RECEIVED
FEB 11 2019
OFFICE OF
FOOD ADDITIVE SAFETY

Fermentalg

01 February 2019

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

Re: DHA Algal Oil from *Schizochytrium* sp. FCC-3204 for Use in Select Food Categories

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 (GRAS) under the intended conditions of use described in the Notice. The Notice is contained in a binder. In addition, we have included 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
Fermentalg
Email: hgriffiths@fermentalg.com

GRAS Notice for DHA Algal Oil from *Schizochytrium* sp. FCC-3204 for Use in Select Food Categories

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

DATE
01 February 2019

GRAS Notice for DHA Algal Oils from *Schizochytrium* sp. FC-3204 for Use in Select Food Categories

TABLE OF CONTENTS

List of Appendices

List of Figures and Tables

GRAS Notice for DHA Algal Oil from *Schizochytrium* sp. FCC-3204 for Use in Select Food Categories

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 food.

In accordance with 21 CFR § 170.270, Fermentalg authorizes the FDA to share all necessary information included in this Notice to the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture.

Signed, [redacted]

(b) (6)

Hywel Griffiths

1ST FEBRUARY 2019

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 intends to market DHA 550 as an ingredient in the U.S. for use as a direct food ingredient in the food categories listed in 21 CFR § 184.1472(a)(3) at levels adjusted for total DHA content (U.S. FDA, 2017a). Use-levels will be adjusted to account for the higher DHA content of Fermentalg's oil (55%) 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, 2017a). The individual proposed food uses and use-levels for DHA 550 are summarized in Table 1.3-1.

Table 1.3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for DHA 550 in the United States (U.S.)

Category of Food	Maximum Level of Use in Food (as served)	
	Menhaden [21 CFR § 184.1472(a)(3)] (U.S. FDA, 2017a)	**DHA 550**
Baked goods, baking mixes, § 170.3(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[a]	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, § 1 70.3(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] Safety and suitability for use in meat products is discussed in Appendix 1.

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 specified conventional food and beverage products, as described in Table 1.3-1 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

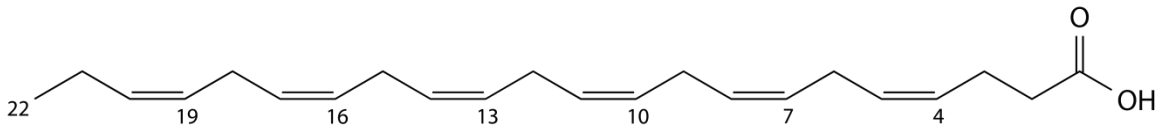
2.1.2 Molecular Formula

$C_{22}H_{32}O_2$

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

DHA 550 is produced *via* fermentation using the single cell marine micro-algae *Schizochytrium* strain FCC-3204. This strain is a natural variant of strain FCC-1324, from which the DHA 350 oil (the subject of GRN 776) oil is produced (U.S. FDA, 2018a). FCC-3204 was selected without the use of any mutagenic agents and has not been subjected to any form of deliberate genetic modification. Oil from *Schizochytrium* strain FCC-3204 was the subject of GRN 777 (U.S. FDA, 2018b).

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 *Thraustochytriaceae* family. Initially, this family was composed of 7 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*, *Parietichytrium*, and *Sicyoidochytrium*, emended from *Ulkenia* or *Aurantiochytrium* and *Oblongichytrium* emended from *Schizochytrium* (Yokohama, Honda 2007; Yokohama, Salleh, Honda 2007).

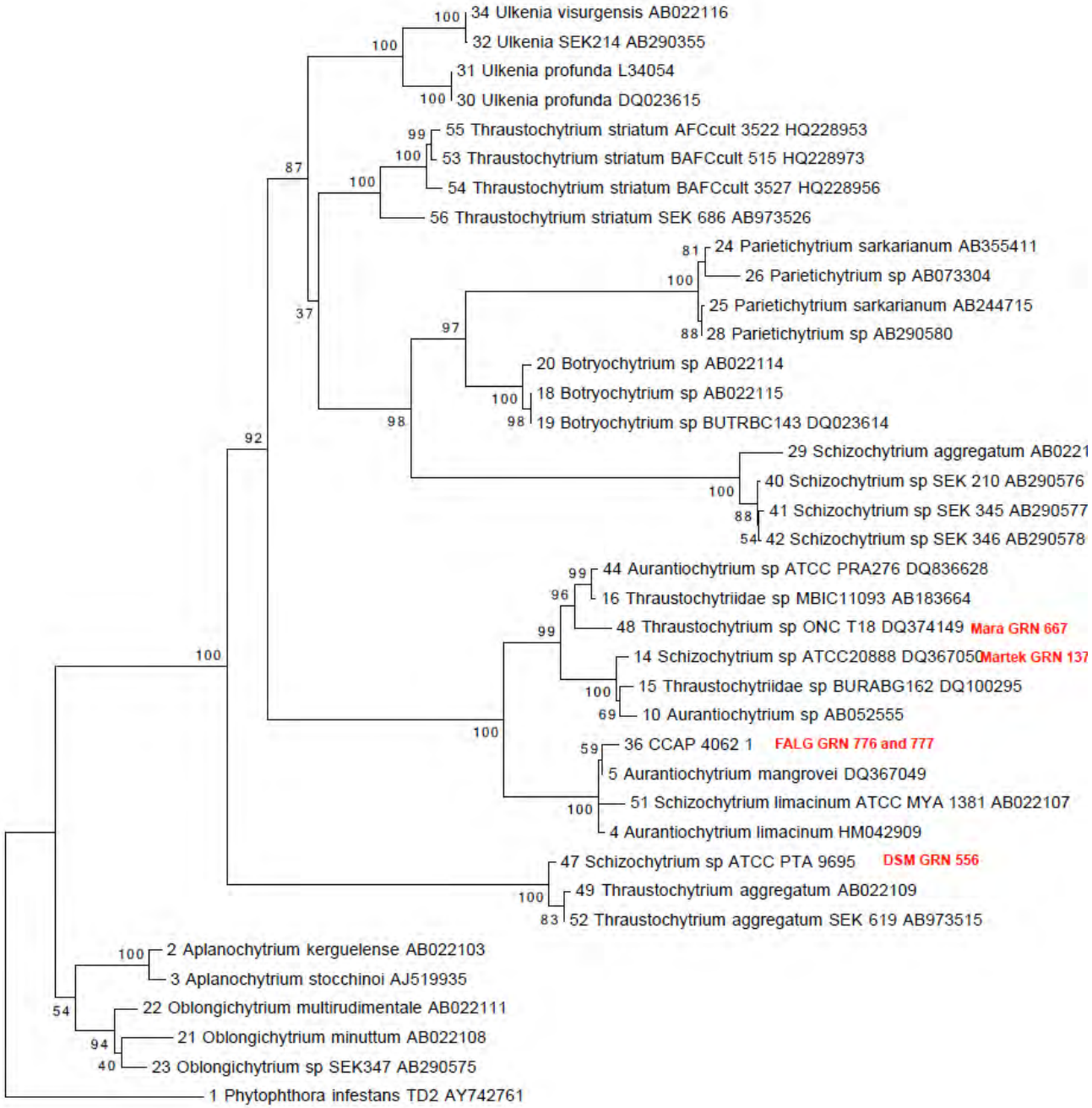
Fermentalg collected a *Schizochytrium*-related strain in estuarine environment and undertook a characterization at a genetic and biochemical level. This study revealed that the parent strain of FCC-3204 (*i.e.,* FCC-1324) could be assigned to the genus *Schizochytrium*. An example of a phylogenetic tree that has been constructed by comparison of sequences of the small subunit of ribosomal DNA (18S SSU-rDNA) is depicted in Figure 2.2.1-1. This figure demonstrates that Fermentalg's production strain, FCC-3204, as well as its parent strain FCC-1324 (corresponding to CCAP 4062 1 in Figure 2.2.1-1) is closely related to the production organisms used to manufacture of other DHA-rich oils that have been the subject of GRAS Notifications to FDA. All of these Notices have received 'no questions' letters from FDA, including GRAS Notice 777, which detailed the intended for use of DHA 550 as an ingredient in exempt and non-exempt infant formula in accordance with good manufacturing practices and in combination with a source of arachidonic acid.

