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Spherix Consulting Group

May 7, 2021

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
CPK-2 Building, Room 2092
5001 Campus Drive, HFS-225
College Park, MD 20740

Dear GRAS Filing Team:

It is our opinion that the attached "GRAS Determination for the Use of Algal Oil from *Schizochytrium limacinum* TKD-1 in Exempt and Non-exempt Infant Formulas for Preterm and Term Infants and Selected Conventional Foods" constitutes a new notification. The production of Algal oil from *Schizochytrium limacinum* TKD-1 described in this Notice utilizes a new strain of *Schizochytrium* sp. in the production of a docosahexaenoic acid-rich oil.

We thank you for taking the time to review this GRAS Determination. Should you have additional questions, please let us know.

Sincerely,

[redacted]

Dietrich B. Conze, PhD, DABT
Managing Partner

Enclosure:

CD containing:

- Form 3667
- Cover Letter
- GRAS Notification
- Appendices
- References

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GRAS Determination for the Use of Algal Oil from *Schizochytrium limacinum* TKD-1 in Exempt and Non-exempt Infant Formulas for Preterm and Term Infants and Selected Conventional Foods

Prepared for:

TK Biohealth Co., Ltd.
109 Husong Road
Shitan Industrial Park
Shizi Town, Quanjiao County
Chuzhou City, China

Prepared by:

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May 7, 2021

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LIST OF ABBREVIATIONS

2-MCPD: 2-monochloro-propanol-1,2-diol

3-MCPD: 3-monochloro-propanol-1,2-diol

AOCS: American Oil Chemists Society

ARfD: Acute Reference Dose

ASP: Amnesic Shellfish Poisoning

BW: Body Weight

CCP: Critical Control Points

CFR: Code of Federal Regulations

CONTAM: European Food Safety Authority Panel on Contaminants in the Food Chain

DHA: Docosahexaenoic Acid

DL-PCBs: Dioxin-like Polychlorinated Biphenyls

DSP: Diarrhetic Shellfish Poisoning

FCC: Food Chemicals Codex

FDA: United States Food and Drug Administration

FFDCA: Federal Food, Drug, and Cosmetic Act

FSANZ: Food Standards Agency of Australia and New Zealand

FSSC: Food Safety System Certification

GC-ECD: Gas Chromatography with an Electron Capture Detector

GC-FPD: Gas Chromatography with a Flame Photometric Detector

GC-MS: Gas Chromatography with Mass Spectroscopy

GRAS: Generally Recognized As Safe

GRN: GRAS Notification

HACCP: Hazard Analysis and Critical Control Point

HPLC/MS/MS: High Performance Liquid Chromatography with Tandem Mass Spectroscopy

HPLC-DAD: High Performance Liquid Chromatography with a Diode-Array Detector

HPLC-FLD: High Performance Liquid Chromatography with a Fluorescence Detector

HPLC-MS: High Performance Liquid Chromatography Mass Spectroscopy

ISO: International Organization for Standardization

Kcal: Kilocalorie

KOH: Potassium Hydroxide

LD_{50}: 50% of the Lethal Dose

LOD: Limit of Detection

LOQ: Limit of Quantitation

Meq: Milliequivalents

MPN: Most Probably Number

MS: Mass Spectroscopy

NA: Not Applicable

ND: Not Detected

NOAEL: No Observed Adverse Effect Level

NR: Not Reported

PCDD: Polychlorinated Dibenzo-p-dioxins

PCDF: Polychlorinated Dibenzofurans

PSP: Paralytic Shellfish Poisoning

QPS: Qualified Presumption of Safety

TEF: Toxic Equivalence Factor

TEQ: Toxic Equivalent

WHO: World Health Organization

I. SIGNED STATEMENT OF THE CONCLUSION OF GENERALLY RECOGNIZED AS SAFE (GRAS) AND CERTIFICATION OF CONFORMITY TO 21 CFR §170.205-170.260

A. SUBMISSION OF GRAS NOTICE

TK Biohealth Co., Ltd (formerly Jiangsu Tiankai Biotechnology Co., Ltd.) is hereby submitting a GRAS notice in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

TK Biohealth Co., Ltd. (formerly Jiangsu Tiankai Biotechnology Co., Ltd.)
109 Husong Road
Shitan Industrial Park
Shizi Town, Quanjiao County
Chuzhou City, China

C. COMMON OR USUAL NAME

Algal oil from *Schizochytrium limacinum* TKD-1

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

This notification does not contain any trade secret or confidential information.

