Fermentalg

March 20, 2018

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 550 from *Schizochytrium* sp. strain FCC-3204 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

#777

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MAR 30 2018
OFFICE OF
FOOD ADDITIVE SAFETY

GRAS Notice for DHA Algal Oil from *Schizochytrium* sp. FCC-3204 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. FC-3204 for Use in Infant Formula

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GRAS Notice for DHA Algal Oil from *Schizochytrium* sp. FCC-3204 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-3204 (referred to as DHA 550 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 550 as an ingredient for addition to infant formula.

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 550 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 that providing 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 550 manufactured by Fermentalg, has been concluded to have GRAS status for use as an ingredient for addition to in 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 550 oil is extracted and refined from *Schizochytrium* sp. FCC-3204. It is a mixture of triglycerides containing polyunsaturated fatty acids (PUFA) in which DHA represents more than 55% of total fatty acids. Information about DHA, the major component of DHA 550, 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

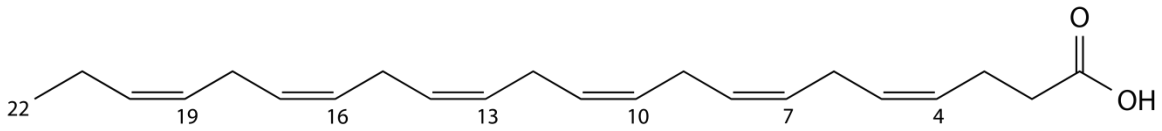
2.1.2 Molecular Formula

$C_{22}H_{32}O_2$

2.1.3 Chemical Abstract Service (CAS) Number

6217-54-5

2.1.4 Chemical Structure



2.2 Source Organism

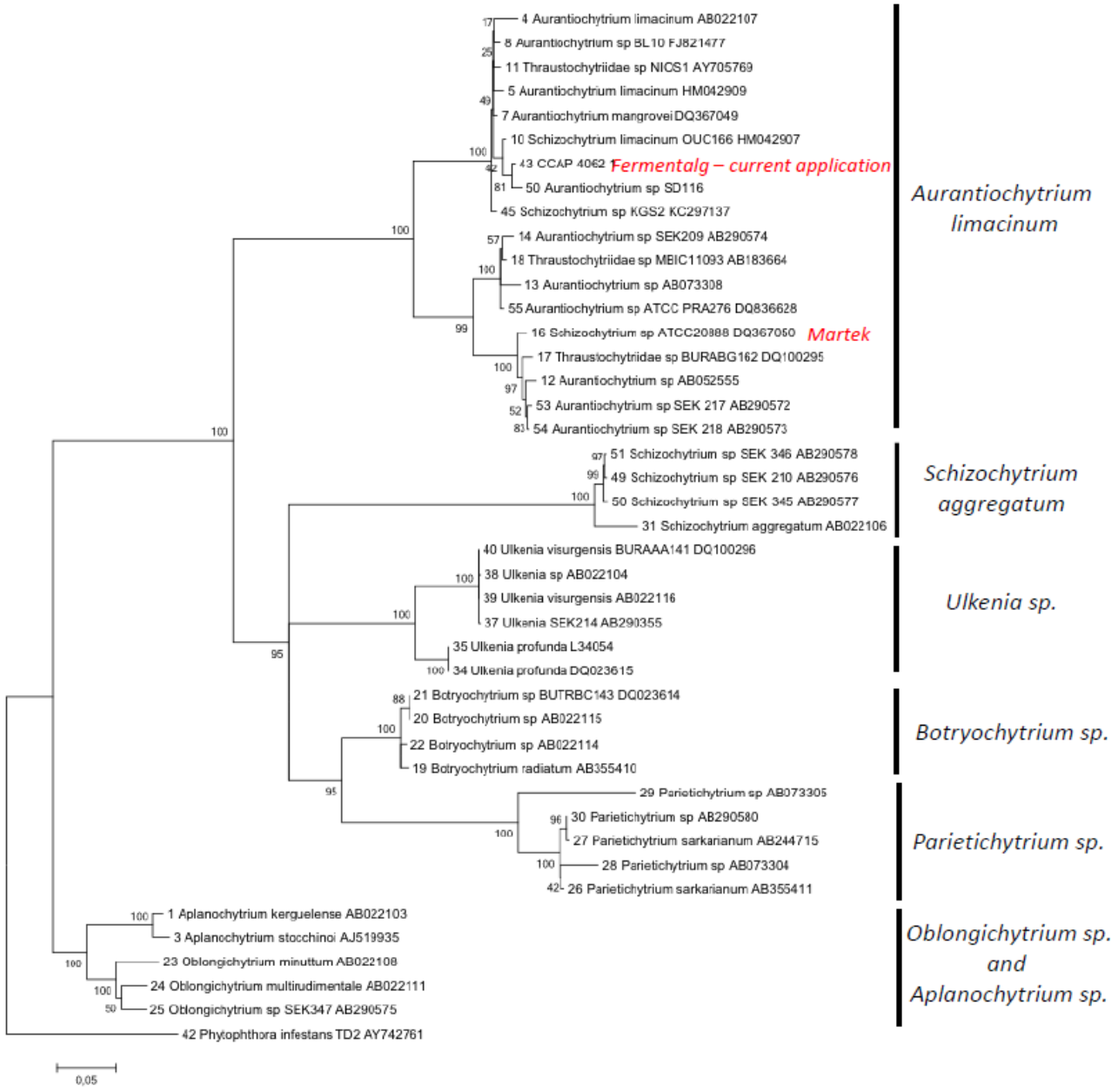
2.2.1 Phenotypic Identity

Fermentalg's DHA 550 is produced *via* fermentation using the single cell marine micro-algae, *Schizochytrium* strain FCC-3204. 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*., (which has an identical 18S SSU sequence to FCC-1324 and corresponds 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-3204

Fermentation Medium for FCC-3204		
	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)
Mineral salts/Nitrogen/ Chelator	$MgSO_{4,}$ $7H_20$	21 CFR § 184.1443 (U.S. FDA, 2017a)
	H_3BO_3	21 CFR § 176.180 (U.S. FDA, 2017a)
	$Na_4EDTA \cdot 2\ H_20$	21 CFR § 184.1315 (U.S. FDA, 2017a)
	$FeSO_4 \cdot 7\ H_2O$	21 CFR § 184.1315 (U.S. FDA, 2017a)
	$(NH_4)_2SO_4$	21 CFR § 184.1143 (U.S. FDA, 2017a)
	$MnCl_2 \cdot 4\ H_20$	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	--
	Na_2SeO_3	--
Vitamins	$CuSO_4$, 5 H_20	21 CFR § 184.1261 (U.S. FDA, 2017a)
	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)
Anti-foam	BIOSPUMEX 153K	

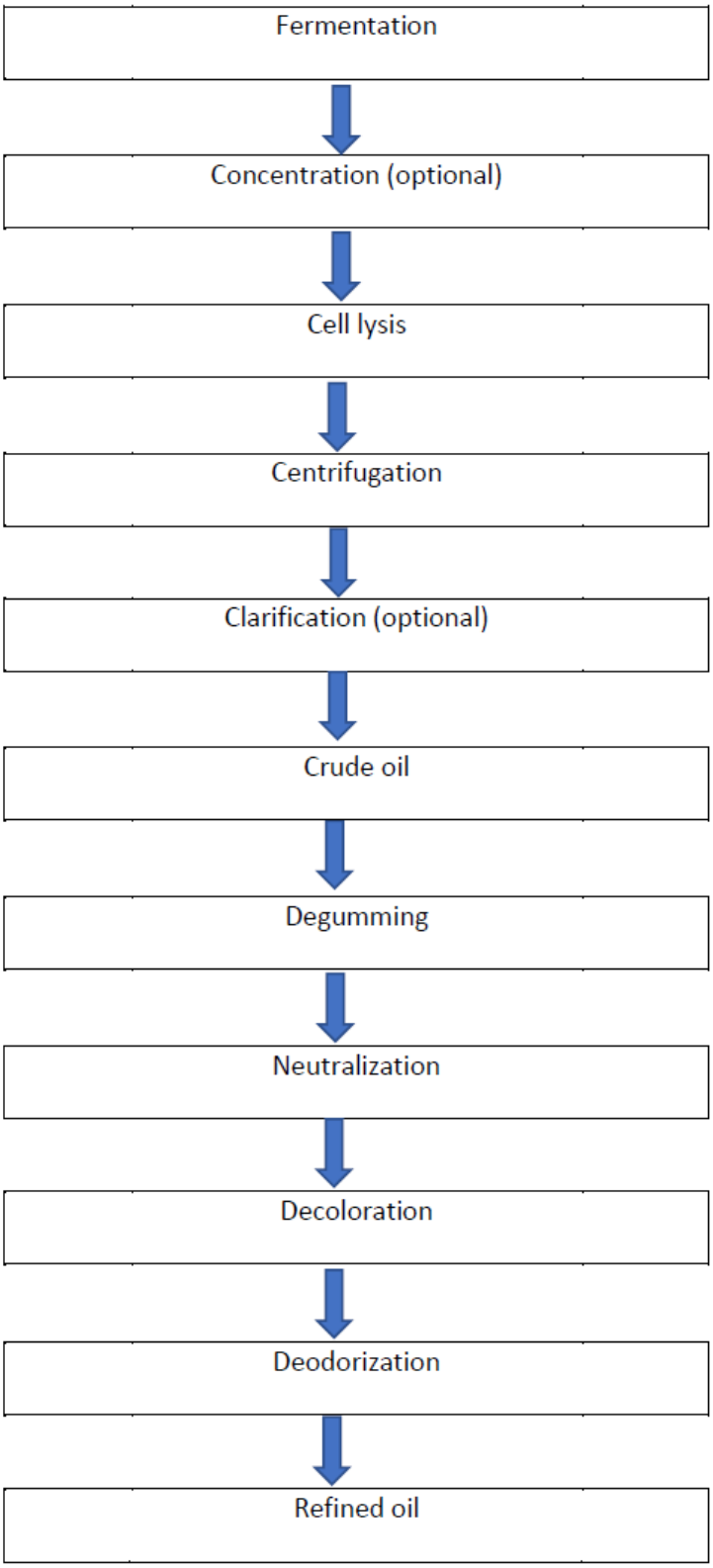
CFR = Code of Federal Regulations.

2.3 Manufacturing

Fermentalg's DHA 550 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 550 is shown in Figure 2.3-1. Additional details follow.

Figure 2.3-1 Schematic of the Production Process of DHA 550



The production process for DHA 550 consists of 3 distinct stages (*i.e.,* contained fermentation, oil extraction, and oil refining). DHA 550 is produced with a fermentation process using a single cell marine micro-alga, *Schizochytrium* sp. FCC-3204. 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. The components used in the preparation of the initial fermentation medium are listed in Table 3.1-1. During the process, the fermentation is fed further with a solution of glucose, ammonium sulfate and potassium dihydrogen phosphate. The pH is controlled with ammonium hydroxide. All ingredients used in the preparation of the culture medium are food grade and are sterilized before use, except for ammonium hydroxide, which is considered auto-sterilizing.

To extract the oil, cells (biomass) from the liquid fermentation medium are (optionally) concentrated by centrifugation or filtration, and treated using food-grade, non-genetically modified organism (GMO) enzymes 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 (*i.e*., mixed tocopherols, ascorbyl palmitate). 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 are 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 Product Specifications and Batch Analyses

2.4.1 Proposed Product Specifications

The proposed product specifications for DHA 550 is provided in Table 2.4.1-1

Table 2.4.1-1 Chemical Specifications for DHA 550

Specification Parameter	Specification	Method
Color[a]	Report	Lovibond/Gardner
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. 55%	NF EN ISO 12966-2 and NF EN ISO 5508
mg/g	Min. 550 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
Mercury	< 0.04 mg/kg	Internal method

Table 2.4.1-1 Chemical Specifications for DHA 550

Specification Parameter	Specification	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 550 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 550

Specification Parameter	Specification	Method
Aerobic microorganisms	< 1,000 CFU/g	NF EN ISO 4833-1
Yeasts	< 100 CFU/g	NF EN ISO VO8-59
Molds	< 100 CFU/g	NF EN ISO VO8-59
Coliforms	< 10 MPN/g	NF EN ISO V08-50
Thermotolerant coliforms	< 10 CFU/g	NF EN ISO V08-60
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 550 shows 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 550

Parameter	Specification	Manufacturing Lot		
		Batch ITE_17_0001	Batch ITE_17_0002	Batch ITE_17_0023
Color[a]	Report	10.9R; 70.0 Y	2.2 R; 24.0Y	10.7R; 70.0Y
Acid Value	Max 0.5 mg KOH/g	0.28 ± 0.10 mg KOH/g	0.16 ± 0.10 mg KOH/g	0.08 ± 0.10 mg KOH/g
Peroxide value (PV)	Max. 5.0 meqO_2/kg	2.8 ± 1.1 meqO_2/kg	2.6 ± 1.0 meqO_2/kg	0.4 ± 1.0 meqO_2/kg
Moisture and volatiles	Max 0.05%	< 0.05%	< 0.05%	< 0.05%
Unsaponifiables	Max 3.5%	1.36 ± 0.3%	1.77 ± 0.3%	1.22 ± 0.3%

Table 2.4.3-1 Summary of the Chemical Product Analysis for 3 Lots of DHA 550

Parameter	Specification	Manufacturing Lot		
		Batch ITE_17_0001	Batch ITE_17_0002	Batch ITE_17_0023
Trans fatty acids	Max 1%	< 0.25%	< 0.2%	< 0.02%
DHA				
Area %	Min. 55%	59.8%	60.4%	63.1%
mg/g	Min. 550 mg/g	566 mg/g	565 mg/kg	560 mg/kg
Elemental Analysis				
Arsenic	< 0.1 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg
Copper	< 0.05 mg/kg	< 0.006 mg/kg	< 0.005 mg/kg	< 0.005 mg/kg
Iron	< 0.2 mg/kg	< 0.015 mg/kg	0.010 mg/kg	0.060 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 550 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 550

Parameter	Specification	Manufacturing Lot		
		ITE_17_001	ITE_17_002	ITE_17_0023
Aerobic Microorganisms	< 1,000 CFU/g	< 1 /g	< 1 /g	29 /g
Yeasts	< 100 CFU/g	< 1 /g	< 1 /g	< 1 /g
Mold	< 100 CFU/g	< 1 /g	< 1 /g	< 4 /g
Coliforms	< 10 CFU/g	< 1 /g	< 1 /g	< 1 /g
Thermotolerant coliforms	< 10 CFU/g	< 1 /g	< 1 /g	< 1 /g
E. coli	Negative/g	< 1 /g	< 1 /g	< 1 /g
Coagulase positive *Staphylococci*	< 10 CFU/g	< 10 /g	< 10 /g	< 10 /g

CFU = colony forming units.

