.2-5.7%	0.9-2.9%
Delta 5,24 Stigmastadienol	<0.1%	<0.1%	0.2%	3.9-7.0%	0.4-0.5%
Delta 7-Stigmasterol	7.7%	0.9%	1.2%	<0.1-26.1%	1.6-2.5%
Delta7-Avenasterol	0.4%	0.4%	0.3%	1.4-9.1%	0.3-3.2%
Sum of non-identified peaks[2]	3.6%	7.0%	3.2%	Not reported	Not reported
Total Sterol Content	9,377 mg/kg of fat	8,482 mg/kg of fat	10,011 mg/kg of fat	831-2310 mg/kg fat	5100-5600 mg/kg fat

*Mara Renewable's oil and DSM's oil also contained 24-methylene cholesterol. [1] Two sterol compounds that have the same retention time during analysis. [2] Non-identified peaks have not been seen in previous analyses such as those submitted with the original notification. It is probable that these are sterols that have been incompletely derivatized (AOCS DOI:10.21748/lipidlibrary/40384).

The sterols present in Fermentalg's DHA 550 oil and the inter-batch variation are comparable to those present in other DHA algal oils currently used in infant formula, and other ingredients used in the manufacture of infant formula. They are also present in human milk and in the human diet.

Nine sterols comprise at least 5% of at least one of the oils, (Cholesterol, Brassicasterol, Stigmasterol, Chlerosterol, Beta-sitosterol, Delta-7-stigmasterol, Delta-5,23-stigmasterol, Delta 5-avenasterol, and Delta-7-avenasterol). These sterols are ubiquitous in the food supply and commonly used as sources of essential fatty acids in infant formula including corn, palm, safflower, soybean, and sunflower oil.

Fermentalg's oil contains a significantly higher amount of Cholesterol and Chlerosterol when compared to the other two oils, whereas the other sterols are either found in roughly equivalent proportions or are found in higher levels in Mara's oil and/or DSM's oil.

Cholesterol is the most significant sterol in the DHA 350 oil at 44-55%. Human breast milk contains significant quantities of cholesterol, whereas infant formulas contain up to ten times less (Claumarchirant *et al.* 2015). Nonetheless, given the expected levels of incorporation of the algal oil into infant formula,

the amounts of cholesterol provided by the algal oil will not significantly increase the total amount of cholesterol provided by the infant formula since the sterols provided in the algal oil will represent 1/10th to 1/20th of the total sterol.

As reviewed in GRN 000553, the other sterols are also reported in human milk, infant formula, or common foods and dietary oils. Various plant stanols have been evaluated by competent authorities world-wide and approved for use in a variety of foods, beverages and dietary supplements (Cantrill and Kawamura, 2008).

In the event that the unidentified unsaponifiable components of the oil are *not* the result of a partial derivitization during analysis, they would represent, at maximum, 0.7% of sterols provided in the infant formula.

Fermentalg's specifications for unsaponifiables (max. 3.5%) is the same as that of similar DHA algal oils, including the oils notified in GRN 000553 and GRN 000667. While the level in Fermentalg' DHA 350 is higher than the values presented in the representative batches of these oils, the levels are within the specification for all oils. Under the intended conditions of use, the total sterol intake from DHA algal oil would be minimal.

6) Sterols are not addressed in GRN 000777 beyond a general comment that there are:

"…slight differences in the relative proportions of various sterols which are not expected to be affect [sic] safety."
Please provide the results of sterol analyses from three non-consecutive batches for the DHA algal oil that is the subject of GRN 000777 and provide additional discussion explaining the aforementioned statement.

The sterol profile of 3 non-consecutive batches of DHA 550 is shown in Table 4.