E. INTENDED USE

TK Biohealth Co., Ltd intends to use the algal oil from *Schizochytrium limacinum* TKD-1 as an ingredient in exempt and non-exempt cow's milk and soy-based infant formulas for preterm and term infants and as an ingredient in the food categories listed in 21 CFR 184.1472(a)(3).

F. BASIS FOR GRAS DETERMINATION

The use of Algal oil from *Schizochytrium limacinum* TKD-1 in exempt and non-exempt cow's milk and soy-based infant formulas for preterm and term infants, and general foods has been determined to be GRAS using scientific procedures, and generally available and accepted information specified under 21 CFR §170.30 (a) (b). The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part VII of this GRAS notice contains the citations for the published studies. These publicly available data and information fulfill the requirement of the GRAS standard for general availability of the scientific data, information, and methods relied on to establish the safety of DHA Algal Oil for its intended conditions of use. The peer-review of the published studies and lack of Letters to the Editor or other dissenting opinions provide ample evidence of general recognition among qualified experts that there is reasonable

certainty that consumption of Algal Oil for its intended use is not harmful. The general availability and acceptance of these scientific data, information, and methods satisfy the criterion of the GRAS standard that general recognition of safety requires common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food that there is reasonable certainty that the substance is not harmful under the conditions of its intended use.

As presented in this Notice, the scientific basis for GRAS is based on the following:

1. The subject of this GRAS Notice, Algal oil from *Schizochytrium limacinum* TKD-1, is derived from *S. limacinum* TKD-1, which is a strain of *S. limacinum.*
    a. *Schizochytrium limacinum* is a microalgae known to produce large amounts of DHA, as well as other omega-3 fatty acids.
    b. The 18s rRNA sequence of *S. limacinum* TKD-1 is 88% identical to *Schizochytrium* sp. ONC-T18, which is the subject of GRN 677.
2. The subject of this GRAS Notice is manufactured according to current Good Manufacturing Practices using processing aids, and food contact substances that conform to the conditions of use specified in Title 21 of the United States Code of Federal Regulations and/or Food Chemicals Codex specifications.
    a. Process procedures and product specifications are in place to control pivotal quality attributes that ensure a consistent, safe, food-grade finished ingredient.
    b. The available stability studies support a shelf-life of 1 year at 4-5°C and 2 years at -18°C.
3. There is a long history of safe use of *Schizochytrium* sp.-derived, DHA-rich oils in infant formulas and general foods worldwide.
4. The subject of this GRAS Notice is quantitatively and qualitatively equivalent with the subject of GRN 677. Therefore, the toxicology studies conducted by Schmitt et al. (2012a) and Schmitt et al. (2012b) with *Schizochytrium* sp. ONC-T18 support the safe use of the subject of this GRAS Notice in infant formula and general foods.
5. The published toxicology studies conducted by Schmitt et al. (2012a) and Schmitt et al. (2012b) demonstrate that the DHA-rich oil derived from *Schizochytrium* sp. ONC-T18 is not genotoxic and does not result in adverse effects at the highest levels tested (3305 and 3679 mg/kg body weight/day in male and female rats, respectively).

6. Numerous toxicology studies of *Schizochytrium* sp.-derived DHA-rich oils conducted over a period of more than a decade, include acute, subacute, and subchronic toxicity, a battery of genotoxicity studies, and developmental and reproductive toxicity studies. In these reports, no evidence of toxicity was noted at up to 5,000 mg/kg bw/day. Therefore, *Schizochytrium* sp.-derived, DHA-rich oils are not genotoxic or toxigenic.