2.4.4 Additional Analytical Information

The fatty acid profiles of Fermentalg's DHA 550 is shown in Table 2.4.4-1. These data demonstrated a good repeatability of the fermentation process.

Table 3.5-2 Fatty Acid Profile of DHA 550

Fatty Acid	Manufacturing Lot			
	ITE_17_001	ITE_17_002	ITE_18_0023	Mean
12:0	0.1	0.1	0.1	0.10
14:0	1.5	1.2	1.1	1.27
14:1	0.3	0.5	0.3	0.37
15:0	< 0.05	< 0.05	0.1	0.10
16:0	22.3	20.7	18.7	20.57

Table 3.5-2 Fatty Acid Profile of DHA 550

Fatty Acid	Manufacturing Lot			
	ITE_17_001	ITE_17_002	ITE_18_0023	Mean
16:1	0.3	0.4	0.2	0.30
16:2	< 0.05	< 0.05	N.D.	< 0.05
16:3	0.4	0.3	N.D.	0.23
16:4	< 0.05	< 0.05	N.D.	< 0.05
17:0	< 0.05	0.1	0.1	0.10
17:1	0.1	0.2	0.1	0.13
18:0	0.8	0.8	0.7	0.77
18:1	0.6	0.8	0.7	0.70
18:2n-6	0.2	0.1	0.1	0.13
18:3n-6	0.1	0.1	0.1	0.10
18:3n-3	0.2	0.2	0.2	0.20
18:4n-3	0.3	0.4	0.3	0.33
20:0	0.1	0.1	0.1	0.10
20:1	< 0.05	< 0.05	N.D.	< 0.05
20:2n-6	-	-	N.D.	N.D
20:3n-6	0.1	0.1	0.2	0.13
20:3n-3	-	-	N.D.	N.D.
20:4n-6	0.1	0.1	0.1	0.10
20:4n-3	0.6	0.6	0.6	0.60
20:5n-3	0.5	0.9	0.6	0.67
22:0	0.1	0.1	0.1	0.10
22:1	0.9	1.3	0.9	1.03
22:4n-6	< 0.05	< 0.05	N.D.	< 0.05
22:5n-6	10.3	10.0	11.2	10.50
22:5n-3	0.2	0.4	0.2	0.27
22:6n-3	**59.8**	**60.4**	**63.1**	**61.10**
24:0	< 0.05	< 0.05	0.1	0.10
24:1	0.1	0.1	0.1	0.10

N.D. = not detected.

Proximate analysis demonstrates that Fermentalg's DHA 550 is free from protein and carbohydrate (limit of detection of 0.1%). Chemical elements have been assessed in three batches of Fermentalg's DHA 550. Results are provided in Appendix 2.

Although there are no reports of toxin production by any members of the *Thraustochytriaceae* family, member, Fermentalg has analyzed three samples of DHA 550 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 550

Toxin	Limit of Detection	Manufacturing Lot		
		ITE_17_001	ITE_17_002	ITE_17_0023
Azaspiracids	5 µg/kg	< 5 µg/kg	< 5 µg/kg	0 µg/kg
Pectenotoxins	5 µg/kg	< 20 µg/kg	< 20 µg/kg	0 µg/kg
Yessotoxins	20 µg/kg	< 5 µg/kg	< 5 µg/kg	0 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	< 5.0 µg/kg	< 5.0 µg/kg	0 µg/kg
Paralytic Shellfish Poison, saxitoxin	20 µg/kg	< 20 µg/kg	< 20 µg/kg	< 20 µg/kg

The sterol composition of Fermentalg's DHA 550 is expected to be similar to DHA algal oil derived from *Schizochytrium* sp. which have attained GRAS status (GRN No. 553 and 677) (U.S. FDA, 2015a, 2017b), with slight differences in the relative proportions of various sterols which are not expected to be affect safety.

2.5 Stability

Stability analysis of DHA 550, under both accelerated and real-time storage conditions, is ongoing. Approved antioxidants (*e.g.*, mixed tocopherols, ascorbyl palmitate) are used to enhance stability. Due to the high level of DHA present in DHA 550, this oil may be sensitive to oxidation compared to other available algal oils; however, under proper packaging and storage conditions, exposure to oxygen is limited and this should not present a significant real-world risk.

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 550

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 550 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 550.

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 infant formula as described herein is based on scientific procedures. Much of the 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 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, 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 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: 4hr +/- 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

bw = body weight; DHA = ; LD_{50} = ; NOAEL = no-observed-adverse-effect level; ppm = parts per million.

[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 for 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 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.

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 550, manufactured consistent with cGMP and meeting food-grade specifications, is GRAS for use as in select food categories and infant formula as described in Part 1.3, on the basis of scientific procedures. Fermentalg's conclusion on the GRAS status of DHA 550 under the conditions of its intended use is based its similarity in its source, composition, nutritional value, and metabolism to other GRAS-notified DHA algal oils. 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 550 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 550, meeting appropriate food-grade specifications and manufactured consistent with cGMP, is safe and suitable for use as an ingredient in select food categories and infant formula, 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 550 is presented in Appendix 2.

6.7 Conclusions

Based on data and information presented herein Fermentalg has concluded that DHA 550 can be determined to be Generally Recognized as Safe (GRAS) on the basis of scientific procedures.

The GRAS status of DHA 550 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 550, as described herein, is GRAS.

Therefore, the intended use of DHA 550 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)
	170.205	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
176—Indirect food additives: paper and paperboard components	176.180	Components of paper and paperboard in contact with dry food
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
	184.1857	Corn sugar
	184.1945	Vitamin B 12

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U.S. FDA (2017c). *Agency Response Letter GRAS Notice No. GRN 000731 [Docosahexaenoic acid oil produced in Schizochytrium sp., Shangdong Province, China: Linyi Youkang Biology Co., Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=731 [FDA's Letter: Pending; Page Last Updated: Dec. 31, 2017].

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APPENDIX 1
Certificates of Analysis

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Pessac, 10/04/2017

n/réf. : FERMENTALG – D2A08 – FA36

ANALYSIS CERTIFICATE

Contract followed by: LEITNER Loïc

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 LIQUID CHROMATOGRAPHY (NF EN ISO 9936) (*)

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

DETERMINATION OF 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 OF 4 PAH (Polycyclic Aromatic Hydrocarbons) BY HIGH PERFORMANCE LIQUID PERFORMANCE (ITERG method)

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

MICROBIOLOGICAL DETERMINATIONS (subcontracted analysis)

DETERMINATION OF METALS AND MINERALS BY ICP (ASTDM ASTDM D 5185 : Al, Ar, Ba, B, Ca, Cu, Sn, Fe, Mg, Mn, Mo, Ni, P, Pb, K, Si, Na, Ti, V et Zn) AND BY ICP-MS (RW/MA/01015 : Sb, Co, Li) (LQ = 1 mg/kg) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF MERCURY BY ICP-MS (LQ = 0.01 mg/kg) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF CADMIUM BY ICP-MS (LQ = 0.02 mg/kg) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF DIOXINELIKE PCB, PCDD/F and PCB (ICES-6) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF CALORIC VALUE – subcontracted analysis – COFRAC accreditation

SAMPLE

Reception date : 19/01/2017
Nature : **Refined algae oil ITE_17_001 (ITERG CODE E17-2931)**

The Institut des Corps Gras warrants that the essays have been performed according to the General Quality Procedure in use, and that the results of the essays pertain to the samples analysed.
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RESULTS

Refined algae oil ITE_17_001

FATTY ACIDS : composition and content	
FATTY ACIDS	**RESULTS (% of TOTAL FATTY ACIDS)**
12 : 0	0,1
14 : 0	1,5
14 : 1	0,3
15 : 0	<0,05
16 : 0	22,3
16 : 1	0,3
16 : 2	<0,05
16 : 3	0,4
16 : 4	<0,05
17 : 0	<0,05
17 : 1	0,1
18 : 0	0,8
18 : 1	0,6
18 : 1 trans	<0,05
18 : 2 (n-6)	0,2
18 : 2 trans	0,1
18 : 3 (n-6)	0,1
18 : 3 (n-3)	0,2
18 : 3 trans	<0,05
18 : 4 (n-3)	0,3
20 : 0	0,1
20 : 1	<0,05
20 : 3 (n-6)	0,1
20 : 4 (n-6)	0,1
20 : 4 (n-3)	0,6
20 : 5 (n-3)	0,5
22 : 0	0,1
22 : 1	0,9
22 : 4 (n-6)	<0,05
22 : 5 (n-6)	10,3
22 : 5 (n-3)	0,2
22 : 6 (n-3)	**59,8**
22 : 6 trans	<0,05
24 : 0	<0,05
24 : 1	0,1
TOTAL FA (g/100g)	**94,7**
DHA CONTENT (g/100g)	**56,6**
TOTAL trans FA (% of TOTAL FA)	**< 0,25**

Refined algae oil ITE_17_001

Determination	Results
ACID VALUE (NF EN ISO 660)	0,28 ± 0,10 mg KOH/g
OLEIC ACIDITY (NF EN ISO 660)	0,14 ± 0,05 % (m/m)
PEROXIDE VALUE (NF EN ISO 3960)	2,8 ± 1,1 méqO_2/kg
COLOR (NF ISO 27608)	Lovibond 5"1/4 : 10,9 R ; 70,0 Y
WATER AND VOLATIL CONTENT (NF EN ISO 662)	<0,05 %
ANISIDINE VALUE (NF EN ISO 6885)	83,3
TOCOPHEROL CONTENT (NF EN ISO 9936)	6 mg/kg ± 1
UNSAPONIFIABLE CONTENT (NF EN ISO 3596)	1,36 % ± 0,30
Arsenic (method ITERG)	<0,01 mg/kg
Lead (NF EN ISO 12193)	<0,01 mg/kg
Iron (NF EN ISO 8294)	0,015 mg/kg
Copper (NF EN ISO 8294)	0,006 mg/kg
Mercury (subcontracted determination)	<0,005 mg/kg
Cadmium (subcontracted determination)	<0,01 mg/kg
4 PAH* content (method ITERG) among B(a)P	<0,4 µg/kg <0,2 µg/kg

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

Refined algae oil ITE_17_001

Determination	Results
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

Determination	Results
Content in PCB NDL (6PCB) Subcontracted determination	0,6 (**) ng/g
Content in PCB " dioxinlike " Subcontracted determination	0,139 (**) pg/g
Content in PCDD/F Subcontracted determination	0,164 (**) pg/g
Content in PCDD/F + PCB "dioxinlike" Subcontracted determination	0,303 (**) pg/g

(**) For each individual result beyond the limit of detection, the value of the limit of detection is taken into account for the calculation of the sum. For each individual result between the limit of detection and the limit of quantification, the value of the limit of quantification is taken into account for the calculation of the sum.

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Pessac, 10/04/2017

n/réf. : FERMENTALG – D2A08 – FA36

ANALYSIS CERTIFICATE

Contract followed by: LEITNER Loïc

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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 LIQUID CHROMATOGRAPHY (NF EN ISO 9936) (*)

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

DETERMINATION OF 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

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DETERMINATION OF MERCURY BY ICP-MS (LQ = 0.01 mg/kg) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF CADMIUM BY ICP-MS (LQ = 0.02 mg/kg) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF DIOXINELIKE PCB, PCDD/F and PCB (ICES-6) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF CALORIC VALUE – subcontracted analysis – COFRAC accreditation

SAMPLE

Reception date : 19/01/2017
Nature : **Refined algae oil ITE_17_002 (ITERG CODE E17-2932)**

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RESULTS

Refined algae oil ITE_17_002

FATTY ACIDS (FA) : composition and content	
FATTY ACIDS	**RESULTS (% of TOTAL FATTY ACIDS)**
12 : 0	0,1
14 : 0	1,2
14 : 1	0,5
15 : 0	<0,05
16 : 0	20,7
16 : 1	0,4
16 : 2	<0,05
16 : 3	0,3
16 : 4	<0,05
17 : 0	0,1
17 : 1	0,2
18 : 0	0,8
18 : 1	0,8
18 : 1 trans	<0,05
18 : 2 (n-6)	0,1
18 : 2 trans	<0,05
18 : 3 (n-6)	0,1
18 : 3 (n-3)	0,2
18 : 3 trans	<0,05
18 : 4 (n-3)	0,4
20 : 0	0,1
20 : 1	<0,05
20 : 3 (n-6)	0,1
20 : 4 (n-6)	0,1
20 : 4 (n-3)	0,6
20 : 5 (n-3)	0,9
22 : 0	0,1
22 : 1	1,3
22 : 4 (n-6)	<0,05
22 : 5 (n-6)	10,0
22 : 5 (n-3)	0,4
22 : 6 (n-3)	**60,4**
22 : 6 trans	<0,05
24 : 0	<0,05
24 : 1	0,1
TOTAL FA (g/100g)	**93,5**
DHA CONTENT (g/100g)	**56,5**
TOTAL trans FA (% of TOTAL FA)	**< 0,2**

Refined algae oil ITE_17_002

Determination	Results
ACID VALUE (NF EN ISO 660)	0,16 ± 0,10 mg KOH/g
OLEIC ACIDITY (NF EN ISO 660)	0,08 ± 0,05 % (m/m)
PEROXIDE VALUE (NF EN ISO 3960)	2,6 ± 1,0 méqO_2/kg
COLOR (NF ISO 27608)	Lovibond 5"1/4 : 2,2 R ; 24,0 Y
WATER AND VOLATIL CONTENT (NF EN ISO 662)	<0,05 %
ANISIDINE VALUE (NF EN ISO 6885)	48,8
TOCOPHEROL CONTENT (NF EN ISO 9936)	2 mg/kg ± 1
UNSAPONIFIABLE CONTENT (NF EN ISO 3596)	1,77 % ± 0,30
Arsenic (method ITERG)	<0,01 mg/kg
Lead (NF EN ISO 12193)	< 0,01 mg/kg
Iron (NF EN ISO 8294)	0,010 mg/kg
Copper (NF EN ISO 8294)	< 0,005 mg/kg
Mercury (subcontracted determination)	<0,005 mg/kg
Cadmium (subcontracted determination)	<0,01 mg/kg
4 PAH* content (method ITERG) among B(a)P	<0,4 µg/kg <0,2 µg/kg

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

Refined algae oil ITE_17_002

Determination	Results
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

Determination	Results
Content in PCB NDL (6PCB) Subcontracted determination	1,6 (**) ng/g
Content in PCB " dioxinlike " Subcontracted determination	0,139 (**) pg/g
Content in PCDD/F Subcontracted determination	0,169 (**) pg/g
Content in PCDD/F + PCB "dioxinlike" Subcontracted determination	0,308 (**) pg/g

(**) For each individual result beyond the limit of detection, the value of the limit of detection is taken into account for the calculation of the sum. For each individual result between the limit of detection and the limit of quantification, the value of the limit of quantification is taken into account for the calculation of the sum.