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

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 550 is a highly purified oil.

Figure 2.2.1-1 Phylogeny of *Aurantiochytrium, Schizochytrium, Sicyiodochytrium,* and *Thraustochytrium* 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[a]	-

CFR = Code of Federal Regulations.

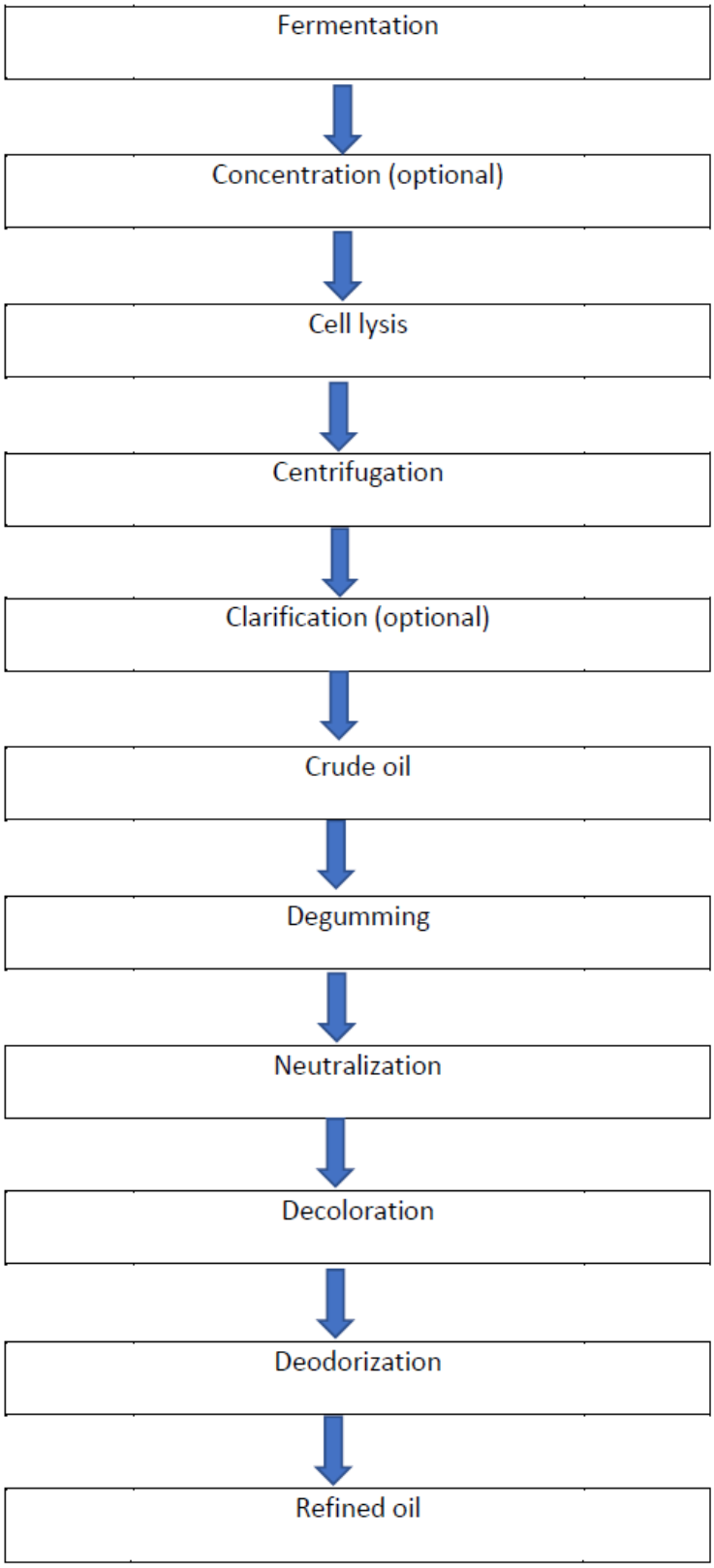
[a] Biospumex 153K is a proprietary mix of modified polyalkoxyesters which are nonionic and contain no silicone. The product is used in a wide range of food processes including fermentation and extraction. A data sheet and certificate regarding its safety in the production of foodstuffs are included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

2.3 Manufacturing

Fermentalg's DHA 550 is produced in accordance with Hazard Analysis Critical Control Point (HACCP) and current Good Manufacturing Practices (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 2.2.1-1. 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 (*e.g.,* Alcalase from Novazyme) so that the cells are lysed and oil is liberated. The enzyme is a protease (subtilisin) produced by *Bacillus licheniformis* (CAS Number: 9014-01 -1) and it is used in accordance with 21 CFR § 184.1027. 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.

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. 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. 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

Table 2.4.1-1 Chemical Specifications for DHA 550

Specification Parameter	Specification	Method
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[b]
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[b]
Lead	< 0.01 mg/kg	NF EN ISO 12193
Cadmium	< 0.01 mg/kg	Internal method[b]

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

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
Escherichia 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 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
Color[a]	Report	10.9R; 70.0 Y	2.2R; 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%
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
Escherichia 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 2.4.4-1 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
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%).

Although there are no reports of toxin production by any members of the *Thraustochytriaceae* family, member, Fermentalg has analyzed 3 samples of DHA 550 for the presence of algal toxins. As demonstrated in Table 2.4.4-2, no toxins were detected. 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 Consumer Protection and Food Safety. The entire range was tested for completeness although none were expected to be found in the production organism or to be resistant to the refining process.

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 a representative batch of Fermentalg's DHA 550 is presented in Table 2.4.4-3. This table also provides comparisons to other *Schizochytrium* sp.-derived DHA oils already in the food supply. The sterol composition of Fermentalg's DHA 550 is similar to that of other DHA algal oil derived from *Schizochytrium* sp. which have attained GRAS status (GRN 553, 677, and 777) (U.S. FDA, 2015a, 2017b, 2018b). As shown in Table 2.4.4-3, Fermentalg's product does not contain new components, and the slight differences in the relative proportions of various sterols between Fermentalg's DHA 550 and other DHA oil products are not expected to be affect safety under the proposed conditions of use. 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's DHA 550 is higher than the values presented in the representative batches of these oils, the levels are within the specification for all oils.