Table 4 Sterol Profile of DHA 550

Sterol	Lot # 0403019	Lot # 0419022	Lot #0419028-A	Mara Renewables GRN 000677* (Range)	DSM Nutritional Products GRN 000553* (Range)
Cholesterol	40.7%	49.9%	53.9%	12.6-32.9%	9.8-14.4%
Brassicasterol and/or Ergosterol[1]	10.4%	10.1%	9.2%	<0.1-6.5%	0.9-1.7%
Campesterol	< 0.1%	< 0.1%	<0.1%	1.2-3.9%	1.5-2.2%
Campestanol	< 0.1%	<0.1%	<0.1%	<0.1%	0.1
Stigmasterol	1.5%	5.2%	3.6%	<0.1-23.1%	60.6-65.3%
Delta 7-Campesterol	<0.1%	< 0.1%	< 0.1%	<0.1-7.0%	0.4-0.6%
D5,23 Stigmastadienol	<0.1%	<0.1%	< 0.1%	<0.1-7.7%	0.8-1.0%
Chlerosterol and/or fucosterol[1]	33.5%	21.9%	19.7	6.3-19.3%	1.6%
Beta-sitosterol	9.6%	5.2%	4.6%	9.4-14.8%	9.7-14.6%
Sitostanol	<0.1%	< 0.1%	< 0.1%	<0.1-0.5%	0.5-0.6%
Delta5-Avenasterol	0.3%	0.4%	0.6%	1.2-5.7%	0.9-2.9%
Delta 5,24 Stigmastadienol	<0.1%	<0.1%	<0.1%	3.9-7.0%	0.4-0.5%
Delta 7-Stigmasterol	0.6%	0.4%	0.8%	<0.1-26.1%	1.6-2.5%
Delta7-Avenasterol	0.4%	0.2%	0.6%	1.4-9.1%	0.3-3.2%
Sum of non-identified peaks[2]	3.0%	6.8%	7.1%	Not reported	Not reported
Total Sterol Content	20,381 mg/kg fat	12,894 mg/kg fat	10210 mg/kg fat	831-2310 mg/kg fat	5100-5600 mg/kg fat

*Mara Renewable's oil and DSM's oil also contained 24-methylene cholesterol. [1] Two sterol compounds that have the same retention time during analysis. [2] Non-identified peaks have not been seen in previous analyses. It is probable that these are sterols that have been incompletely derivatized (AOCS DOI:10.21748/lipidlibrary/40384).

The sterols present in Fermentalg's DHA 550 oil and the inter-batch variation are comparable to those present in other DHA algal oils currently used in infant formula, and other ingredients used in the manufacture of infant formula. They are also present in human milk and in the human diet.

Nine sterols comprise at least 5% of at least one of the oils, (Cholesterol, Brassicasterol, Stigmasterol, Chlerosterol, Beta-sitosterol, Delta-7-stigmasterol, Delta-5,23-stigmasterol, Delta 5-avenasterol, and Delta-7-avenasterol). These sterols are ubiquitous in the food supply and commonly used as sources of essential fatty acids in infant formula including corn, palm, safflower, soybean, and sunflower oil.

Fermentalg's oil contains a significantly higher amount of Cholesterol and Brassicasterol when compared to the other two oils, whereas the other sterols are either found in roughly equivalent proportions or are found in higher levels in Mara's oil and/or DSM's oil.

Cholesterol is the most significant sterol in the DHA 550 oil at 40-54%. Human breast milk contains significant quantities of cholesterol, whereas infant formulas contain up to ten times less (Claumarchirant *et al.* 2015). Nonetheless, given the expected levels of incorporation of the algal oil into infant formula,

the amounts of cholesterol provided by the algal oil will not significantly increase the total amount of cholesterol provided by the infant formula since the sterols provided in the algal oil will represent 1/10th to 1/20th of the total sterol.

As reviewed in GRN 000553, the other sterols are also reported in human milk, infant formula, or common foods and dietary oils. Various plant stanols have been evaluated by competent authorities world-wide and approved for use in a variety of foods, beverages and dietary supplements (Cantrill and Kawamura, 2008).