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CANCELS AND REPLACES*

Sample code Nr. 370-2017-00076638 **Report Date** 21/04/2017

Analytical Report Nr. AR-17-AA-078437-02 / 370-2017-00076638

(*this report cancels and replaces the previous one, numbered AR-17-AA-078437-01/370-2017-00076638 dated 11/04/2017 which must be destroyed)

FERMENTALG SA

For the attention of **Mme. Catherine MEAR**
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Copy to : Madame LETERRIER (mleterrier@fermentalg.com), Mme. MEAR (Mail)

Your contact for Customer Service : Charles Victoire

Our reference :	370-2017-00076638/ AR-17-AA-078437-02	**Type :**	EX
Client reference :	**E17-3628**		
Sample described as :	Huile raffinée		
Packaging :	NonCommercial : 2x125ml=250ml		
Your purchase order date :	30/03/2017	**Your purchase order reference :**	ANA_20170329_01 / (EOL) 518-581985
Sample reception date :	31/03/2017 10:30:00	**Analysis starting date :**	03/04/2017
Sampling/Transport :	DHL		
Analyses requested :	JJ0F5 : Toxines coquillages azaspiracides, pectenotoxines JJL3K : Toxine coquillages ASP,PSP,acide okadaique,Dinophy JJ0F6 : Toxines coquillages yessotoxines		

Contaminants			Results	
JJ0F5	**ext**	**Shellfish poisons azaspiracids, pectenotoxins**	**Method : BVL L 12.03/04-4**	
	Azaspiracids		0.0	µg/kg
	AZA-1		<5	µg/kg
	AZA-2		<5	µg/kg
	AZA-3		<5	µg/kg
	Pectenotoxins		0.0	µg/kg
	PTX-1		<5	µg/kg
	PTX-2		<5	µg/kg
	PTX-2sa		<5	µg/kg
JJ0F6	**ext**	**Shellfish poisons yessotoxins**	**Method : BVL L 12.03/04-4**	
	Yessotoxins		0.0	µg/kg
	YTX		<20	µg/kg
	45-OH-YTX		<20	µg/kg
	Homo-YTX		<20	µg/kg
	45-OH-homo-YTX		<20	µg/kg
JJL3K	**ext**	**Shellfish Poisons ASP, PSP, Okadaic acid, Dinophy**	**Method : BVL L 12.03/04-1/3/4**	
	Amnesic Shellfish Poison, Domoic acid		<1000	µg/kg
	Paralytic Shellfish Poison, Saxitoxin		<20	µg/kg
	DSP		0.0	µg/kg
	Okadaic acid		<5	µg/kg
	DTX-1		<5	µg/kg

CONCLUSION

As judged by the results of the analyses performed, and in comparison with industry standards, scientific litterature, and values at our disposal :

To the quantification limits of the methods involved, none of the screened parameters was observed.

SIGNATURE (b) (6)

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Analytical Service Manager

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Pessac, 10/04/2017

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

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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

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MICROBIOLOGICAL DETERMINATIONS (subcontracted analysis)

DETERMINATION OF METALS AND MINERALS BY ICP (ASTDM ASTDM D 5185 : Al, Ar, Ba, B, Ca, Cu, Sn, Fe, Mg, Mn, Mo, Ni, P, Pb, K, Si, Na, Ti, V et Zn) AND BY ICP-MS (RW/MA/01015 : Sb, Co, Li) (LQ = 1 mg/kg) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF MERCURY BY ICP-MS (LQ = 0.01 mg/kg) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF CADMIUM BY ICP-MS (LQ = 0.02 mg/kg) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF DIOXINELIKE PCB, PCDD/F and PCB (ICES-6) – subcontracted analysis – COFRAC accreditation

DETERMINATION OF CALORIC VALUE – subcontracted analysis – COFRAC accreditation

SAMPLE

Reception date : 28/03/2017
Nature : **Refined algae oil ITE_17_0023 (ITERG CODE E17-3628)**

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RESULTS

Refined algae oil ITE_17_0023

FATTY ACIDS : composition and content	
FATTY ACIDS	**RESULTS (% of TOTAL FATTY ACIDS)**
12 : 0	0,1
14 : 0	1,1
14 : 1	0,3
15 : 0	0,1
16 : 0	18,7
16 : 1	0,2
16 : 2	nd
16 : 3	nd
16 : 4	nd
17 : 0	0,1
17 : 1	0,1
18 : 0	0,7
18 : 1	0,7
18 : 1 trans	< 0,05
18 : 2 (n-6)	0,1
18 : 2 trans	< 0,05
18 : 3 (n-6)	0,1
18 : 3 (n-3)	0,2
18 : 3 trans	< 0,05
18 : 4 (n-3)	0,3
20 : 0	0,1
20 : 1	nd
20 : 2 (n-6)	nd
20 : 3 (n-6)	0,2
20 : 3 (n-3)	nd
20 : 4 (n-6)	0,1
20 : 4 (n-3)	0,6
20 : 5 (n-3)	0,6
22 : 0	0,1
22 : 1	0,9
22 : 4 (n-6)	Nd
22 : 5 (n-6)	11,2
22 : 5 (n-3)	0,2
22 : 6 (n-3)	**63,1**
22 : 6 trans	< 0,05
24 : 0	0,1
24 : 1	0,1
TOTAL FA (g/100g)	**88,7**
DHA CONTENT (g/100g)	**56,0**
TOTAL trans FA (% of TOTAL FA)	**< 0,02**

Refined algae oil ITE_17_0023

Determination	Results
ACID VALUE (NF EN ISO 660)	0,08 ± 0,10 mg KOH/g
OLEIC ACIDITY (NF EN ISO 660)	0,04 ± 0,05 % (m/m)
PEROXIDE VALUE (NF EN ISO 3960)	0,4 ± 1,0 méqO_2/kg
COLOR (NF ISO 27608)	Lovibond 5"1/4 : 10,7 R ; 70,0 Y
WATER AND VOLATIL CONTENT (NF EN ISO 662)	<0,05 %
ANISIDINE VALUE (NF EN ISO 6885)	57,4
TOCOPHEROL CONTENT (NF EN ISO 9936)	1341 mg/kg ± 201
UNSAPONIFIABLE CONTENT (NF EN ISO 3596)	1,22 % ± 0,30
Arsenic (method ITERG)	<0,01 mg/kg
Lead (NF EN ISO 12193)	<0,01 mg/kg
Iron (NF EN ISO 8294)	0,060 mg/kg
Copper (NF EN ISO 8294)	<0,005 mg/kg
Mercury (subcontracted determination)	<0,005 mg/kg
Cadmium (subcontracted determination)	<0,01 mg/kg
4 PAH* content (method ITERG) among B(a)P	<0,4 µg/kg <0,2 µg/kg

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

Refined algae oil ITE_17_0023

Determination	Results
Research for Aerobic microorganisms 30°C (NF EN ISO 4833-1)	29/g
Research for Yeast (NF V08-059)	<1/g
Research for moulds (NF V08-059)	<4/g Presence of microorganisms but unquantifiable
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

Determination	Results
Content in PCB NDL (6PCB) Subcontracted determination	0,6 (**) ng/g
Content in PCB " dioxinlike " Subcontracted determination	0,139 (**) pg/g
Content in PCDD/F Subcontracted determination	0,208 (**) pg/g
Content in PCDD/F + PCB "dioxinlike" Subcontracted determination	0,347 (**) pg/g

(**) For each individual result beyond the limit of detection, the value of the limit of detection is taken into account for the calculation of the sum. For each individual result between the limit of detection and the limit of quantification, the value of the limit of quantification is taken into account for the calculation of the sum.

APPENDIX 2
Expert Panel Consensus Statement

Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of DHA 550 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 550 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 550 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 550 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 550 oil is extracted and refined from *Schizochytrium sp.* FCC-3204. It is a mixture of triglycerides containing polyunsaturated fatty acids (PUFA) in which DHA represents more than 55% of total fatty acids.

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 550 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, 2016). Analysis of 3 non-consecutive lots each of DHA 550 demonstrated that this process produces oils that reproducibly meet appropriate food-grade specifications. Fermentalg has demonstrated the absence of algal toxins in DHA 550.

In addition to DHA, Fermentalg's oils contain other fatty acids, as well as sterols. There fatty acids present in Fermentalg's DHA 550 are all common dietary fatty acids. 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 550 and other DHA oil products are not expected to affect safety. Proximate analysis demonstrates that Fermentalg's DHA 550 is free from protein and carbohydrates (limit of detection of 0.1%).

Due to the higher level of DHA present in DHA 550, this oil may be more sensitive to oxidation than algal oils; however, under proper packaging and storage conditions, exposure to oxygen is limited and this should not present a significant real-world risk. Stability analysis of DHA 550, under both accelerated and real-time storage conditions, is ongoing. Approved antioxidants (*e.g.*, mixed tocopherols, ascorbyl palmitate) are used to enhance the stability of the oil.

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 550 will be used at roughly 35% 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 550
Baked goods, baking mixes, § 170.3[a](n)(1) of this chapter	5.0 percent	1.8 percent
Cereals, § 170.3(n)(4) of this chapter	4.0 percent	1.4 percent
Cheese products, § 170.3(n)(5) of this chapter	5.0 percent	1.8 percent
Chewing gum, § 170.3(n)(6) of this chapter	3.0 percent	1.1 percent
Condiments, § 170.3(n)(8) of this chapter	5.0 percent	1.8 percent
Confections, frostings, § 170.3(n)(9) of this chapter	5.0 percent	1.8 percent
Dairy product analogs, § 170.3(n)(10) of this chapter	5.0 percent	1.8 percent
Egg products, § 170.3(n)(11) of this chapter	5.0 percent	1.8 percent
Fats, oils, § 170.3(n)(12) of this chapter, but not in infant formula	12.0 percent	4.2 percent
Fish products, § 170.3(n)(13) of this chapter	5.0 percent	1.8 percent
Frozen dairy desserts, § 170.3(n)(20) of this chapter	5.0 percent	1.8 percent
Gelatins, puddings, § 170.3(n)(22) of this chapter	1.0 percent	0.4 percent
Gravies, sauces, § 170.3(n)(24) of this chapter	5.0 percent	1.8 percent
Hard candy, § 170.3(n)(25) of this chapter	10.0 percent	1.8 percent
Jams, jellies, § 170.3(n)(28) of this chapter	7.0 percent	2.45 percent
Meat products, § 170.3(n)(29) of this chapter	5.0 percent	1.8 percent
Milk products, § 170.3(n)(31) of this chapter	5.0 percent	1.8 percent
Nonalcoholic beverages, § 170.3(n)(3) of this chapter	0.5 percent	0.18 percent
Nut products, § 170.3(n)(32) of this chapter	5.0 percent	1.8 percent
Pastas, §170.3(n)(23) of this chapter	2.0 percent	0.7 percent
Plant protein products, § 170.3(n)(33) of this chapter	5.0 percent	1.8 percent
Poultry products, § 170.3(n)(34) of this chapter	3.0 percent	1.1 percent
Processed fruit juices, § 170.3(n)(35) of this chapter	1.0 percent	0.4 percent
Processed vegetable juices, § 170.3(n)(36) of this chapter	1.0 percent	0.4 percent
Snack foods, § 170.3(n)(37) of this chapter	5.0 percent	1.8 percent
Soft candy, § 170.3(n)(38) of this chapter	4.0 percent	1.4 percent
Soup mixes, § 170.3(n)(40) of this chapter	3.0 percent	1.1 percent
Sugar substitutes, § 170.3(n)(42) of this chapter	10.0 percent	3.5 percent
Sweet sauces, toppings, syrups, § 170.3(n)(43) of this chapter	5.0 percent	1.8 percent
White granulated sugar, § 170.3(n)(41) of this chapter	4.0 percent	1.4 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 550 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 550 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-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-Dahms *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.,* 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, 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, 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 550, 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

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From:	Erica Cermak Intertek
To:	Morissette, Rachel; Hywel Griffiths
Cc:	West-Barnette, Shayla
Subject:	RE: follow-up to phone call for GRNs 000776 and 000777
Date:	Friday, August 24, 2018 11:41:30 AM
Attachments:	image002.png image007.png image020.png GRN 000776 and GRN 000777 supplement August 24 2018.docx Appendix 1 Comparative Fatty Acid Analysis – ITERG report.pdf Appendix 2 Processing Aid Certificates.pdf

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

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Total Quality. Assured.