Table 2.4.4-3 Comparative Sterol Profile

Sterols	Fermentalg's DHA 550 Manufacturing Lot #0419028-A	DSM Nutritional Products GRN 553 Manufacturing Lot #VY00266521	Mara Renewables Corporation GRN 677 Manufacturing Lot: N-2-006-C
Cholesterol	39.2%	14.4%	24.3%
Ergosterol and derived products or Brassicasterol [a]	11.7%	4.7%	< 0.1%
24-Methylcholesterol	NA	0.6%	3.9%
Campesterol	0.5%	1.0%	1.2%
Campestanol	<0.1%	<0.1%	< 0.1%
Stigmasterol	5.7%	31.4%	< 0.1%
Delta 7-Campesterol and iso-fucosterol	6.9%	3.1%	3.4%
Fucosterol	26.3%	40.2%	NA
Beta-sitosterol	6.1%	6.9%	13.4%
Sitostanol	<0.1%	<0.1%	< 0.1%
Delta 5-Avenasterol	0.2%	2.2%	1.4%

Table 2.4.4-3 Comparative Sterol Profile

Sterols	Fermentalg's DHA 550 Manufacturing Lot #0419028-A	DSM Nutritional Products GRN 553 Manufacturing Lot #VY00266521	Mara Renewables Corporation GRN 677 Manufacturing Lot: N-2-006-C
Delta 5,24 Stigmastadienol	0.5%	1.4%	7.0%
Delta7-Stigmasterol	0.8%	6.4%	26.1%
Delta7-Avenasterol	0.4%	2.9%	3.6%
Delta-5,23-stigmastadienol	NA	NA	6.9%
Clerosterol	NA	NA	8.8%
Non-identified sterols [b]	1.8%	0.4%	NA
Total sterol content	9,581 mg/kg of oil	6,070 mg/kg of oil	2,310 mg/kg fat

NA = not applicable.

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

[b] 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).

2.5 Stability

Preliminary data for the accelerated storage (at 25°C and 60% relative humidity) of 3 batches of DHA 550 are provided in Table 2.5-1, along with a comparison to stability results when stored under the ideal condition of -20°C. Results show that the product remains within specifications when stored under both conditions for up to 16 weeks. When stored at 4°C, the recommended shelf life is also 16 weeks (4 months). 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.

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 therefore should not present a significant real-world risk. The limits for total levels of oxidation products per gram of oil for DHA 550 are the same as those for other DHA-containing algal oils currently used in food. Given that less oil will be used to provide the same amount of DHA, actual exposure to oxidation products could be reduced. The specifications for oxidation products set by Fermentalg are stricter than the FCC monograph specifications for anisidine value of NMT 20.0 and total oxidation (NMT 26). Likewise, free fatty acids [acid value of not more than (NMT) 0.5 mg KOH/g, approximately equivalent to 0.25% free fatty acids] are also stricter than the FCC monograph specifications.

Table 2.5-1 Stability of DHA 550 Under Accelerated Storage Conditions *vs.* Ideal Condition

Fatty acid	Specification	Accelerated Studies Carried Out at 25°C and 60% Relative Humidity				Study Carried Out Under Ideal Conditions of -20°C
		Time 0	8 Weeks	14 Weeks	16 Weeks	16 Weeks
Batch 0403019						
DHA (%)	NLT 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 0413022-A						
DHA (%)	NLT 55	72.1	69.4	69.4	70.5	70.9

Table 2.5-1 Stability of DHA 550 Under Accelerated Storage Conditions *vs.* Ideal Condition

Fatty acid	Specification	Accelerated Studies Carried Out at 25°C and 60% Relative Humidity				Study Carried Out Under Ideal Conditions of -20°C
		Time 0	8 Weeks	14 Weeks	16 Weeks	16 Weeks
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 041028-A						
DHA (%)	NLT 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

DHA = docosahexaenoic acid; NLT = not less than.

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 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 U.S. 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, 776, and 777) (U.S. FDA, 2001, 2004a,b, 2010, 2012, 2013, 2015a,b, 2018a,b). DHA algal oils from *Schizochytrium* strains related to Fermentalg's production organisms were described in GRN 137, 553, and 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

As with the use of menhaden oil and other fish and algal oils containing the omega-3 fatty acids DHA or EPA, the maximum levels of use of DHA 550 (Table 1.3-1) are designed to assure that the combined daily intake of EPA/DHA would not exceed 3 g/person/day. DHA 550 is intended for use in an identical manner and same foods as the currently marketed oil. Therefore, Fermentalg's oil will replace, rather than add to, intake from the currently marketed 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 foods 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, and 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, and 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 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 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 F_1 rats for 90 days (Fedorova-Dahms *et al*., 2011b). In a second such study with the same exposure duration, the NOAEL for F_0 male and female and F_1 male systemic toxicity was considered to be 50,000 ppm (highest concentration administered) and 25,000 ppm for F_1 female systemic toxicity (higher mean body weight, body weight gain, and food consumption). No adverse effects on reproduction or development were seen (Schmitt *et al.*, 2012b). Furthermore, the FDA has reviewed numerous GRNs for substantially equivalent or similar products, including 3 for DHA algal oils from closely related *Schizochytrium* strains (GRN 137, 553, and 677), and has issued "no questions" letters to these notifications (U.S. FDA, 2004a, 2015a, 2017b).

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

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

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

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

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

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

Reference	Study Type	Test System	Exposure	Findings/Comments
Schmitt *et al.* (2012a)	Acute Toxicity	Female Sprague-Dawley rats	5,000 mg/kg bw	Acute oral LD_{50} was greater than 5,000 mg/kg of body weight.
Schmitt *et al.* (2012a)	Subchronic Toxicity	Sprague-Dawley rats	TOX: Basal diet, tuna oil control (50,000 ppm), or 10,000, 25,000 ppm, or 50,000 ppm DHA-rich oil in the diet REC: Vehicle control or 5,000 mg/kg bw/day for 90-days, 28-day recovery period	DHA-rich algal oil was well-tolerated at these dietary levels as evidenced by the absence of major treatment-related changes in the general condition and appearance of the rats, neurobehavioral endpoints, growth, feed and water intake, ophthalmoscopic examinations, routine hematology and clinical chemistry parameters, urinalysis, or necropsy findings. The no observed adverse effect level (NOAEL), the highest level fed, was determined to be 50,000 ppm, the highest dose tested, and equivalent to at least 3,305 and 3,679 mg/kg bw/day, for male and female rats, respectively.
Schmitt *et al.* (2012a)	Ames +/- S9	*S. 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	*S. 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-hr exposure): 0.00 (negative control), 0.00 (vehicle control), 1.25, 2.5, and 5.0 mg DHA-rich oil/mL Phase 2 (24-hr 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 = docosahexaenoic acid; hr = hour(s); LD_{50} = medium lethal dose ; 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 1 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 1 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. Overall, the published scientific literature continues to support the safety EPA/DHA intakes of up to 3 g/day from use in foods.

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 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, 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 4.