7. DHA-rich oils from numerous sources including *Schizochytrium* sp., *Crypthecodinium cohnii*, *Ulkenia* sp. SAM2179, *Chlorella protothecoides* strain S106, *Prototheca moriformis* strain S2532, tuna and other fish are GRAS for use in general foods and exempt and non-exempt infant formulas for preterm and term infants (GRN 41; GRN 137; GRN 138; GRN 319; GRN 384; GRN 469; GRN 527; GRN 553; GRN 677; GRN 731; GRN 732; GRN 776; GRN 777; GRN 836; GRN 843; GRN 844; GRN 862; GRN 913; GRN 933).

8. The publicly available scientific literature on the consumption and the safety of DHA-rich oils in clinical studies with both term and pre-term infants is extensive and sufficient to support the safety and GRAS status of the proposed DHA-rich oil ingredients. Published clinical studies that show that *Schizochytrium* sp.-derived, DHA-rich oils are also safe and well-tolerated in adults.

9. Literature searches did not identify safety/toxicity concerns related to the *Schizochytrium* sp.-derived DHA-rich oil ingredient.

10. The use of Algal oil from *Schizochytrium limacinum* TKD-1 in exempt and non-exempt preterm and term infant formulas will be used in conjunction with a safe and suitable source of arachidonic acid (ARA).

11. The estimated exposure to the subject of the GRAS Notice from its addition to exempt and non-exempt infant formulas for preterm and term infants is based on a target DHA concentration of 0.5% of total fat. Assuming infants consume approximately 100 to 120 kcal/kg body weight/day of which fat comprises about 50%, the corresponding DHA intake will be 27 to 33 mg DHA/kg body weight/day at the target DHA concentration of 0.5 % of total fat and approximately 43 to 62 mg Algal oil from *Schizochytrium limacinum* TKD-1/kg body weight/day, assuming the oil contains 53 to 62 % DHA. This DHA intakes are 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).

12. The estimated exposure to the subject of the GRAS Notice from its addition to general foods will result in a maximum dietary exposure of less than 1.5 grams of DHA/day.

    a. The proposed uses of the subject of this GRAS Notice are identical to those for other *Schizochytrium* sp.-derived, DHA-rich oils.

In all the studies summarized in this Notification, there were no significant adverse effects/events or tolerance issues attributable to *Schizochytrium* sp.-derived DHA or DHA-rich oils. Because this safety evaluation was based on generally available and widely accepted data and information, it satisfies the so-called "common knowledge" element of a GRAS determination. In addition, the intended uses of DHA-rich oil have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination.

Algal oil from *S. limacinum* TKD-1 has been the subject of a thorough safety assessment as described above. The general availability and general acceptance, throughout the scientific community of qualified experts, of the data and information that establish the safety of DHA Algal Oil under its intended conditions of use establish the general recognition of this data and information. Together, the establishment of safety based on scientific procedures and its general recognition form the basis for TK Biohealth Co, Ltd. conclusion of GRAS status of DHA Algal Oil for its intended uses.

Therefore, Algal oil from *S. limacinum* TKD-1 is safe and GRAS at the proposed levels of addition to exempt and non-exempt cow's milk and soy-based infant formulas for preterm and term infants, and general foods. The subject of this GRAS Notice is therefore excluded from the definition of a food additive and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

G. PREMARKET APPROVAL

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the substance is GRAS under the conditions of intended use.

H. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Dietrich Conze, PhD, Managing Partner, Spherix Consulting Group Inc., at 751 Rockville Pike, Unit 30-B, Rockville, MD 20852; Telephone: 240-367-6089; Email: dconze@spherixgroup.com; or be sent to FDA upon request.