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

FDA U.S. FOOD & DRUG ADMINISTRATION [fda.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

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
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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

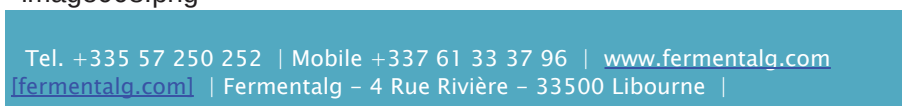
From: Hywel Griffiths [mailto:hgriffiths@fermentalg.com]
Sent: Wednesday, July 18, 2018 11:31 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Erica Cermak Intertek <erica.cermak@intertek.com>
Subject: Re: questions for GRNs 000776 and 000777 (DHA algal oil)

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



> 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>

1) For both GRNs 000776 and 000777, please clarify the intended use to indicate that the DHA oil will be used with a safe and suitable source of arachidonic acid (ARA) within the range indicated from 1:1 to 1:2 DHA:ARA.

Fermentalg confirms that its DHA algal oil is intended for use as a direct ingredient in exempt (pre-term) and nonexempt (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 similar to all other approved uses for incorporation of DHA in infant formula.

2) For both GRNs 000776 and 000777, the DHA algal oil derived from *Schizochytrium* sp. that is the subject of these notices does not meet the specifications for fatty acid levels (with the exception of DHA), expressed in area %, as listed in the Food Chemicals Codex (FCC) 11 monograph for this ingredient. Please address this issue for both GRNs.

The upper and lower limits of fatty acids present in the FCC monograph are narrow and inconsistent with the values reported in GRAS Notices for other DHA algal oils intended for use in infant formula. In several cases, the levels of various fatty acids present in these oils likewise fall outside the ranges specified in the monographs.

Fatty acid	Shorthand notation	Lower Limit (area %)	Upper limit (area %)	Martek DHASCO-B Lot # 08-6530 (GRN 553)	Mara Renewables Lot #16039 (GRN 677)	Fermentalg DHA Algal Oil from *Schizochytrium* sp. FCC-1324 Lot # NF1 (GRN 776)	Fermentalg DHA Algal Oil from *Schizochytrium* sp. FCC-3204 Lot # ITE_17_001 (GRN 777)
Dihomo-gamma-linolenic acid	20:3 n-6	1.7	2.8	< 0.1	<0.1	0.1	0.1
Arachidonic acid	20:4 n-6	0.6	1.3	0.67	0.75	0.3	0.1
Eicosapentanoic acid	20:5 n-3	1.3	3.9	5.90	1.08	0.2	0.5
Docosapentaenoic acid	22:5 n-6	10.5	16.5	2.63	7.21	8.3	10.3
Docasahexaenoic acid	22:6 n-3	30	40	44.3	37.10	38.2	59.8

Fermentalg's oils are not intended as a source of dihomo-gamma-linolenic acid. With regards to arachidonic acid, although Fermentalg's oils are below the FCC monograph, this fatty acid will be added to infant formula separately as noted in the response to Question #1. Manufacturers will compensate to achieve the desired ratio in the finished product. Recommendations are to use less EPA than DHA in infant formula, as a result, levels below the monograph minimum are not considered to be detrimental. DHA550 is higher than FCC11 specification for DHA, but since incorporation of these oils into infant formula is by weight of DHA and not by weight of oil this autocontrols.

It is likely that the limits included in the monograph were representative of the oil produced by the industry member that originally petitioned FCC for the monograph and, as demonstrated in Table 1, are not

representative of all currently available DHA algal oils derived from *Schizochytrium*, including those marketed for use in infant formula.

3) In GRN 000776, Fermentalg provides a comparison of the fatty acid content of its oil with Martek's GRN 000137 oil. Fermentalg also notes:
"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)."
However, the intended uses considered in GRN 000137 and by FSAI did not include infant formula. Please compare Fermentalg's DHA algal oil to oils currently used in infant formula or oils that were the subject of relevant developmental and clinical studies cited in the notice in order to support the claim of "substantial equivalence" for use in infant formula. If there are appreciable differences in the concentration of one or more fatty acids between Fermentalg's DHA algal oil and other oils used in infant formula or studies, explain why such differences are not a safety concern by discussing the absorption/distribution/metabolism/excretion of fatty acids and the requirement of fatty acids in infants, including pre-term infants.

As GRN 000137 did not include use in infant formula, Fermentalg presents the Table 2 below comparing the fatty acid profile of other DHA oils derived from *Schizochytrium* that were notified for use in formula, namely the oil produced by DSM Nutritional Products (GRN 000553) and the oil produced by Mara Renewables (GRN 000677).

Fatty Acid	DSM DHASCO-B Lot # 08-6530 (GRN 553)	Mara Renewables Lot #16039 (GRN 667)	Fermentalg DHA Algal Oil from *Schizochytrium* sp. FCC-1324 (DHA 350) Lot # NF1 (GRN 776)	Fermentalg DHA Algal Oil from *Schizochytrium* sp. FCC-3204 (DHA 550) Lot # ITE_18_023 (GRN 777)
12:0	<0.1	0.92	0.2	0.1
14:0	1.30	12.30	4.0	1.1
14.1	<0.1	<0.10		0.3
15:0	0.24	0.68		0.1
16:0	13.9	22.67	42.8	18.7
17:0	<0.1	0.15		0.1
16:1 n-7	<0.1	6.16	0.1	0.2
17:0				0.1
17:1				0.1
18:0	1.64	0.77	1.1	0.7
18:1 n-9	24.5	7.49	0.6	0.7
18:1 n-7	0.22		Sum with oleate	
18:2 n-6	2.05	0.34	0.8	0.1
20:0	0.31	<0.1		0.1
18:3 n-3	0.10	0.24		0.2
18:3 n-6				0.1
18:4 n-3		0.24	0.2	0.3
20:1 n-9	<0.1	<0.1		
20:2 n-6	0.12			
20:3 n-6	<0.1	<0.1	0.1	0.2
22:0	0.32	<0.1		0.1
20:3 n-3	<0.1			
20:4 n-6	0.67	0.65	0.3	0.1
20:4 n-3			0.5	0.6
20:5 n-3	5.90	1.08	0.2	0.6
24:0	0.12	<0.1		0.1
22:1				0.9
22:2 n-6	0.54			
24:1 n-9	<0.1			0.1
22:4 n-3	<0.1			
22:4 n-6				
22:5 n-6	2.63	7.21	8.3	11.2
22:5 n-3			0.1	0.2
22:6 n-3	44.35	37.10	39.2	63.1

As shown in Table 2, the overall composition of DHA350 is similar to that of Mara Renewable's oil (GRN 000677). With the exception of some fatty acids present at low levels (≤1%), Fermentalg's oils do not contain fatty acids not present in other sources of DHA algal oils used in infant formulas. These other fatty acids are naturally present in either human breast milk (Yuhas *et al.* 2006) or infant formula components such as cow's milk (Blaško *et al.* 2010).
DHA550 contains similar fatty acids to the other oils but the polyunsaturates are present at higher levels, whereas the saturates are reduced. The ratio of DHA:DPAn-6 and DHA to other polyunsaturates is very similar between DHA550, DHA350 and Mara Renewable's oil. Levels of incorporation of the oil into infant formula are mandated by DHA level and not oil level and so the levels of polyunsaturates provided from DHA550 will be nearly identical to that provided by DHA350 and Mara Renewable's oil. The higher level of DHA in DHA550 will result in the use of less oil and lower levels of saturates incorporated from the DHA550. However, the milk component, not the DHA oil, is the major source of lipids in infant formula.

DHA, along with the other fatty acids present in Fermentalg's DHA 350 and DHA 550, are present in breast milk. The triglyceride structures of the fatty acids that are chemically equivalent to those delivered to infants from mother's milk, and as a result, the metabolic fate of the fatty acids present in DHA 350 and DHA 550 is similar. As reviewed by Kroes *et al.* (2003), dietary triglycerides, regardless of the source, undergo enzymatic hydrolysis in the upper intestine to free fatty acids and 2-monoglycerides, which are integrated into bile acid micelles for diffusion into the interior of the intestinal epithelial cells and subsequent incorporation into new or reconstituted triglycerides.

Comprehensive summaries of the clinical study literature regarding DHA or long-chain polyunsaturated fatty acids (LC-PUFA) relevant to supplementation of infant formula from fish and algal oil sources have been included in previous GRAS Notices (FDA, 2011, 2014, FDA 2015). As reviewed in GRN 553 (US FDA, 2015a) , there are no adverse effects associated with 1% DHA in infant formulas, with some studies suggesting a benefit to stature and body composition. Preterm infants fed 1.0% DHA and weighing ≥ 1250 g at birth were up to 1.7 cm longer at 18 months corrected age compared to infants fed 0.35% DHA. In those weighing <1250 g at birth, head circumference was greater in response to 1% DHA at expected delivery date (Collins *et al.*, 2011). Pittaluga and co-workers (2011) note that preterm infants fed 0.25-0.12% DHA throughout the first year of life are leaner and have lower fasting insulin levels at 1 and 2 years of age than infants consuming formula devoid of tong chain polyunsaturated fatty acids (LCPUFA). Data on supplementation of DHA up to 1% in term and preterm infants appears to improve certain cardiovascular and respiratory health outcomes. Improvements in visual acuity and mental acuity have also been reported in infants fed DHA supplemented formulas.

4) Please provide the source of the data presented in Table 2.4.4-1 on pp. 12-13 of GRN 000776 for "Martek Oil Analysis (Composition by Area %)". The data does not appear to match the composition data for fatty acids provided in GRN 000137. The mean reported for 20:4 n3 appears to be in error compared to the individual data points. Please confirm these values.

The fatty acid composition performed on Fermentalg's DHA-rich oil and on the approved Martek DHA-rich oil presented in table 2.4.4-1 were analyzed for Fermentalg at an accredited external laboratory located in Europe (ITERG, the French Institute for Fats and Oils). Results of this analysis are provided as Appendix 1 to this response. This was an analysis performed by the same laboratory at the same time as the Fermentalg batches NF1-NF3 on a sample of oil obtained commercially. Part of the difference in values between Table 2.4.4-1 of our notice and Table 3 of GRN000137 may arise from the different methods of expressing content (the first is as % area – effectively the % of total fatty acids, the second is as mg/g which generally gives a lower value (x10) since it takes into account other components of the oil such as unsaponifiables).

Furthermore, while Fermentalg cannot comment on other differences in detail since this is not our oil, there has been a tendency within the industry towards higher and higher DHA contents in basic oils, and subsequent dilution with high-oleic sunflower oil back to specification. This may explain the presence of higher levels of C18:1 and C18:2.

The mean value for 20:4n3 should be 0.4 rather than 0.2 as originally indicated in the Notice.

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.

Response to follow pending receipt of additional analytical data.

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.

Response to follow pending receipt of additional analytical data.

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.

Response to follow pending receipt of additional analytical data.

8) For both GRNs 000776 and 000777, Fermentalg notes the trade name of the antifoam reagent. Please provide the chemical identity of the antifoam reagent and a statement regarding its safety for the intended use.

Biospumex 153K is a proprietary mix of modified polyalkoxyesters which are nonionic and contain no silicone. The product is used in a wide range of food processes including fermentation and extraction. A data sheet and certificate regarding its safety in the production of foodstuffs are included in Appendix 2.

9) For the optional clarification by filtration step, please cite an effective Food Contact Notification or relevant regulation for the filtration material.

If filtered, the oils are mixed with a filter-aid Clarcel DICB (a diatomaceous earth) and then filtered on a Fibrafix filter plate (bleached cellulose and perlite). Certificates confirming the suitability of these materials for contact with foods are included in Appendix 2.

10) On p. 9 of GRN 000776, the method of manufacture flow chart shows refining (discoloration/deodorization) as a single step. Please clarify the following: Is the oil degummed before refining? Is the oil alkali refined? Is the oil subjected to a separate bleaching step or is it partially decolorized by heat bleaching during the deodorization process? If bleaching clay or other material is used, please briefly describe.

No degumming step is carried out on the oils as the levels of gums are sufficiently low that they can be removed during the bleaching step without need for a separate process. The bleaching step is carried out prior to, and separately from the deodorization step. Bleaching earths Trisyl and Tonsyl are used. Certificates confirming the suitability of these materials for contact with foods are included in Appendix 2

11) The enzyme used when extracting the DHA algal oil was not identified in GRN 000777, but was identified as "alcalase" (from Novazyme) in GRN 000776. We note that this appears to be a trade name. Please indicate the accepted or recommended name of this enzyme, including the production organism and EC or CAS number for this enzyme. Is the same enzyme used in the production of both GRNs 000776 and GRN 000777 DHA algal oils? Is this the same enzyme preparation that was described in GRN 000667 (and used in accordance with 21 CFR 184.1027)?

The enzyme is a protease (subtilisin) produced by *Bacillus licheniformis*. The CAS number is 9014-01 -1 and It is used in accordance with 21 CFR § 184.1027. It would appear to be the same enzyme used in GRN000667.

12) Please provide a brief statement about the removal or inactivation of the alcalase enzyme after it is used to extract the DHA algal oil from the biomass.

The vast majority of the (water soluble) enzyme is expected to be separated from the oil immediately after the lysis reaction along with the cellular material and aqueous fractions. Any proteinaceous compounds remaining associated with the crude oil are removed during the standard processes of refining, but if any doubt remains, the enzymatic activity would be destroyed by the elevated temperatures to which the oil is exposed during deodorization.