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

Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL, Willett WC (1995) Dietary intake of marine n-3 fatty acids, fish intake, and the risk of coronary disease among men. N Engl J Med 332(15):977-982.

DSM Nutritional Products (2014) *GRAS Determination of DHA Algal Oil (DHASCO®-B) Produced from a New Production Strain of Schizochytrium sp. for Use as an Ingredient in Infant Formula (Pre-Term and Term)*. (Submitted as U.S. FDA, 2014 - GRN No. 553). Submitted by Columbia (MD): DSM Nutritional Products to College Park (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: http://wayback.archive-it.org/7993/20171031040128/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM456952.pdf.

Falk MC, Zheng X, Chen D, Jiang Y, Liu Z, Lewis KD (2017). Developmental and reproductive toxicological evaluation of arachidonic acid (ARA)-Rich oil and docosahexaenoic acid (DHA)-Rich oil. Food Chem Toxicol 103:270-278. DOI:10.1016/j.fct.2017.03.011.

Fedorova-Dahms I, Marone PA, Bailey-Hall E, Ryan AS (2011a). Safety evaluation of Algal Oil from *Schizochytrium* sp. Food Chem Toxicol 49(1):70-77. DOI:10.1016/j.fct.2010.09.033.

Fedorova-Dahms I, Marone PA, Bauter M, Ryan AS(2011b). Safety evaluation of DHA-rich Algal Oil from *Schizochytrium* sp. Food Chem Toxicol 49(12):3310-3318. DOI:10.1016/j.fct.2011.08.024.

Fedorova-Dahms I, Thorsrud BA, Bailey E, Salem N Jr. (2014). A 3-week dietary bioequivalence study in preweaning farm piglets of two sources of docosahexaenoic acid produced from two different organisms. Food Chem Toxicol 65:43-51.

Hammond BG, Mayhew DA, Holson JF, Nemec MD, Mast RW, Sander WJ (2001b). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. II. Developmental toxicity evaluation in rats and rabbits. Regul Toxicol Pharmacol 33(2):205-217.

Hammond BG, Mayhew DA, Naylor MW, Ruecker FA, Mast RW, Sander WJ (2001a). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. I. Subchronic rat feeding study. Regul Toxicol Pharmacol 33(2):192-204. DOI:10.1006/rtph.2001.1458.

Hammond BG, Mayhew DA, Robinson K, Mast RW, Sander WJ (2001c). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. III. Single-generation rat reproduction study. Regul Toxicol Pharmacol 33(3):356-362.

Hammond BG, Mayhew DA, Kier LD, Mast RW, Sander WJ (2002). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. IV. Mutagenicity studies. Regul Toxicol Pharmacol 35(2, Part 1):255-265. DOI:10.1006/rtph.2002.1535.

Kris-Etherton M, Grieger JA, Etherton D (2009) Dietary reference intakes for DHA and EPA. Prostaglandins, Leukotrienes and Essential Fatty Acids 81:99-104.

Lewis KD, Huang W, Zheng X, Jiang Y, Feldman RS, Falk MC (2016). Toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. Food Chem Toxicol 96:133-144. DOI:10.1016/j.fct.2016.07.026.

Mara Renewables Corporation (2016) *GRAS Determination of DHA Algal Oil for Use in Infant Formula* (Submitted as U.S. FDA, 2016 - GRN No. 677). Submitted by ToxStrategies, Inc. on behalf of Dartmouth (NS): Mara Renewables Corporation to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm530169.pdf.

Martek Biosciences Corporation (2003). *GRAS Notification for DHA Algal Oil Derived from Schizochytrium sp.* (Submitted as U.S. FDA, 2004 - GRN No. 137). Prepared by Columbia (MD): Martek Biosciences Corporation for Submission to Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://wayback.archive-it.org/7993/20171031055555/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM264032.pdf.

Sanders, TAB (1989) Effects of fish oils on lipid metabolism. Nutrition 5(4):248-250.

Schmitt D, Tran N, Peach J, Bauter M, Marone P (2012a). Toxicologic evaluation of DHA-rich algal oil: genotoxicity, acute and subchronic toxicity in rats. Food Chem Toxicol 50(10):3567-3576. DOI:10.1016/j.fct.2012.07.054.

Schmitt D, Tran N, Peach J, Edwards T, Greeley M (2012b). Toxicologic evaluations of DHA-rich algal oil in rats: developmental toxicity study and 3-month dietary toxicity study with an *in utero* exposure phase. Food Chem Toxicol 50(11):4149-4157. DOI:10.1016/j.fct.2012.08.035.

U.S. FDA (2001). *Agency Response Letter GRAS Notice No. GRN 000041 [DHASCO (docosahexaenoic acid-rich single-cell oil) and ARASCO (arachidonic acid-rich single-cell oil), Columbia (MD): Martek Biosciences Corporation]*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Premarket Approval. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=41 [May 17, 2001].

U.S. FDA (2004a). *Agency Response Letter GRAS Notice No. GRN 000137 [Algal oil (Schizochytrium sp.), Columbia (MD): Martek Biosciences Corporation]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=137 [Feb. 12, 2004].

U.S. FDA (2004b). *Agency Response Letter GRAS Notice No. GRN 000138 [Fish Oil, Bedford (NS): Ocean Nutrition Canada Ltd.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=138 [Apr. 20, 2004].

U.S. FDA (2010). *Agency Response Letter GRAS Notice No. GRN 000319 [Micro-algal oil, Ulkenia sp. SAM2179, Basel, Switz: Lonza Ltd.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=319 [Aug. 4, 2010].

U.S. FDA (2012). *Agency Response Letter GRAS Notice No. GRN 000384 [Algal oil derived from Chlorella protothecoides strain S106 (Cp algal oil), South San Francisco (CA): Solazyme, Inc.]*. 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=384 [Jun. 13, 2012].

U.S. FDA (2013). *Agency Response Letter GRAS Notice No. GRN 000469 [Chlorella protothecoides strain S106 flour with 40-70% lipid (algal flour), South San Francisco (CA): Solazyme Roquette Nutritionals, LLC]*. 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=469 [Jun. 7, 2013].

U.S. FDA (2015a). *Agency Response Letter GRAS Notice No. GRN 000553 [Algal oil (40% docosahexaenoic acid) derived from Schizochytrium sp., Columbia (MD): DSM Nutritional Products]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=553 [Jun. 19, 2015].

U.S. FDA (2015b). *Agency Response Letter GRAS Notice No. GRN 000527 [Algal oil (87% oleic acid) derived from Prototheca moriformis strain S2532, South San Francisco (CA): Solazyme, Inc.]*. 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=527 [Feb. 6, 2015].

U.S. FDA (2017a). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Part	Section §	Section Title
170—Food additives	170.3	Definitions
	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
	184.1472	Menhaden oil
	184.1857	Corn sugar
	184.1945	Vitamin B 12

U.S. FDA (2017b). *Agency Response Letter GRAS Notice No. GRN 000677 [Docosahexaenoic acid oil produced in Schizochytrium sp., Dartmouth (NS): Mara Renewables Corporation]*. 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: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=667 [Date of filing: Sep. 14, 2016; FDA's Letter: Pending].

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. 20, 2018].