In the event that the unidentified unsaponifiable components of the oil are *not* the result of a partial derivitization during analysis, they would represent, at maximum, 0.7% of sterols provided in the infant formula.

Fermentalg's specifications for unsaponifiables (max. 3.5%) is the same as that of similar DHA algal oils, including the oils notified in GRN 000553 and GRN 000667. While the level in Fermentalg' DHA 550 is higher than the values presented in the representative batches of these oils, the levels are within the specification for all oils. Under the intended conditions of use, the total sterol intake from DHA algal oil would be minimal (see response to question #19).

7) Fermentalg does not provide a comparison of the fatty acid or sterol content of its GRN 000777 DHA algal oil with the *Schizochytrium* sp.-derived DHA algal oils that were the subjects of published studies cited in the notice. Please provide this comparison and a discussion comparing the identity of the subject of GRN 000777 to *Schizochytrium* sp.-derived DHA-algal oils currently used in infant formulas for term and pre-term infants or oils that were the subject of relevant developmental and clinical studies cited in the notice.

Please see the Table 2 in the response of August 24, 2018 in response to question #3, which compares the fatty acid profile of Fermentalg's DHA 550 to those marketed by DSM (GRN 553) and Mara Renewables (GRN 677). A comparison of the sterol profile is provided in Tables 3 and 4 in response to questions #5& 6 above.
Given the phylogenic relationship between strains used in the safety studies, along with the comparative fatty acid and sterol profile, Fermentalg's DHA 550 may be considered sufficiently similar to the other GRAS-notified oils such that the developmental and clinical studies safety data generated for these oils can be considered supportive of the safety of DHA 550.

19) Please provide an estimate of total sterol intake from the intended uses in GRNs 000776 and 000777. Please discuss how these estimates compare to the exposure from consuming *Schizochytrium* sp. oils that are the subjects of published studies cited in GRNs 000776 and 000777 that are relevant to infant formula uses in term and pre-term infants.

As noted in GRN 000776 and GRN 000777, it is assumed that infants consume about 100 to 120 kcal/kg body weight (bw)/day, of which fat constitutes approximately 50% of calories, or approximately 5.5 to 6.7 g fat/kg bw/day (1 g of fat is equivalent to 9 kcal). Assuming incorporation of the proposed DHA ingredient at a maximum use level of 0.5% of fatty acids, the intake of DHA would be 27 to 33 mg/kg bw/day. These levels would be associated with exposure to 77-94 mg of DHA350 and 49-60 mg of DHA 550.

The unsaponifiable content of the three batches of DHA 350 included in GRN 000776 ranged from 1.40% to 1.86%.

As a result, sterol consumption resulting from the proposed use of DHA 350 would range from 1.1 to 1.7 mg/kg bw/day, roughly half of which would be cholesterol.

The unsaponifiable content of the three batches of DHA 550 included in GRN 000777 ranged from 1.22% to 1.77%.
As a result, sterol consumption resulting from the proposed use of DHA 550 would range from 0.6 to 1.1 mg/kg bw/day, roughly half of which would be cholesterol.

In comparison, total sterol consumption from an infant formula diet would be around 15-30mg/kg bw/day (Claumarchirant *et al.* 2015), with higher levels possible in breast fed infants.

As the maximum specification for sterol content for DHA 350 and DHA 550 is the same as that for DSM and Mara's oils, maximum exposure to sterols would be the same.

References

Cantrill R and Kawamura Y. (2008). Phytosterols, phytostanols and their esters. Chemical and technical assessment for the 69th JECFA. Available online at:
http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/69/Phytosterols.pdf

Claumarchirant, L.; Matencio, E.; Sanchez-Siles, L.M.; Alegria, A.; & Lagarda, M.J. (2015). Sterol Composition in Infant Formulas and Estimated Intake. J Agric Food Chem 63(32):7245-7251.