13) Fermentalg states that there are no reports of toxin production by the *Thraustochytriaceae* family, but they provide the results of analysis for a number of algal toxins, including: azaspiracids, pectinotoxins, yessotoxin, okadaic acid, domoic acid, diarrhetic shellfish poison, and paralytic shellfish poison. GRN 000137 noted that two toxic compounds are produced in Chromista (domoic acid in *Pseudo-nitzschia* and prymnesin in *Prymnesium* spp.). Please comment on the reasoning behind the selection of algal toxins that Fermentalg presented in GRNs 000776 and 000777. Are they all lipophilic? Are they resistant to removal by common oil refining processes? Please provide a brief

statement on the method(s) of analysis for these toxins in GRN 000776 (this information is found in GRN 000777, but not GRN 000776).

The toxins tested are the complete list of toxins in the standard tests for seafood and analysis was performed by an independent laboratory (Eurofins) using method BVL L 12.03/04-4 of the German Federal Office of Consumr Protection and Food Safety

The complete range was tested for completeness but none would be expected a) to be found in the production organism or b) to be resistant to the refining process.

14) PCB and dioxin analyses are presented in GRN 000777. We would not anticipate finding these substances in algal oil produced in accordance with current good manufacturing practices. Please provide the reasoning behind including these analyses and whether Fermentalg expects to find these substances in the final product.

Fermentalg does not anticipate finding these substances in the algal oil, but wished to confirm and demonstrate this fact. Fermentalg will not be analyzing each batch for the absence of these contaminants, but will continue to perform periodic verification as this is a standard demand of the market place

15) For both GRNs 000776 and 000777, please verify that validated analytical methods were used for the "internal methods" mentioned for arsenic, mercury, and cadmium specifications.

The analytical methods used for the analysis of arsenic, mercury and cadmium were based on the method EN NF 15763 (French Standards Agency) but minor variation from the Standard cause this to be noted as a "internal method". The subcontractor who carried out the analyses validated that the deviation from the Standard did not significantly impact the result.

16) The FCC 11 monograph includes limits for anisidine value (NMT 20.0), free fatty acids (NMT 0.4%), and total oxidation value (NMT 26). For both GRNs 000776 and 000777, what is the limit for free fatty acids in Fermentalg's DHA algal oil? Are the limits in the FCC monograph for anisidine value and total oxidation met?

Specifications for the anisidine value (NMT20.0) and total oxidation (NMT 26) defined by the FCC monograph are respected. Our specification for free fatty acids (Acid value of NMT 0.5mg KOH/g, which is roughly equivalent to 0.25% free fatty acids) is ~~more strict~~stricter than the monograph.

17) While stability data is provided in GRN 000776, it is not provided in GRN 000777. Rather, there is a statement in GRN 000777 that the stability analysis is ongoing. Also, Fermentalg notes:

"Due to the high level of DHA present in DHA 550, this oil may be sensitive to oxidation compared to other available algal oils; however, under proper packaging and storage conditions, exposure to oxygen is limited and this should not present a significant real-world risk."
Please provide data from the accelerated storage studies for GRN 000777 if they are available. Please provide a statement that total oxidation products arising from the use of this DHA algal oil are the same as those for other DHA-containing algal oils currently used in infant formula.

Preliminary data for the accelerated storage study of three batches of DHA 550 are provided in Table 5. Product remains within specification after 4 months in accelerated conditions, which is the recommended shelf-life at 4°C. Results at -20°C (the preferred storage temperature) appear consistent with a shelf-life of at least 12 months based on comparison with other commercial algal oils.

Batch	Parameter	Specification	Start	Accelerated stability studies carried out at 25°C and 60% RH			Study at -20 °C
			Time (weeks)				
			0	8	14	16	16
0403019	DHA (%)	Min 55	71.4	67.7	67.1	69.3	69.3
	Peroxide Value (meq KOH/g)	< 5	<1	< 1	< 1	< 1	< 1
	Anisidine Value	< 20	3.9	10.7	6.8	12.5	5.1

Batch	Parameter	Specification	Start	Accelerated stability studies carried out at 25°C and 60% RH			Study at -20 °C
			Time (weeks)				
			0	8	14	16	16
0413022-A	DHA (%)	Min 55	72.1	69.4	69.4	70.5	70.9
	Peroxide Value (meq KOH/g)	< 5	<1	1.5	1	<1	1.1
	Anisidine Value	< 20	2.2	6.4	3.1	3.6	2.8

Batch	Parameter	Specification	Start	Accelerated stability studies carried out at 25°C and 60% RH			Study at -20 °C
			Time (weeks)				
			0	8	14	16	12
041028-A	DHA (%)	Min 55	68.7	67.1	67.9	-	68.0
	Peroxide Value (meq KOH/g)	< 5	2.2	2.0	<1	-	1
	Anisidine Value	< 20	4.6	6.8	7.0	-	2.8

The limits for total levels of oxidation products per gram of oil for the product of GRN 000777 are the same as those for other DHA-containing algal oils currently used in infant formula. Given that less oil will be used to provide the same amount of DHA, actual exposure to oxidation products could be reduced.

18) For both GRNs 000776 and 000777, Fermentalg provides an estimate of intake based on the percent DHA. Please provide an estimate of intake of the total DHA algal oil ingredient, based on the range of DHA in the oil. We note that no upper limit is indicated for the DHA algal oil ingredients, but this information would be relevant for an exposure estimate.

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

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.

Response to follow pending receipt of additional analytical data.

20) For GRN 000776, please provide more background information on the *Schizochytrium* sp. organism, including whether or not *Schizochytrium* sp. FCC-1324 is pathogenic or toxigenic.

The taxonomic classification of the source organism is as follows:
Kingdom: Chromista
Phylum: Bigyra
Class: Labyrinthulea
Order: Thraustochytriida
Family: Thraustochytriaceae
Genus: Schizochytrium

Schizochytrium is a genus of unicellular protist that belongs to the *Throustochytrioceoe* family. Initially, this family was composed of seven genera (*Althornia*, *Aplanochytrium*, *Diplophrys*, *Japonochytrium*, *Schizochytrium*, *Thraustochytrium* and *Ulkenia*). Recent studies based on genetic and phenotypic analysis proposed changes in the classification, with the erection of new genera like *Botryochytrium*, *Porietichytrium* and *Sicyoidochytrium*, emended from *Ulkenio* or *Aurantiochytrium* and *Oblongichytrium* emended from *Schizochytrium* (Yokohama, Honda 2007; Yokohama, Salleh, Honda 2007).

Fermentalg collected a *Schizochytrium*-related strain in estuarine environment and undertook a characterization at a genetic and biochemical level. This study revealed that this strain (FCC-1324) could be assigned to the genus *Schizochytrium*. An example of a phylogenetic tree that has been constructed by comparison of sequences of the small subunit of ribosomal DNA (18S SSU-rDNA) is depicted on figure 1.

There are no reports of pathogenicity or toxigencity associated with *Schizochytrium* FCC-1324 or the other related *Schizochytrium* strains used in the production of DHA algal oils.

21) For GRN 000777, please provide more background information on the Schizochytrium sp. organism, including whether or not Schizochytrium sp. FCC-3204 is pathogenic or toxigenic.

Schizochytium strain FCC-3204 is a natural variant of the strain FCC-1324, from which the DHA350 oil is produced. FCC-3204 was selected without the use of any mutagenic agents and has not been subjected to any form of deliberate genetic modification. The taxonomic classification of the source organism is as follows:
Kingdom: Chromista
Phylum: Bigyra
Class: Labyrinthulea
Order: Thraustochytriida
Family: Thraustochytriaceae
Genus: Schizochytrium

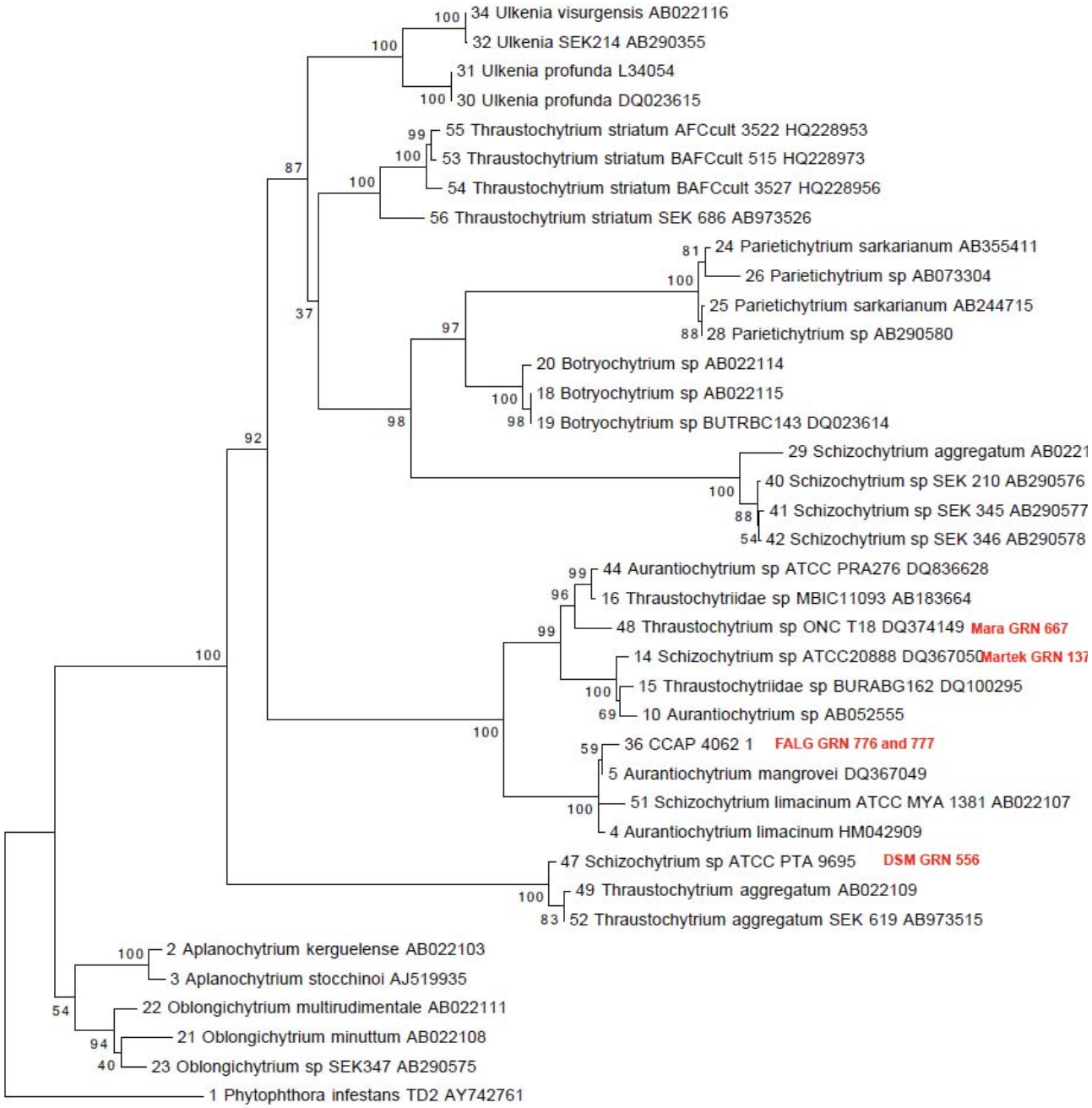
There are no reports of pathogenicity or toxigencity associated with *Schizochytrium* FCC-3204 or the other related *Schizochytrium* strains used in the production of DHA algal oils.

22) For both GRNs 000776 and 000777, please provide a narrative describing how the *Schizochytrium* sp. strains used in the toxicity studies you presented can be used to support the safety of your specific strains. The strains from your GRAS notices were not used in the mentioned toxicity studies and the connection between your strains and the published studies is not clear.

The source microalgae for all of these oils, *Schizochytrium*, are thraustochytrids, members of the kingdom Chromista (stramenopiles) which includes the heterokont algae. *Schizochytrium* sp. occurs widely in the aquatic environment and is an indirect component of the human food chain through indirect consumption of fish and other marine animals which feed on the microalgae. Figure 1 shows the phylogenic relationship between the strains used to produce other GRAS Notified DHA algal oils and the subject of the previous GRAS Notices covering infant formula.

The close taxonomic relationship between these species of micro-algae and Fermentalg's *Schizochytrium* strains is further evidenced by the close compositional similarity of the oil products derived from them. In addition, DHA 350 and DHA 550 are highly purified oils. Proximate analysis demonstrates that Fermentalg's DHA 350 is free from protein and carbohydrate (limit of detection of 0.1%), indicating that the remnants of production organism are not present in the purified oils.

Figure 1 **Updated Phylogeny of *Aurantiochytrium, Schizochytrium, Sicyiodochytrium* and *Thraustochytrium* Genera, Collectively Referred to as *Schizochytrium***



23) Please clarify how the presented safety studies in both GRNs 000776 and 000777 apply to the safe use of DHA algal oil in pre-term infant populations. Please specify which studies support the safe use of DHA algal oil in pre-term infants.

GRN 000776 and GRN000777 do not provide detail related to the safety of DHA algal oil in pre-term infants. However, such studies are available. For example, Carlson *et al.* (2013) reported no safety concerns following consumption of a marine algae-oil source of DHA (600 mg/day) from < 20 wk of gestation to birth. However, beneficial effects were observed. Specifically, DHA supplementation resulted in higher maternal and cord RBC-phospholipid-DHA, longer gestation duration (and greater birth weight, length, ad head circumference. In addition, there were fewer infants born at <34 wk of gestation and shorter hospital stays were required for infants born preterm in the DHA group compared to the placebo group.