U.S. FDA (2017d). *Agency Response Letter GRAS Notice No. GRN 000732 [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=732 [FDA's Letter: Pending; Page Last Updated: Dec. 20, 2018].

U.S. FDA (2018a). *Agency Response Letter GRAS Notice No. GRN 000776 [Algal oil (35% docosahexaenoic acid) from Schizochytrium sp. FCC-1324 Libourne (France)]*. 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.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625983.pdf

U.S. FDA (2018b). *Agency Response Letter GRAS Notice No. GRN 000777 [Algal oil (55% docosahexaenoic acid) from Schizochytrium sp. FCC-3204 Libourne (France)]*. 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.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625998.pdf

Yokohama R, Honda D (2007) Taxonomic rearrangement of the genus *Schizochytrium*sensu lato based on morphology, chemotaxonomic characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for *Schizochytrium*and erection of *Aurantiochytrium* gen. nov. Mycoscience 48:199–211.

Yokoyama R, Salleh B, Honda D (2007) Taxonomic rearrangement of the genus Ulkenia sensu lato based on morphology, chemotaxonomical characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for Ulkenia and erection of Botryochytrium, Parietichytrium , and Sicyoidochytrium gen. nov. Mycoscience, 48: 329-341.

APPENDIX 1
Safety and Suitability for Use in USDA Regulated Products

Safety and Suitability for Use in USDA Regulated Products

As one of the proposed conditions of use [*i.e*., meat products, § 170.3(n)(29) of this chapter] is a United States Department of Agriculture (USDA) regulated category, consideration of the suitability of DHA 550 in this application was considered. As detailed in this Notice, Fermentalg's DHA 550 oil is considered similar in its source, composition, nutritional value, and metabolism to the Generally Recognized as Safe (GRAS)-Notified substance described in GRN 137. Martek Biosciences Corporation (Martek)'s oil is listed on the table of Safe and Suitable Ingredients available on USDA's website[1]. This listing indicates Martek's oil is safe and suitable for use as an alternative edible oil in the production of various meat and poultry products (at a level not to exceed 1.45 percent by weight of the product formulation for meat products and 0.87 percent by weight of the product formulation for poultry products). The oil is required to be listed by is common or usual name in the ingredients statement.

The intended use of Fermentalg's DHA 550 in meat products is not expected to adversely affect the wholesomeness of the product. The organoleptic properties (*e.g*., color, odor, taste) of DHA 550 are comparable to the DHA algal oil and menhaden oil currently approved for use in meat products). The safety of DHA 350 is addressed in Part 6 (§ 170.250 Narrative and Safety Information) of this Notice.

DHA 550 is intended to serve as a source of DHA. It is not intended for use as a processing aid as defined under 21 CFR § 101.100(a)(3)(ii). As such, the presence of DHA 550 will be listed by its common or usual name (DHA algal oil) in the ingredients statement of any resultant product.

[1] https://www.fsis.usda.gov/wps/wcm/connect/ce40e7ae-3d55-419e-9c68-a1b6fefcd4de/7120.1_Table_2.pdf?MOD=AJPERES

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
info.ouvrie@ouvrie.com

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

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
15 Avenue Edouard Belin, 92500 Rueil-Malmaison
www.chemviron.eu

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.
Apart from the right to use this information in relation to the use of this product and to make a limited number of copies of this document for such purpose, no right of intellectual property in or in relation to this document or this product is hereby granted by us.
Chemviron's products are continuously being improved and changes may have taken place since this publication went to press.

Avertissement

Se reporter à la fiche de sécurité (FDS) pour les considérations relatives à la santé et à la sécurité.
Les déclarations, informations techniques et recommandations contenues dans le présent document sont spécifiques à ce produit et présumées exactes à la date des présentes. Les conditions et les procédés d'utilisation du produit et des informations visés aux présentes étant indépendants de notre volonté, nous déclinons expressément toute responsabilité quant aux résultats obtenus ou découlant de l'utilisation du produit ou de ces informations. Aucune garantie quant aux performances, adéquation, commercialité, aptitude à l'emploi, conformité aux lois ou aux exigences d'utilisation finale ou tout autre garantie n'est formulée concernant le produit, les applications ou informations fournies par ce document.
A part le droit d'utiliser cette information en relation à l'utilisation de ce produit et de faire un nombre limité de copies de ce document à cette fin, aucun droit à la propriété intellectuelle dans ou en relation avec ce document n'est accordé par Chemviron.
Les produits de Chemviron étant améliorés continuellement des modifications peuvent avoir eu lieu depuis l'impression de ce document.

CHEMVIRON France- European Operations of CALGON CARBON Corporation
15 Avenue Edouard Belin, 92500 Rueil-Malmaison
www.chemviron.eu

KAL
Kantonales Amt für Lebensmittelkontrolle

Blarerstrasse 2, CH-9001 St.Gallen
Telefon 071 229 28 00, Fax 071 229 28 01
http://www.kal.ch

05 août 2003

Contact Klaus Luczynski
Tél. direct +41 (0)71 229 28 60

SMTP klaus.luczynski@gd-kal.sg.ch

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

(b) (6)

(b) (6)

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

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

Dietmar Richter
Manager Toxicology and Regulatory Affairs

T +49 6241 403-1461
F +49 6241 403-8118
dietmar.richter@grace.com
www.grace.com

Grace GmbH
In der Hollerhecke 1
67547 Worms, Germany

Grace GmbH · 67545 Worms, Germany

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.

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
Telefax 089 / 14 71 83 - 35

E-Mail service@Labor-Dr-Boehm.de
Internet www.Labor-Dr-Boehm.de

HypoVereinsbank München
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

APPENDIX 3
Certificates of Analysis

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.

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.

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 4
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

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

Date

(b) (6)

March 1, 2018

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

Date

(b) (6)

March 3, 2018

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

Date

REFERENCES

DSM Nutritional Products (2014) *GRAS Determination of DHA Algal Oil (DHASCO®-B) Produced from a New Production Strain of Schizochytrium sp. for Use as an Ingredient in Infant Formula (Pre-Term and Term)*. (Submitted as U.S. FDA, 2014 - GRN No. 553). Submitted by Columbia (MD): DSM Nutritional Products to College Park (MD): U.S. Food and Drug Administration (U.S. FDA). Available at: http://wayback.archive-it.org/7993/20171031040128/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM456952.pdf.

Falk MC, Zheng X, Chen D, Jiang Y, Liu Z, Lewis KD (2017). Developmental and reproductive toxicological evaluation of arachidonic acid (ARA)-Rich oil and docosahexaenoic acid (DHA)-Rich oil. Food Chem Toxicol 103:270-278. DOI:10.1016/j.fct.2017.03.011.

Fedorova-Dahms I, Marone PA, Bailey-Hall E, Ryan AS (2011a). Safety evaluation of Algal Oil from *Schizochytrium* sp. Food Chem Toxicol 49(1):70-77. DOI:10.1016/j.fct.2010.09.033.

Fedorova-Dahms I, Marone PA, Bauter M, Ryan AS (2011b). Safety evaluation of DHA-rich Algal Oil from *Schizochytrium* sp. Food Chem Toxicol 49(12):3310-3318. DOI:10.1016/j.fct.2011.08.024.