Similarly, Clandinin *et al.* (2005) evaluated safety and benefits of feeding preterm infants formulas containing docosahexaenoic acid (DHA) and arachidonic acid (ARA) in pre-term infants. Preterm infants (n=361) were randomized across three formula groups: (1) control, no supplementation; (2) algal-DHA (DHA from algal oil, ARA from fungal oil); and (3) fish-DHA (DHA from fish oil, ARA from fungal oil). Term infants breast-fed <4 months (n = 105) served as a reference group. Infants receiving formula with algal DHA weighed significantly more than the control group from 66 to 118 weeks postmenstrual age (PMA) and significantly more than infants in the fish-DHA group at 118 weeks PMA. Likewise, the algal-DHA group was significantly longer than the control group at 48, 79, and 92 weeks PMA and the fish-DHA group at 57, 79, and 92 weeks. Weight and length were comparable to term infants. In addition, supplemented groups had higher Bayley mental and psychomotor development scores at 118 weeks PMA compared to the control group. Supplementation did not increase morbidity or adverse events. There were no differences in caloric intake from formula, daily gastric residuals, stool frequency, stool consistency, or abdominal distention among the preterm groups during hospitalization. Likewise, there were no differences among preterm groups with respect to parental reports of fussiness, diarrhea, or constipation. There were no adverse effects on hematology, serum glucose, cholesterol, high-density lipoproteins, triglyceride, mineral, and electrolyte measurements; and liver and kidney function tests. As noted in this supplement, the DHA algal oils used in these studies is comparable to that of Fermentalg's oils.

24) In both GRNs 000776 and 000777, two different dates are provided for when an updated literature search was conducted. Please clarify the correct date through which an updated literature search was done for these two notices.

Both GRN 000776 and 00077 state that the published scientific literature was reviewed in several previous GRAS Notices through May of 2017. In addition to reviewing information contained in these GRAS Notices, Fermentalg performed an updated search of the published scientific through August 2017.

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Collins CT, Makrides M, Gibson RA, et al. (2011). Pre- and post-term growth in pre-term infants supplemented with higher-dose DHA: a randomised controlled trial. Br J Nutr 105(11):1635-1643

Kroes R, Schaefer EJ, Squire RA, Williams GM. (2003) . A review of the safety of DHA45-oil. Food and Chemical Toxicology 41:1443-1446.

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U.S. FDA (2016 GRN 000677 [*Dacosahexaenoic acid oil produced in Schizochytrium sp., Dartmouth (Canada) Mara Renewables Corporation.* Available at: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm530169.pdf

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Appendix 1 Comparative Fatty Acid Analysis – ITERG report

Appendix 2 Processing Aid Certificates

BIOSPUMEX 153 K

Technical Data Sheet

BPX0001

Revision date: 15/11/2017 Supersedes TDS of : 23/06/2017 TDS version: 1.5

DESCRIPTION

Defoamer BIOSPUMEX 153 K is a blend based on polyether polyol and a natural fatty acid.

PHYSICO-CHEMICAL HAZARD DATA

Appearance	Viscous Colourless,light yellow
Relative density	≈ 1
Viscosity, dynamic	≈ 800 mPa.s 20°C
Solubility	In water, the material disperses.
Active matter	< 100 %

APPLICATION

Defoamer BIOSPUMEX 153 K is recommended to cure the foaming problems in aqueous media.
It can be used in various processes such as:

- Sugar
- Yeast

SAFE HANDLING ADVICE

Our technical team is at your disposal to optimize the point of introduction and dosage.
It can be implemented continuously or localy, either manually operated or by metering pump.
The expected maximum dose is of 80 g/T cossettes for transforming sugar beets in white crystallised sugar.
In general it is advisable to use it at 50 to 500 ppm for fermentation process. For other process at a level not higher than is necessary to achieve the intended purpose.

ADDITIONAL TECHNICAL DATA

The French order dated 19th October 2006 regarding use of processing aids in foodstuffs manufacture allows components of BIOSPUMEX 153 K mixture to be used as defoaming agent for processing :
yeast
sugar

PURITY CRITERIA	Yes	Heavy Metals : Pb<5ppm, As<1ppm, Cd<1ppm, Cr<1ppm, Hg<1ppm
	Yes	Residual monomers (EO+PO) <25ppm
CONFESSIONAL STATUTE	Yes	Kosher product: only upon request
	Yes	Halal product: only upon request
	Yes	This product doesn't contain ingredients of animal origin (including oils, grease or gelatin) or ethyl alcohol.
CONTAMINANTS	Yes	Do not contain BSE/TSE
	Yes	Do not contain pesticides.
	Yes	Have not been treated by ionizing radiation.
	Yes	Doesn't contain nanomaterial according to definition in recommandation 2011/696/EU.
GMO STATUS	Yes	Does not contain any genetically modified organism and is not produced from genetically modified organisms.

ALLERGEN STATUS	PRESENCE	CROSS-CONTAMINATION
Cereals containing gluten	No	No

Crustaceans and products thereof	No	No
Eggs et products thereof	No	No
Fish and products thereof	No	No
Peanuts / Groundnut and products thereof	No	No
Soybeans and products thereof	No	No
Milk and products thereof	No	No
Nuts and products thereof	No	No
Celery and products thereof	No	No
Mustard and products thereof	No	No
Sesame and products thereof	No	No
Sulphur dioxide and sulfites >10 ppm	No	No
Lupin and products thereof	No	No
Molluscs and products thereof	No	No

HANDLING AND STORAGE

Before use, it is recommended to read the safety data sheet.
Protect from freeze. Store in dry, cool, well-ventilated area.
After a long storage time a little phase displacement could appear. Original properties could be recovered by simple mixing. Shelf life : 2 years

PACKAGING

- Bulk
- Container of 1000 litres
- Drums of 200 litres
- Can of 25 litres

Contact address
PMC OUVRIE
Rue Albert Einstein, 44
F-62220 CARVIN - France
T +33 3 91.83.71.71 - F +33 3 91.83.71.91
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cognis
www.cognis.com

functionalproducts

Biospumex 153 K

Composition

Modified polyalkoxyesters - Non ionic.

Quality Control Data

(These data are used for quality release and are certified for each batch.)

Item		Value	Method / Remarks
Appearance:		At 25°C, clear colourless to yellow liquid - In 5% deionised water dilution : opalescent emulsion + cream after 15 minutes	
Acid Value:		< 3 mg KOH/g	ISO 660
Density:	20 °C	1.015 - 1.025 g/l	ISO 6883
Viscosity:	20 °C-2-12-SG	0 - 1500 mPas	ISO 2555 - Brookfield

Properties & Use

BIOSPUMEX 153 K is particularly suitable to eliminate foam that builds-up in food processes like fermentation & extraction. This product is mainly used in biochemical media.

Food industry:

- Starch extraction from corn flour.
- Protein extraction from vegetables.

Bio-chemistry:

- Production of citric and amino acids.
- Production of natural flavours and biomass.

BIOSPUMEX 153 K contains 100% of active matter. Its main features are as follows:

- has a very low toxicity towards a wide range of micro-organisms and does not interfere with their growth,
- does not affect the dissolved oxygen rate,
- is not affected by sterilisation (either neat or in aqueous emulsions),
- is economical because of its effectiveness/concentration,
- mixes easily in water/aqueous medium,
- is used at temperatures ranging from 0°C to 100°C,
- is totally silicone free.

Recipies & Dosage

BIOSPUMEX 153 K is generally used neat. When a dilution is needed, it has to be stirred during storage and introduced into the foaming medium at the last minute. In fermentation processes, the dosage usually varies from 50 to 500 ppm. The rate is 10 to 20 times lower in other food processes.

Additional Technical Data

The freezing point of BIOSPUMEX 153 K is below - 20°C.

Its viscosity ranges from 415 cSt. at 40°C, 670 cSt. at 30°C, 1155 cSt. at 20°C to 2120 cSt. at 10°C.

BIOSPUMEX 153 K is free of ethanol and animal origin product. It is Kosher approved.

BIOSPUMEX 153 K is compliant with the decree of 19 October 2006 concerning the application of technological auxiliaries in the manufacturing of certain foodstuffs **and in particular of sugar(semi-)white crystallized.**

Remarks

Handling & Safety:	Please refer to the safety data sheet for details.
Storage:	BIOSPUMEX 153 K properties are not affected by low temperatures. Nevertheless, it should be stored at room temperature.

Revision-No. 2.1-07.2008 Effective July 8, 2008

Cognis France - Ponthierry (Paris) - Phone 33 -1- 60 65 21 39 **F_A_S**

Made in Paris, on December 01, 2016
Expires on December 31, 2017

Food Chemical Codex Statement

La diatomite (terre de diatomées) est listée dans la X^{ème} édition du Food Chemical Codex (2016) en tant qu'auxiliaire technologique.

CHEMVIRON FRANCE, filiale du groupe CALGON CARBON, certifie par la présente que ses diatomées naturelles, calcinées et calcinées activées commercialisées sous la marque CLARCEL® respectent les critères de pureté décrits dans la monographie FCC correspondante, et notamment les teneurs maximales en Arsenic et Plomb mentionnées ci-dessous. Les Diatomées commercialisées ne sont pas des additifs alimentaires.

The diatomite (Diatomaceoussilica) islistedin the Xthedition of Food Chemical Codex (2016) as filteraids in foodprocessing.

CHEMVIRON FRANCE, a subsidiary of CALGON CARBON corporation, hereby certifies that its natural diatomite, calcined and flux-calcined diatomite marketed under the trademarks CLARCEL® comply with the specifications of the FCC monograph, in particular the following maximum content in Arsenic and Lead. The Marketed Diatomite are not food additives.

Impurities	Typical content	Acceptance criteria NMT
Arsenic	< 8 mg / kg	10 mg / kg
Lead	< 3 mg / kg	10 mg / kg

Product Manager

Laurent Bertrand

(b) (6)

Regulatory Affairs & Product engineer

Mara Campagnolle

(b) (6)

CHEMVIRON France- European Operations of CALGON CARBON Corporation
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CLARCEL: 78 CBL, CBL3, CBR, CBR3, F, FD, DIC, DICB, DICS, DIC3, DIFBO, DITR, DIT2R, DIT3R, DIFB, DIFN, DIFD, DIFC, DIFR

Disclaimer

See the product's safety data sheet (SDS) for health & safety considerations.
The statements, technical information and recommendations contained herein are specific to this product and are believed to be accurate as of the date hereof. Since the conditions and methods of use of the product and of this information are beyond our control, we expressly disclaims any and all liability as to the consequences resulting from or relating to their use or reliance. No warranty or guarantee whether on performance, suitability, merchantability, fitness for purpose, compliance to laws or to end-use requirements or otherwise, is made concerning the product, its applications or the information that is provided by this document.
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Kantonales Amt für Lebensmittelkontrolle

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05 août 2003

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FILTROX AG
Moosmühlestrasse 6
9001 St. Gallen

Confirmation relative à la conformité des couches filtrantes aux lois sur les denrées alimentaires

Suivant l'examen du dossier déposé concernant les couches filtrantes identifiées ci-après, nous arrivons à la conclusion qu'après un rinçage adéquat avant la première utilisation, une contamination des boissons filtrées par des substances insalubres n'est pas identifiable à l'état actuel des connaissances.

Les filtres sont conformes à la recommandation XXXVI/1 du BgVV et satisfont aux exigences de la Lebensmittel- und Bedarfsgegenständegesetz LMBG [loi sur les denrées alimentaires et les objets usuels], en particulier §§5, 30 et 31. Les produits peuvent être utilisés comme papiers filtres d'eau chaude et bouillante et couches filtrantes pour denrées alimentaires.

Les paramètres de test sont basés sur ces dispositions et les directives de la loi suisse sur les denrées alimentaires.

Cette confirmation concerne les filtres suivants:

FibraFix:

							TecnaFix:
AF 6	AF 30	AF 21H	AF Steril 110	W-Steril	AF 03	AF 103	TS 4
AF 9	AF 50	AF 41H	AF Steril 130	W-Steril S	AF 23	AF 113	TS 5
AF 15	AF 70	AF 71H	AF Steril 140	FKV	AF 43	AF 133	TS 6
AF 15 S	AF 100	AF 71S	AF Steril 150	FKS	AF 73	AF 143	TS 15
AF 20	U3	AF 101 H WS				AF 153	TS 30
							TS 70

AMT FÜR LEBENSMITTELKONTROLLE
[OFFICE DU CONTRÔLE DES DENRÉES ALIMENTAIRES]
ST. GALLEN

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Dr. P. Kölbener
Leiter Abt. Chemie
[Direction Dép. Chimie]

(b) (6)

K. Luczynski
Sachbearbeiter
[Coll.compétent]

FILTROX AG, CH-9001 St.Gallen / Switzerland

St. Gallen, 24.03.2014

Declaration of Conformity for Filter Sheets

To whom it may concern

FILTROX AG is a producer of filter sheets for applications in the food and beverage industry as well as in the pharmaceutical and chemical industry.

These filter sheets are manufactured of specially selected raw materials such as purified and bleached cellulose, inorganic natural filter aids, like Kieselguhr, Perlite and Polyamidoamine resin as wet strength agent.

The filter sheets are in line with recommendation XXXVI/1 of BfR and comply with the requirements of the "Lebensmittel-, Bedarfsgegenstände- und Futtermittelgesetzbuch LFGB" (German Food and Feed Code). The products also comply with the requirements of U.S.P. (Safety Test) as weil as F.D.A. regulations CFR21, § 177.2260 e,f,g,h,i,j,k, and I. All our products are made according to the rules of Quality Management System EN ISO 9001 as well as to the Environmental Management System EN ISO 14001.

Furthermore, we confirm that the filter sheets are in conformation with the regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27th October 2004 on materials and articles intended to come into contact with food, as well as regulation (EC) No 2023/2006.

FILTROX filter sheets and all raw materials contain no live organism or animal based extracts. Therefore these filter sheets can be used for HALAL certified foodstuffs.

FILTROX filter sheets do not contain alcohol or raw materials that were in contact with alcohol. The raw materials of all products we supply are GMO free. There is no contact with any animal based material during the whole production process.

Best regards

FILTROX AG

(b) (6)

Markus Saurer
General Sales Manager Filter Media

GRACE
Talent | Technology | Trust™

Dietmar Richter
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To Whom It May Concern

May 2, 2017
DRI/MZF
Version 002
replaces Version 001

Food Application Status

TRISYL® 300 Silica

for Edible Oil Refining

Table of Content

General information

TRISYL® 300 Silica for Edible Oil Refining consists of synthetic amorphous silicon dioxide with citric acid treatment. Synthetic amorphous silicon dioxide is manufactured from a controlled mixture of sulfuric acid with sodium silicate solution. The hydrogel is generated from an acid-catalyzed condensation reaction. During the subsequent washing process excess salts are removed. Thereafter the product is dried and milled.

Harmonized Tariff Schedule:	**38249996**
Nature of the raw materials:	Silicon dioxide: Inorganic Citric acid: Organic
Country of origin (product):	Germany

National Inventories

Synthetic amorphous silicon dioxide and Citric acid are registered as follows:

Inventory	**Silicon dioxide**	**Citric acid**
Australien, AICS CAS No.	7631-86-9	77-92-9
Canada, DSL CAS No.	7631-86-9	77-92-9
Canada, NDSL CAS No.	7631-86-9	77-92-9
China, IECSC CAS No.	7631-86-9	listed
EU, EINECS	231-545-4	201-069-1
EU, REACH	01-2119379499-16-XXXX	01-2119457026-42-XXXX
Japan, ENCS MITI No.	1-548	2-1318
Japan, ISHL	Not listed	Not listed
Korea, KECI (ECL) KE No.	KE-31032	KE-20831
New Zealand, NZIoC CAS No.	7631-86-9	77-92-9
Philippines, PICCS CAS No.	7631-86-9	77-92-9
Switzerland (Produkteregister Chemikalien)	Not applicable	Not applicable
Taiwan	EPEP4A01648271	EPEP4A01713947
Turkey EC No.	231-545-4	201-069-1
USA, TSCA CAS No.	7631-86-9	77-92-9

Nanomaterials registered (French- Décret No. 2012-232)	Synthetic amorphous silica (SAS) BK Notification Number: BK 484-2017-07665889	Not applicable

Europe

COMMISSION REGULATION (EU) No 231/2012
Silicon dioxide (E 551) and citric acid (E 330) meet the purity requirements according to COMMISSION REGULATION (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives, last amended by COMMISSION REGULATION (EU) 2017/234 of 24 February 2017. TRISYL® 300 Silica Gel is suitable for human consumption.

TRISYL® 300 Silica for Edible Oil Refining is used as a processing aid for the adsorptive cleaning of edible oils and fats. The clarification step ends with a filtration, where TRISYL® Silica for Edible Oil Refining is completely removed from the oil except for unintentional but technically unavoidable traces. Processing aids are particularly excluded from the European Regulation (EC) No 1333/2008 on food additives according to the scope and the definitions given therein. Since processing aids do not need to be approved or labeled in line with the current vertical EU provisions, horizontal and national legislations have to be considered as well.

Regulation (EU) No 1308/2013
Silicon dioxide and citric acid can be used in the processing of refined olive oil and refined olive-pomace oil according to Regulation (EU) No 1308/2013 establishing a common organization of the markets in agricultural products, last amended by COMMISSION DELEGATED REGULATION (EU) 2016/1226 of 04 May 2016.

Germany

According to the Guidelines on edible fats and edible oils (Leitsätze für Speisefette und Speiseöle) silicon dioxide and citric acid can be used as inert filter aids in the manufacturing process of cold pressed edible oil and refined edible fats.

For further information on the use of silicon dioxide and citric acid as processing aids for edible oils and edible fats please consider also national provisions and obligations.

USA

Silicon dioxide is approved as a direct food additive and as a stabilizer in the production of beer according to the Code of Federal Regulations 21, § 172.480 (revision date: April, 2016). Similarly it is referenced as a technological adjuvant for clarifying wine and juice in the Code of Federal Regulations 27, § 24.246 (revision date: April, 2016). Silicon dioxide meets the Food Chemicals Codex monograph requirements for INS 551, which are referenced by the U.S. Food and Drug Administration.

Citric acid is classified as Affirmed as Generally Recognized as Safe (GRAS) by the FDA (Food and Drug Administration) when used in accordance with 21 CFR, § 184.1033 and when used in accordance with good manufacturing practises.

Treatment with adsorptive materials is a common procedure for removing color producing substances from edible oil. The adsorbents have to be completely removed by filtration. Silicon dioxide can be considered as safe for this application.

TRISYL® 300 Silica for Edible Oil Refining can be applied as processing aid in refining of edible oil or fat. The before-mentioned product is appropriate to be used for, or be in contact with foodstuff and is not hazardous for human health.

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Should further information be required on this subject, please do not hesitate to contact us via our local Grace Business Representative.

Yours sincerely,

Grace GmbH

(b) (6)

Dietmar Richter
Manager Toxicology and Regulatory Affairs

Disclaimer:
The above statement(s) are based on our current knowledge and experience and on legislation in effect on the date above. This compliance statement does not warrant against modifications of this product resulting from its processing or from the addition of other products, nor against any inadequate use and/or storage of this product or the materials and articles containing it. The present statement also does not warrant compliance with legislation changed after the date above.

Akkreditiertes Prüflabor gemäß DIN EN ISO / IEC 17025

Chemische und mikrobiologische Untersuchungen
von Lebensmitteln und Wasser
Beratung · HACCP-Konzepte

LABOR DR. BÖHM

Trinkwasseruntersuchungsstelle nach § 15 Abs. 4 der TrinkwV 200

Andreas Böhm, Staatl. geprüfter Lebensmittelchemiker

Telefon 089 / 14 71 83 - 0
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E-Mail service@Labor-Dr-Boehm.de
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IBAN: DE93700202706410059361
SWIFT (BIC): HYVEDEMMXXX

Postbank München
IBAN: DE54700100800027757807
SWIFT (BIC): PBNKDEFF

UID: DE 294 230 008

Gerichtsstand München
Steuer-Nr. 144/154/01109

Labor Dr. Böhm · Inh. Andreas Böhm · Schragenhofstr. 35 · 80992 München

15. Januar 2018
AB/asw

Health Certificate

Article:

Tonsil Supreme 110 FF	Tonsil Standard 315 FF	Tonsil 7118-X FF
Tonsil Supreme 111 FF	Tonsil Standard 3151 FF	Tonsil 7120-X FF
Tonsil Supreme 112 FF	Tonsil Standard 316 FF	Tonsil 7125-X FF
Tonsil Supreme 113 FF	Tonsil Standard 317 FF	Tonsil 7127-X FF
Tonsil Supreme 114 FF	Tonsil Standard 318 FF	Tonsil 7130-X FF
Tonsil Supreme 115 FF	Tonsil Standard 510 FF	Tonsil 7132-X FF
Tonsil Supreme 116 FF	Tonsil Standard 512 FF	Tonsil 7134-X FF
Tonsil Supreme 117 FF	Tonsil 4110-X FF	Tonsil 7136-X FF
Tonsil Supreme 118 FF	Tonsil 4111-X FF	Tonsil 813-X FF
Tonsil Supreme 119 FF	Tonsil 4112-X FF	Tonsil 8114-X FF
Tonsil Supreme 516 FF	Tonsil 4114-X FF	Tonsil 8118-X FF
Tonsil Supreme 158 FF	Tonsil 4118-X FF	Tonsil 8120-X FF
Tonsil Optimum 208 FF	Tonsil 4120-X FF	Tonsil 8125-X FF
Tonsil Optimum 210 FF	Tonsil 4122-X FF	Tonsil 8132-X FF
Tonsil Optimum 212 FF	Tonsil 4124-X FF	Tonsil 919 FF
Tonsil Optimum 213 FF	Tonsil 4125-X FF	Tonsil 9191 FF
Tonsil Optimum 214 FF	Tonsil 4127-X	Tonsil 9192 FF
Tonsil Optimum 215 FF	Tonsil 413-X FF	Tonsil 9194 FF
Tonsil Optimum 216 FF	Tonsil 4130-X FF	Tonsil 9195 FF
Tonsil Optimum 217	Tonsil 4132-X FF	Tonsil 9196 FF
Tonsil Optimum 218 FF	Tonsil 4134-X FF	Tonsil 9198 FF
Tonsil Optimum 254 FF	Tonsil 4136-X FF	Tonsil EX 501
Tonsil Optimum 258 FF	Tonsil 4137-X FF	Tonsil EX 722
Tonsil Optimum 514 FF	Tonsil 4150-X FF	Tonsil EX 1707
Tonsil Optimum 515 FF	Tonsil 4192-X FF	
Tonsil Optimum 558 FF	Tonsil 713-X FF	
Tonsil Standard 310 FF	Tonsil 7110-X FF	
Tonsil Standard 312 FF	Tonsil 7112-X FF	
Tonsil Standard 314 FF	Tonsil 7114-X FF	

DAkkS Deutsche Akkreditierungsstelle D-PL-18257-01-00

Die Akkreditierung durch die DAkkS gilt für die Untersuchung von Lebensmitteln und Wasser für die in der Urkunde aufgeführten Prüfverfahren

Labor Dr. Böhm, zugelassen für
- Untersuchungen von amtlichen Gegenproben nach § 43 LFGB
- mikrobiologische Untersuchungen nach § 44 Infektionsschutzgesetz
- Trinkwasseruntersuchungen nach § 15 Abs. 4 TrinkwV 2001

After examination of the documents and dates given by the manufacturer we certify, that the above mentioned products can be used in food processing (especially refining vegetable and animal oils and fats).

As far as obvious out of the documents there are no health risks in using. Precondition is, that the products will be used appropriate and in accordance to the specific legal regulations.

(b) (6)

Andreas Böhm
General management, technical management

Labor Dr. Böhm
Schragenhofstraße 35
80992 München

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
Health, Environmental & Regulatory Services (HERS)

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

Total Quality. Assured.

Intertek, New Jersey, USA

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

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

[fda.gov]

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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)

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www.intertek.com

intertek
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Intertek, New Jersey, USA

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

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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

<image008.png>

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

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.

From:	Hywel Griffiths
To:	Morissette, Rachel
Cc:	Erica Cermak Intertek
Subject:	Re: response letters for GRNs 000776 and 000777
Date:	Friday, October 26, 2018 2:26:29 PM
Attachments:	image001.png

Dear Dr Morissette,

Thank you very much for these letters.

Have a good weekend!

Best wishes

Hywel

Sent from my phone
Envoyé de mon phone
+33 7 61 33 37 96

On 26/10/2018, at 8:13 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

> Dear Dr. Griffiths,
>
> Please see attached the response letters for GRNs 000776 and 000777. Please let me know if you have any questions.
>
> 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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> <2018-10-24 Response letter GRN000776_transmittal.pdf>
>
> <2018-10-24 Response letter GRN000777_transmittal.pdf>

From:	Hywel Griffiths
To:	Morissette, Rachel
Subject:	Re: GRNs 776 and 777 follow-up on amendments provided to FDA and confidentiality
Date:	Tuesday, November 06, 2018 11:44:17 AM
Attachments:	logo201606.png Appendix 1 Comparative Fatty Acid Analysis – ITERG report copy 2.pdf

Dear Dr Morissette,

Appendix 1 in the amendments submitted in response to the agency's questions on GRN 776 and 77 is NOT CONFIDENTIAL.
The "CONFIDENTIAL" footer on these pages was included as an oversight and Fermentalg does not consider this information to be confidential or proprietary. Please find an amended version of these pages attached in which the confidential marking has been removed.

Thank you for bringing this to our attention.

Best wishes

Hywel Griffiths
Directeur Scientifique/Chief Scientist

Fermentalg

> On 31 Oct 2018, at 5:42 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Dear Dr. Griffiths,
>
> As you may be aware, FDA is now posting the amendments to GRAS notices on our GRAS Notice Inventory webpage, along with the notice itself and response letter. Previously, the amendments were only available through a Freedom of Information Act request. We note that some of the pages in the appendices that you provided in response to our safety questions are marked confidential. In Part 1 of the notice, you stated that there was no confidential information contained in the notice. Any data and information that bears on safety cannot be held confidential in a GRAS notice. Can you please provide a statement confirming that the information contained in these two appendices can be made publicly available and, if possible, provide a clean copy without the Confidential markings? We apologize for not catching the confidential designation sooner, but we do need to have this resolved for the record before we can proceed with posting your letter on our webpage and closing out these two notices.
>
> Thank you for your attention to this matter. If you have any questions, please let me know.
>
> 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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Certificates of analysis for Fermentalg FCC-1324 DHA-Oil

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Pessac, le 18/03/2014

v/réf. :
n/réf. : EIMA – D2A02 – Dossier de demande d'équivalence

RAPPORT D'ANALYSE

Dossier suivi par : **JOFFRE Florent**

Méthodes :

Détermination de l'indice d'acide et de l'acidité (NF EN ISO 660) (*)

Détermination de l'indice de peroxyde (NF EN ISO 3960) (*)

Détermination de la teneur en eau et en matières volatiles (NF EN ISO 662) (*)

Détermination de l'indice d'anisidine (NF EN ISO 6885) (*)

Détermination des tocophérols et tocotrienols dans les huiles végétales et les graisses par chromatographie liquide haute performance (NF EN ISO 9936) (*)

Préparation et analyse par chromatographie en phase gazeuse des esters méthyliques d'acides gras (NF EN ISO 12966-2 et NF EN ISO 5508) (*)

Détermination de la teneur en matières insaponifiables (méthode par extraction a l'oxyde diethylique (NF EN ISO 3596)) (*)

Détermination de la teneur en arsenic par spectrométrie d'absorption atomique (méthode ITERG) - LQ = 0,05 mg/kg

Détermination de la teneur en plomb par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 12193) (*) - LQ = 0,01 mg/kg

Détermination de la teneur en fer par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

Détermination de la teneur en cuivre par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

Détermination de 4 HAP (hydrocarbure aromatique polycyclique) par chromatographie liquide haute performance de polarité de phase inversée (méthode interne)

Analyses microbiologiques (sous-traitance)