Fedorova-Dahms I, Thorsrud BA, Bailey E, Salem N Jr. (2014). A 3-week dietary bioequivalence study in preweaning farm piglets of two sources of docosahexaenoic acid produced from two different organisms. Food Chem Toxicol 65:43-51.

Hammond BG, Mayhew DA, Naylor MW, Ruecker FA, Mast RW, Sander WJ (2001a). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. I. Subchronic rat feeding study. Regul Toxicol Pharmacol 33(2):192-204. DOI:10.1006/rtph.2001.1458.

Hammond BG, Mayhew DA, Holson JF, Nemec MD, Mast RW, Sander WJ (2001b). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. II. Developmental toxicity evaluation in rats and rabbits. Regul Toxicol Pharmacol 33(2):205-217.

Hammond BG, Mayhew DA, Kier LD, Mast RW, Sander WJ (2002). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. IV. Mutagenicity studies. Regul Toxicol Pharmacol 35(2, Part 1):255-265. DOI:10.1006/rtph.2002.1535.

Koletzko B, Boey CC, Campoy C, Carlson SE, Chang N, Guillermo-Tuazon MA, et al. (2014). Current information and Asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation, and infancy: systematic review and practice recommendations from an early nutrition academy workshop. Ann Nutr Metab 65(1):49-80. DOI:10.1159/000365767.

Lewis KD, Huang W, Zheng X, Jiang Y, Feldman RS, Falk MC (2016). Toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. Food Chem Toxicol 96:133-144. DOI:10.1016/j.fct.2016.07.026.

Mara Renewables Corporation (2016) *GRAS Determination of DHA Algal Oil for Use in Infant Formula* (Submitted as U.S. FDA, 2016 - GRN No. 677). Submitted by ToxStrategies, Inc. on behalf of Dartmouth (NS): Mara Renewables Corporation to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm530169.pdf.

Martek Biosciences Corporation (2003). *GRAS Notification for DHA Algal Oil Derived from Schizochytrium sp.* (Submitted as U.S. FDA, 2004 - GRN No. 137). Prepared by Columbia (MD): Martek Biosciences Corporation for Submission to Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available at: http://wayback.archive-it.org/7993/20171031055555/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM264032.pdf.

Schmitt D, Tran N, Peach J, Bauter M, Marone P (2012a). Toxicologic evaluation of DHA-rich algal oil: genotoxicity, acute and subchronic toxicity in rats. Food Chem Toxicol 50(10):3567-3576. DOI:10.1016/j.fct.2012.07.054.

Schmitt D, Tran N, Peach J, Edwards T, Greeley M (2012b). Toxicologic evaluations of DHA-rich algal oil in rats: developmental toxicity study and 3-month dietary toxicity study with an *in utero* exposure phase. Food Chem Toxicol 50(11):4149-4157. DOI:10.1016/j.fct.2012.08.035.

U.S. FDA (2004). *Agency Response Letter GRAS Notice No. GRN 000137 [Algal oil (Schizochytrium sp.), Columbia (MD): Martek Biosciences Corporation]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=137 [Feb. 12, 2004].

U.S. FDA (2015). *Agency Response Letter GRAS Notice No. GRN 000553 [Algal oil (40% docosahexaenoic acid) derived from Schizochytrium sp., Columbia (MD): DSM Nutritional Products]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=553 [Jun. 19, 2015].

U.S. FDA (2017a). *Best Practices for Convening a GRAS Panel (Draft).* (Guidance for Industry). Silver Spring (MD): U.S. Food and Drug Agency (U.S. FDA), Center for Food Safety and Nutrition (CFSAN)/Center for Veterinary Medicine (CVM). Available at: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm583856.htm [November 2017].

U.S. FDA (2017b). Part 184—Direct food substances affirmed as general recognized as safe. §184.1472—Menhaden oil. In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2017c). Part 170—Food additives. §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2017d). *Agency Response Letter GRAS Notice No. GRN 000677 [Docosahexaenoic acid oil produced in Schizochytrium sp. Dartmouth (NS): Mara Renewables Corporation].* 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/?set=GRASNotices&id=677 [May 2, 2017].

From: Hywel Griffiths
To: Morissette, Rachel
Subject: Re: GRNs 000843 and 000844 literature search
Date: Wednesday, April 17, 2019 12:06:40 PM
Attachments: DHA 550 GRAS Notice - Updated Safety Narrative - April 16"19.docx
DHA 350 GRAS Notice - Updated Safety Narrative - April 16"19.docx

---

Dear Rachel,

Please find attached updated texts for the Narrative and Safety Information section for GRNs 843 and 844.
Other than the 'no objection' letters for GRNs 776 and 7, no further information was found.
Discussion of Falk *et al.* was, as you suggested, moved to the table/summary.

Please do not hesitate to contact me with any further questions

Best wishes

Hywel

Hywel GRIFFITHS
Directeur Scientifique/Chief Scientist

Tel. +33 5 57 25 02 52 | Mobile +33 761 33 37 96 | www.fermentalg.com | Fermentalg – 4 Rue Riviére – 33500 Libourne – France |

> On 12 Apr 2019, at 7:00 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Dear Dr. Griffiths,
>
> We have begun our review of GRNs 000843 and 000844. Our toxicologist notes the following:
>
> In GRNs 000843 & 000844, the text in the safety narrative is very similar to that in GRNs 000776 & 000777. Further, the updated literature search section in GRNs 000843 & 000844 shows the same text repeated from the prior GRNs and also states that an updated literature search is only current through August 2017. The study that was new at the time (Falk et al., 2017) was already discussed in GRNs 000776 & 000777.
>
> For GRNs 000843 and 000844, please update the safety narrative to reflect that an updated literature search was conducted spanning the period from August 2017 to the time GRNs 000843 & 000844 were submitted. Please indicate the month and year that the literature search was concluded. Please discuss in detail any new studies or state that no new information has been published since August 2017.
>
> Also, in your revised safety narratives, you may include Falk et al. (2017) in the table, along

with a brief discussion, but you do not need to discuss it in detail, as we have already seen this study detailed in GRNs 000776 & 000777.

We cannot complete our review of these notices without this information, so please provide this revised narrative within 10 business days. 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

<image013.png>

<image014.jpg> <image015.jpg> <image016.jpg> <image017.jpg> <image018.jpg>

Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

Fermentalg's determination that its DHA oil are GRAS under the conditions of intended use in foods as described herein is based on scientific procedures. Much of the information related to the safety of algal DHA oils have been previously reviewed (see GRN 137, 553, 677, 776, 777) (U.S. FDA, 2004a, 2015a, 2017b, 2018a,b). 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 October, 2018. An updated search of the published scientific literature was conducted through 15 April 2019 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®. No new toxicological evaluations of DHA-rich algal oils *from Schizochytrium* were identified in the literature.

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, 2018 a,b). 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).

Falk *et al.* (2017) reported the results of a 15-day developmental study and a reproductive study of DHA-rich oil from *Schizochytrium* in Wistar rats. 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.

Furthermore, the FDA responded with 'no questions' to Fermentalg's notification for the same algal oil (35% docosahexaenoic acid from Schizochytrium sp. strain FCC-1324) that is the subject of this current notification (U.S. FDA, 2018a). That notice covered use in exempt and non-exempt infant formula in accordance with good manufacturing practices and in combination with a source of arachidonic acid. In addition, GRAS Notices for other substantially equivalent or similar products from closely related *Schizochytrium* strains (GRN 137, 553, and 677, 777) have received "no questions" letters to these notifications (U.S. FDA, 2004a, 2015a, 2017b, 2018a).

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

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

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

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

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

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

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

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

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

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

Reference	Study Type	Test System	Exposure	Findings/Comments
Lewis *et al*. (2016)	Chromosome aberration +/- S9	Human peripheral blood lymphocytes	Phase I (4-hour exposure) :0.00 (negative control), 0.00 (vehicle control), 1.25, 2.5, and 5.0 mg DHA-rich oil/mL Phase 2 (24-hour exposure) 1.25, 2.5 and 5.0 mg DHA-rich oil/mL culture	Not clastogenic.
Lewis *et al.* (2016)	*In vivo* Micronucleus Test	Wistar rats	1,000, 2,500, or 5,000 mg/kg bw/day	Not clastogenic.
Falk *et al*. (2017)	Reproductive and Developmental toxicity study	Wistar rats	Reproductive: male and female Wistar rats 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.
			Developmental: corn oil (vehicle control), 1000, 2500, or 5000 mg/kg bw/day of DHA-rich oil or ARA-rich oil via gavage from gestation days 6 through 20.	No significant differences in mean litter size, sex ratio, live birth index, weaning index, number of implantation sites, corpora lutea, and pre- and post-implantation loss. 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. NOAEL for embryo/fetal development was 5,000 mg/kg bw/day, the highest dose tested.

[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

Additional References

Falk MC, Zheng X, Chen D, Jiang Y, Liu Z, Lewis KD (2017). Developmental and reproductive toxicological evaluation of arachidonic acid (ARA)-Rich oil and docosahexaenoic acid (DHA)-Rich oil. Food Chem Toxicol 103:270-278. DOI:10.1016/j.fct.2017.03.011.

U.S. FDA (2018a). *Agency Response Letter GRAS Notice No. GRN 000776 [Algal oil (35% docosahexaenoic acid) from Schizochytrium sp. strain FCC-1324, Libourne, France: Fermentalg]*. 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.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625983.pdf

U.S. FDA (2018b). *Agency Response Letter GRAS Notice No. GRN 000777 [Algal oil (55% docosahexaenoic acid) from Schizochytrium sp. strain FCC-3204, Libroune, France: Fermentalg]*. 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.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625998.pdf

From:	Hywel Griffiths
To:	Morissette, Rachel
Cc:	Corinne Aguenou
Subject:	Re: response to GRN 000844 - response from FSIS
Date:	Thursday, July 04, 2019 3:55:33 AM
Attachments:	GRN843 GRAS Notice updated Appendix 1.docx GRN844 GRAS Notice updated Appendix 1.docx

Dear Rachel,

Please find attached modified versions of the Appendix 1 for GRN844 *and* GRN843, since I imagine that FSIS's comments will hold for both notifications.

With best wishes

Hywel GRIFFITHS
Directeur Scientifique/Chief Scientist

Tel. +33 5 57 25 02 52 | Mobile +33 7 61 33 37 96 | www.fermentalg.com | Fermentalg – 4 Rue Rivière – 33500 Libourne |

> On 2 Jul 2019, at 15:06, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Dear Dr. Griffiths,
>
> Please see below FSIS' response to your email regarding their questions. At your earliest convenience, please provide a revised Appendix 1 for GRM 844 as stated below so that we forward it to FSIS. Once we receive that document, we can proceed with moving your response letters forward. Please let me know if you have any questions at this time.
>
> Best regards,
>
> *Rachel*
> ---
> **Rachel Morissette, Ph.D.**
> *Regulatory Review Scientist*
>
> **Center for Food Safety and Applied Nutrition**
> **Office of Food Additive Safety**
> **U.S. Food and Drug Administration**
> rachel.morissette@fda.hhs.gov
>
> <image001.png>
>
> <image002.jpg> <image003.jpg> <image004.jpg> <image005.jpg> <image006.jpg>
>
> ---
>
> **From:** Evans, Peter - FSIS <Peter.Evans@fsis.usda.gov>

Sent: Tuesday, July 02, 2019 8:43 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: response to GRN 000844 - response from FSIS

Hello Rachel, here is FSIS' response to the submitter

FSIS's question 1: The DHA levels for the Fermentalg product "DHA 550" algal oil (55% DHA) are significantly higher than the levels of DHA (35%) in the algal oil that is the subject of GRN 000137. The levels of use arrived at for the algal oil in GRN 000137 were influenced by a concern for limiting the amount of DHA. As per the FDA agency response letter for GRN 000137: *"FDA raised concerns about the consumption of high levels of two fatty acids (i.e., docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)), which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil final rule; 62 FR 30751; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR 184.1472(a)(3))."* The requested levels of use for the "DHA 550" algal oil (55% DHA) are the same as for "DHA 350" algal oil (35%) (a level not to exceed 1.45 percent by weight of the product formulation for meat products and 0.87 percent by weight of the product formulation for poultry products). This would result in a higher level of DHA in the final product, as the concentration of DHA is higher in the "DHA 550" algal oil (55% DHA) product. ***Please explain why this higher level is needed and what the final level of DHA intake would be in grams per person per day***.

Submitter's Response: The usage levels for DHA350 were established with reference to GRN000137 *i.e.* so that overall intake was 1.5g/day, lower than the 3g/day combined intake of EPA and DHA.
We are willing to decrease the proposed incorporation levels for DHA550 proportionally to maintain the same overall intake. Levels of incorporation would therefore be limited to 0.92% by weight of the product formulation for meat products and 0.55% by weight of the product formulation for poultry products.

FSIS's response: Agree with levels of incorporation limited to 0.92% by weight of the product formulation for meat products and 0.55% by weight of the product formulation for poultry products.

FSIS's question 2: This submission is for the use of this ingredient as an alternative edible oil in the production of various meat and poultry products. Appendix 1 of both submissions state these substances are "intended to serve as a source of DHA". DHA is an omega-3 fatty acid. For your information, please note that fortification is not permitted in meat and poultry products; therefore, **this ingredient would not be**

permitted for use in fortification of omega-3 fatty acid content.

Submitter's Response 2: If the phrase "intended to serve as a source of DHA" poses a problem for FSIS, we would be willing to submit a new version of Appendix 1 in which the sentence is removed, or in which it is replaced by "intended to serve as an alternative edible oil in the production of various meat and poultry products". Please advise which solution is preferred.

FSIS's response 2: Prefer second solution, "intended to serve as an alternative edible oil in the production of various meat and poultry products" as we do need an intended use.