ECHANTILLONS

Date de réception	:	07/01/2014
Nature	:	**Huile d'algues raffinée et anti-oxydée NF1**
Remarque	:	code ITERG E14-032

---

0 annexe(s) et 0 fiche de commentaire(s).

cofrac ESSAIS

RESULTATS

Profil et teneur en acides gras (Huile végétale raffinée et anti-oxydée NF1)

ACIDES GRAS	Huile 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
non identifié	0,9

AG. dans l'extrait (g/100g)	93,8
Teneur en DHA (g/100g)	36.77

Détermination	Huile NF1
Acidité oléique (NF EN ISO 660)	0.06 ± 0.05 % (m/m)
Indice de peroxyde (NF EN ISO 3960)	0.7 ± 1.0 méqO_2/kg
Couleur Lovibond (NF ISO 27608)	Lovibond 5"1/4 : 1.5R, 23.0Y
Teneur en eau et en matières volatiles (NF EN ISO 662)	< 0,05 %
Indice d'anisidine (NF EN ISO 6885)	12,6
Teneur en tocophérols (NF EN ISO 9936)	3965 mg/kg ± 595
Teneur en matières insaponifiables (NF EN ISO 6885)	1,28 % ± 0,30
Arsenic (méthode interne)	< 0,01 mg/kg
Plomb (NF EN ISO 12193)	< 0,01 mg/kg
Fer (NF EN ISO 8294)	0,011 mg/kg
Cuivre (NF EN ISO 8294)	< 0,005 mg/kg
Teneur en 4 HAP* (méthode interne) dont B(a)P	0,5 µg/kg <0,2 µg/kg

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

Détermination	Huile NF1
Recherche des microorganismes aérobies 30°C (NF EN ISO 4833-1)	<1/g
Recherche des levures (NF V08-059)	<1/g
Recherche des moisissures (NF V08-059)	<1/g
Recherche des coliformes 30°C présumés (NF V08-050)	<1/g
Recherche des coliformes thermotolérants (NF V08-060)	<1/g
Recherche d'Escherichia coli (NF ISO 16649-2)	<1/g
Recherche de staphylocoque à coagulase positive (NF V08-057-1)	<10/g

Le Technicien coordinateur Analyse

(b) (6)

BLE Fabienne

Responsable projet

(b) (6)

JOFFRE Florent

DETERMINATION DE LA COMPOSITION EN ACIDES GRAS
INCERTITUDE EXPERIMENTALE SELON NORME NF EN ISO 12966-2 et NF EN ISO 5508 *

Acide Gras (%)	Incertitude élargie (%)
≤ 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

* facteur d'élargissement k=2

ITERG
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pcalleja@fermentalg.com

Pessac, 18/03/2014

v/réf. :
n/réf. : EIMA – D2A02 – Dossier de demande d'équivalence

RAPPORT D'ANALYSE

Dossier suivi par : JOFFRE Florent

Méthodes :

Détermination de l'indice d'acide et de l'acidité (NF EN ISO 660) (*)

Détermination de l'indice de peroxyde (NF EN ISO 3960) (*)

Détermination de la teneur en eau et en matières volatiles (NF EN ISO 662) (*)

Détermination de l'indice d'anisidine (NF EN ISO 6885) (*)

Détermination des tocophérols et tocotrienols dans les huiles végétales et les graisses par chromatographie liquide haute performance (NF EN ISO 9936) (*)

Préparation et analyse par chromatographie en phase gazeuse des esters méthyliques d'acides gras (NF EN ISO 12966-2 et NF EN ISO 5508) (*)

Détermination de la teneur en matières insaponifiables (méthode par extraction a l'oxyde diethylique (NF EN ISO 3596)) (*)

Détermination de la teneur en arsenic par spectrométrie d'absorption atomique (méthode ITERG)

Détermination de la teneur en plomb par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 12193) (*) - LQ = 0,01 mg/kg

Détermination de la teneur en fer par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

Détermination de la teneur en cuivre par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg

Détermination de 4 HAP (hydrocarbure aromatique polycyclique) par chromatographie liquide haute performance de polarité de phase inversée (méthode interne)

Analyses microbiologiques (sous-traitance)

ECHANTILLONS

Date de réception	:	20/01/2013
Nature	:	**Huile d'algues raffinée et anti-oxydée NF2**
Remarque	:	code ITERG E14-189

RESULTATS

Profil et teneur en acides gras (Huile végétale raffinée et anti-oxydée NF2)

ACIDES GRAS	Huile 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
non identifié	0,3
AG. dans l'extrait (g/100g)	96,1
Teneur en DHA (g/100g)	35.85

Détermination	Huile NF2
Acidité oléique (NF EN ISO 660)	0.05 ± 0.05 % (m/m)
Indice de peroxyde (NF EN ISO 3960)	1.7 ± 1.0 méqO_2/kg
Couleur Lovibond (NF ISO 27608)	Lovibond 5"1/4 : 1.0R, 16.0Y
Teneur en eau et en matières volatiles (NF EN ISO 662)	< 0,05 %
Indice d'anisidine (NF EN ISO 6885)	12,8
Teneur en tocophérols (NF EN ISO 9936)	4025 mg/kg ± 604
Teneur en matières insaponifiables (NF EN ISO 6885)	1,16 % ± 0,30
Arsenic (méthode interne)	< 0,01 mg/kg
Plomb (NF EN ISO 12193)	< 0,01 mg/kg
Fer (NF EN ISO 8294)	0,024 mg/kg
Cuivre (NF EN ISO 8294)	< 0,005 mg/kg
Teneur en 4 HAP* (méthode interne) dont B(a)P	0,5 µg/kg <0,2 µg/kg

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

Détermination	Huile NF2
Recherche des microorganismes aérobies 30°C (NF EN ISO 4833-1)	<1/g
Recherche des levures (NF V08-059)	<1/g
Recherche des moisissures (NF V08-059)	<1/g
Recherche des coliformes 30°C présumés (NF V08-050)	<1/g
Recherche des coliformes thermotolérants (NF V08-060)	<1/g
Recherche d'Escherichia coli (NF ISO 16649-2)	<1/g
Recherche de staphylocoque à coagulase positive (NF V08-057-1)	<10/g

Le Technicien coordinateur Analyse

(b) (6)

BLE Fabienne

Responsable projet

(b) (6)

JOFFRE Florent

DETERMINATION DE LA COMPOSITION EN ACIDES GRAS INCERTITUDE EXPERIMENTALE SELON NORME NF EN ISO 12966-2 et NF EN ISO 5508 *	
Acide Gras (%)	Incertitude élargie (%)
≤ 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

* facteur d'élargissement k=2

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Tel : + 335 57 257 977
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pcalleja@fermentalg.com

Pessac, 18/03/2014

v/réf. :
n/réf. : EIMA – D2A02 – Dossier de demande d'équivalence

RAPPORT D'ANALYSE

Dossier suivi par : JOFFRE Florent

Méthodes :

Détermination de l'indice d'acide et de l'acidité (NF EN ISO 660) (*)
Détermination de l'indice de peroxyde (NF EN ISO 3960) (*)
Détermination de la teneur en eau et en matières volatiles (NF EN ISO 662) (*)
Détermination de l'indice d'anisidine (NF EN ISO 6885) (*)
Détermination des tocophérols et tocotrienols dans les huiles végétales et les graisses par chromatographie liquide haute performance (NF EN ISO 9936) (*)
Préparation et analyse par chromatographie en phase gazeuse des esters méthyliques d'acides gras (NF EN ISO 12966-2 et NF EN ISO 5508) (*)
Détermination de la teneur en matières insaponifiables (méthode par extraction a l'oxyde diethylique (NF EN ISO 3596)) (*)
Détermination de la teneur en arsenic par spectrométrie d'absorption atomique (méthode ITERG) - LQ = 0,05 mg/kg
Détermination de la teneur en plomb par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 12193) (*) - LQ = 0,01 mg/kg
Détermination de la teneur en fer par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg
Détermination de la teneur en cuivre par spectrométrie d'absorption atomique avec four en graphite (NF EN ISO 8294) (*) - LQ = 0,1 mg/kg
Détermination de 4 HAP (hydrocarbure aromatique polycyclique) par chromatographie liquide haute performance de polarité de phase inversée (méthode interne)
Analyses microbiologiques (sous-traitance)

ECHANTILLONS

Date de réception	:	27/01/2013
Nature	:	**Huile d'algues raffinée et anti-oxydée NF3**
Remarque	:	code ITERG E14-507

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0 annexe(s) et 0 fiche de commentaire(s).

cofrac ESSAIS
ACCRÉDITATION N° 1-0171 PORTÉE DISPONIBLE SUR WWW.COFRAC.FR

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Recherche • Innovation • Qualité

RESULTATS

Profil et teneur en acides gras (Huile végétale raffinée et anti-oxydée NF3)

ACIDES GRAS	Huile 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
non identifié	0,9

AG. dans l'extrait (g/100g)	95,9
Teneur en DHA (g/100g)	36.44

Détermination	Huile NF3
Acidité oléique (NF EN ISO 660)	0.05 ± 0.05 % (m/m)
Indice de peroxyde (NF EN ISO 3960)	0.7 ± 1.0 méqO_2/kg
Couleur Lovibond (NF ISO 27608)	Lovibond 5"1/4 : 1.5R, 20.0Y
Teneur en eau et en matières volatiles (NF EN ISO 662)	< 0,05 %
Indice d'anisidine (NF EN ISO 6885)	13,3
Teneur en tocophérols (NF EN ISO 9936)	4250 mg/kg ± 637
Teneur en matières insaponifiables (NF EN ISO 6885)	1,06 % ± 0,30
Arsenic (méthode interne)	< 0,01 mg/kg
Plomb (NF EN ISO 12193)	< 0,01 mg/kg
Fer (NF EN ISO 8294)	0,018 mg/kg
Cuivre (NF EN ISO 8294)	< 0,005 mg/kg
Teneur en 4 HAP* (méthode interne) dont B(a)P	0,6 µg/kg <0,2 µg/kg

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

Détermination	Huile NF3
Recherche des microorganismes aérobies 30°C (NF EN ISO 4833-1)	<1/g
Recherche des levures (NF V08-059)	<1/g
Recherche des moisissures (NF V08-059)	<1/g
Recherche des coliformes 30°C présumés (NF V08-050)	<1/g
Recherche des coliformes thermotolérants (NF V08-060)	<1/g
Recherche d'Escherichia coli (NF ISO 16649-2)	<1/g
Recherche de staphylocoque à coagulase positive (NF V08-057-1)	<10/g

Le Technicien coordinateur Analyse

(b) (6)

BLE Fabienne

Responsable projet

(b) (6)

JOFFRE Florent

DETERMINATION DE LA COMPOSITION EN ACIDES GRAS INCERTITUDE EXPERIMENTALE SELON NORME NF EN ISO 12966-2 et NF EN ISO 5508 *	
Acide Gras (%)	Incertitude élargie (%)
≤ 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

* facteur d'élargissement k=2

Certificate of analysis for Martek ATCC-20888 DHA-Oil

ITERG
Expertise Corps Gras

FERMENTALG SA
A l'attention de Mr Bourdenx
4, Bis Rue Rivière
33500 LIBOURNE
FRANCE

bbourdenx@fermentalg.com

Pessac, 22/05/2014

v/réf. : Demande d'analyse du 07 octobre 2013
n/réf. : EIMA – D2A02

RAPPORT D'ANALYSE

Dossier suivi par : JOFFRE Florent

Méthodes :
Préparation et analyse par chromatographie en phase gazeuse des esters méthyliques d'acides gras (NF EN ISO 12966-2 ET NF EN ISO 5508) (*)
Détermination du profil glycéridique par CPG-FID (méthode interne dérivée des normes IUPAC6.002 et NFT60704)

ECHANTILLONS

Date de réception : 07/10/2013

Nature : **Huile référence ITE-13-0113 (code ITERG E13-8705)**
Remarque :

L'Institut des Corps Gras engage sa responsabilité pour ce qui est de la bonne conduite des essais qu'il réalise et l'exactitude des résultats qu'il fournit quant aux matières d'œuvre (contenus) analysées.
Il ne peut porter aucun avis sur l'authenticité des références, dénominations et emballages (contenants) des échantillons remis par le Donneur d'Ordre, mentions reprises dans les bulletins d'analyse seulement pour désigner lesdits échantillons.

. Seules certaines prestations rapportées dans ce document sont couvertes par l'accréditation. Elles sont identifiées par le symbole *.
. La reproduction de ce rapport d'essai n'est autorisée que sous sa forme intégrale.
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. Les résultats des déterminations COFRAC (*) sont accompagnés des incertitudes de mesure. Elles sont précisées en italique, directement dans le tableau de résultats ou reportées en fin de rapport.

0 annexe(s) et 0 fiche de commentaire(s).

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Recherche • Innovation • Qualité

RESULTATS

Profil relatif et teneur en acides gras

ACIDES GRAS	Huile ITE-13-0113 E13-8705
12 : 0	0,1
14 : 0	5,1
14 : 1	0,1
15 : 0	0,2
16 : 0	14,6
16 : 1	0,2
17 : 0	0,1
17 : 1	0,2
18 : 0	0,9
18 : 1	16,5
18 : 2 (n-6)	1,4
18 : 3 (n-6)	0,3
18 : 3 (n-3)	0,1
18 : 4 (n-3)	0,3
20 : 0	0,1
20 : 1	<0,1
20 : 2 (n-6)	<0,1
20 : 3 (n-6)	0,4
20 : 3 (n-3)	0,1
20 : 4 (n-6)	1,2
20 : 4 (n-3)	0,8
20 : 5 (n-3)	1,2
22 : 0	<0,1
22 : 1	0,2
22 : 4 (n-6)	<0,1
22 : 5 (n-6)	16,0
22 : 5 (n-3)	0,6
22 : 6 (n-3)	**38,3**
24 : 0	0,2
24 : 1	0,1
non identifié	0,7
total	100
Teneur en AG (g/100g)	**93,2**