Peter Evans
Phone: (202) 690-6272
peter.evans@usda.gov

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Monday, June 17, 2019 7:49 AM
To: Evans, Peter - FSIS <Peter.Evans@fsis.usda.gov>
Subject: response to GRN 000844 - question from FSIS

Hi Peter,

Please see the notifier's response to FSIS' questions below. Please advise how you would like to proceed.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

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

<image019.png>

<image020.jpg> <image021.jpg> <image022.jpg> <image023.jpg> <image024.jpg>

From: Hywel Griffiths <hgriffiths@fermentalg.com>
Sent: Monday, June 17, 2019 7:29 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Erica Cermak Intertek <erica.cermak@intertek.com>; Corinne Aguenou <caguenou@fermentalg.com>
Subject: Re: GRN 000844 - question from FSIS

Dear Dr. Morissette,

Thank you for your message.

The usage levels for DHA350 were established with reference to GRN000137 *i.e.* so that overall intake was 1.5g/day, lower than the 3g/day combined intake of EPA and DHA.
We are willing to decrease the proposed incorporation levels for DHA550 proportionally to maintain the same overall intake. Levels of incorporation would therefore be limited to 0.92% by weight of the product formulation for meat products and 0.55% by weight of the product formulation for poultry products.

If the phrase "intended to serve as a source of DHA" poses a problem for FSIS, we would be willing to submit a new version of Appendix 1 in which the sentence is removed, or in which it is replaced by "intended to serve as an alternative edible oil in the production of various meat and poultry products". Please advise which solution is preferred.

Best wishes

Hywel

> On 14 Jun 2019, at 15:54, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Dear Dr. Griffiths,
>
> FSIS has asked us to forward the following question/comment to you regarding the use level of DHA and information regarding restrictions on omega-3 fatty acid fortification. Please provide your response to me within 5 business days and I will forward it along to FSIS for review. Please let me know if you have any questions at this time.
>
> FSIS:
>
> 1. The DHA levels for the Fermentalg product "DHA 550" algal oil (55% DHA) are significantly higher than the levels of DHA (35%) in the algal oil that is the subject of GRN 000137. The levels of use arrived at for the algal oil in GRN 000137 were influenced by a concern for limiting the amount of DHA. As per the FDA agency response letter for GRN 000137:
>
> *"FDA raised concerns about the consumption of high levels of*

two fatty acids (i.e., docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)), which may increase bleeding time, increase levels of low-density lipoprotein cholesterol, and have an effect on glycemic control in non-insulin dependent diabetics (menhaden oil final rule; 62 FR 30751; June 5, 1997). In affirming the GRAS status of menhaden oil, FDA concluded that the use of menhaden oil as a direct food ingredient is GRAS, provided that the combined daily intake of EPA and DHA from consumption of menhaden oil does not exceed 3 g/p/d. To assure that the combined exposure to EPA and DHA would not exceed 3 g/p/d, FDA established maximum levels of use of menhaden oil that would be permitted in specified food categories (21 CFR 184.1472(a)(3)).”

The requested levels of use for the “DHA 550” algal oil (55% DHA) are the same as for “DHA 350” algal oil (35%) (a level not to exceed 1.45 percent by weight of the product formulation for meat products and 0.87 percent by weight of the product formulation for poultry products). This would result in a higher level of DHA in the final product, as the concentration of DHA is higher in the “DHA 550” algal oil (55% DHA) product. *Please explain why this higher level is needed and what the final level of DHA intake would be in grams per person per day.*

2. This submission is for the use of this ingredient as an alternative edible oil in the production of various meat and poultry products. Appendix 1 of both submissions state these substances are “intended to serve as a source of DHA”. DHA is an omega-3 fatty acid. For your information, please note that fortification is not permitted in meat and poultry products; therefore, this ingredient would not be permitted for use in fortification of omega-3 fatty acid content.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

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

<image002.png>

APPENDIX 1
Safety and Suitability for Use in USDA Regulated Products

Safety and Suitability for Use in USDA Regulated Products

As one of the proposed conditions of use [*i.e*., meat products, § 170.3(n)(29) of this chapter] is a United States Department of Agriculture (USDA) regulated category, consideration of the suitability of DHA 550 in this application was considered. As detailed in this Notice, Fermentalg's DHA 550 oil is considered similar in its source, composition, nutritional value, and metabolism to the Generally Recognized as Safe (GRAS)-Notified substance described in GRN 137. Martek Biosciences Corporation (Martek)'s oil is listed on the table of Safe and Suitable Ingredients available on USDA's website[1]. This listing indicates Martek's oil is safe and suitable for use as an alternative edible oil in the production of various meat and poultry products (at a level not to exceed 1.45 percent by weight of the product formulation for meat products and 0.87 percent by weight of the product formulation for poultry products). The oil is required to be listed by is common or usual name in the ingredients statement.

The intended use of Fermentalg's DHA 550 in meat products is not expected to adversely affect the wholesomeness of the product. The organoleptic properties (*e.g*., color, odor, taste) of DHA 550 are comparable to the DHA algal oil and menhaden oil currently approved for use in meat products). The safety of DHA 350 is addressed in Part 6 (§ 170.250 Narrative and Safety Information) of this Notice.

DHA 550 is intended to serve as an alternative edible oil in the production of various meat and poultry products. It is not intended for use as a processing aid as defined under 21 CFR § 101.100(a)(3)(ii). As such, the presence of DHA 550 will be listed by its common or usual name (DHA algal oil) in the ingredients statement of any resultant product.

[1] https://www.fsis.usda.gov/wps/wcm/connect/ce40e7ae-3d55-419e-9c68-a1b6fefcd4de/7120.1_Table_2.pdf?MOD=AJPERES

From: Hywel Griffiths
To: Morissette, Rachel
Cc: Corinne Aguenou
Subject: Re: response requested for GRNs 000843 and 000844
Date: Friday, September 27, 2019 1:51:31 AM
Importance: High

Dear Rachel,

Thanks for your email,

Yes I can confirm that all three statements are correct.

Best wishes

Hywel GRIFFITHS
Directeur Scientifique/Chief Scientist

Tel. +33 5 57 25 02 52 | Mobile +33 7 61 33 37 96 | www.fermentalg.com | Fermentalg – 4 Rue Rivière – 33500 Libourne |

> On 26 Sep 2019, at 20:43, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Dear Dr. Griffiths,
>
> Can you please confirm that the following statements are correct:
>
> 1. Fermentalg is incorporating the safety data and information pertaining to the safe use of DHA and EPA per 21 CFR 184.1472 (menhaden oil) discussed in GRN 000137 into GRNs 000843 and 000844 to support its GRAS conclusion.
>
> 2. Fermentalg states that algal oil (35% DHA) will be used as the sole added source of DHA in any given food category and, if blended with another source of DHA or eicosapentaenoic acid (EPA), the levels will provide no more than 1.5 g of DHA/person/day and no more than 3.0 g/person/day of DHA and EPA combined.
>
> 3. Fermentalg states that algal oil (55% DHA) will be used as the sole added source of DHA in any given food category and, if blended with another source of DHA or eicosapentaenoic acid (EPA), the levels will provide no more than 1.5 g of DHA/person/day and no more than 3.0 g/person/day of DHA and EPA combined.
>
> Please provide your response as soon as possible.
>
> Best regards,
>
> Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

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

<image013.png>

<image014.jpg> <image015.jpg> <image016.jpg> <image017.jpg> <image018.jpg>