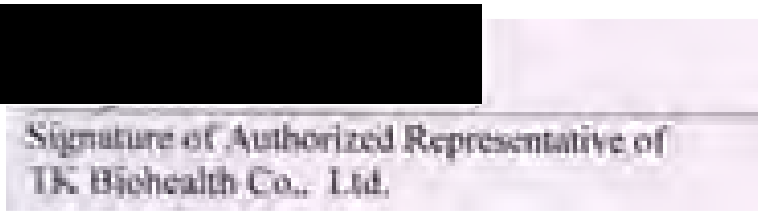
I. FREEDOM OF INFORMATION ACT (FOIA)

Parts 2 through 7 of this notification do not contain data or information that is exempt from disclosure under the FOIA.

J. INFORMATION INCLUDED IN THE GRAS NOTIFICATION

To the best of our knowledge, the information contained in this GRAS notification is complete, representative and balanced. It contains both favorable and unfavorable information, known to TK Biohealth Co., Ltd and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

[redacted]



May 7, 2021
Date

II. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT OF THE NOTIFIED SUBSTANCE

A. COMMON OR USUAL NAME

Algal oil from *Schizochytrium limacinum* TKD-1

B. TRADE NAME

Schizochytrium sp. oil, DHA-rich oil from *Schizochytrium*, DHA-rich oil, Algal oil

C. DESCRIPTION OF THE ALGAL OIL FROM *SCHIZOCHYTRIUM LIMACINUM*

The subject of this GRAS Determination is an algal oil from *Schizochytrium limacinum* TKD-1 that is rich in the omega-3 (n-3) fatty acid docosahexaenoic acid (DHA; 4,7,10,13,16,19-docosahexaenoic acid; $C_{22}H_{32}O_2$; Chemical Abstract Service (CAS) number 6217-54-5). The oil is manufactured by TK Biohealth Co., Ltd (formerly Jiangsu Tiankai Biotechnology Co., Ltd.) by expanding *S. limacinum* TKD-1 and hydrolysing the biomass using an alkaline protease derived from *Bacillus licheniformis*. The hydrolysate is then heated to inactivate the protease and centrifuged. The resulting DHA-containing supernatant is removed from the pelleted cellular debris, refined using processes typical to food oil manufacturing, and mixed with tocopherols and sunflower oil to prevent oxidation and standardize DHA content to not less than 35%, respectively. The specified quality attributes include DHA content, acid value, peroxide value, moisture and volatiles, unsaponifiables, trans-fatty acids, and free fatty acids. Importantly, the resulting algal oil is quantitatively and qualitatively similar to other *Schizochytrium* sp.-derived, DHA-rich oils that are GRAS for use in exempt and non-exempt infant formulas for preterm and term infants and conventional foods in the United States (GRN 137, 2003, Martek Biosciences Corporation; GRN 553, 2014, DSM Nutritional Products; GRN 677, 2017, Mara Renewables Corporation; GRN 731, 2018, Linyi Youkang Biology Co., Ltd.; GRN 732, 2018, Linyi Youkang Biology Co, Ltd.; GRN 776, 2018, Fermentalg; GRN 777, 2018, Fermentalg; GRN 836, 2019, Xiamen Huison Biotech Co., LTD; GRN 843, 2019, Fermentalg; GRN 844, 2019, Fermentalg; GRN 860, Ceased, Algal oil (36% docosahexaenoic acid) from *Schizochytrium* sp. strain DHF; GRN 862, 2020, BASF Corporation; GRN 933, 2020, Hubei Fuxing BioTechnology, Co., Ltd.; GRN 934, Pending, CABIO Biotech (Wuhan) Co., Ltd.).

Docosahexaenoic (22:6n-3) acid is a structural component of human cells, either synthesized endogenously from α-linolenic (18:3n-3) acid or obtained through the diet from ingestion of breast milk, fish, or dietary supplements containing fish oil. Although DHA is the primary component, the oil manufactured by TK Biohealth, Ltd., Co. also contains other fatty

acids including lauric (12:0), myristic (14:0), palmitic (16:0), margaric (17:0), stearic (18:0), behenic (22:0), palmitoleic (16:1n-7), oleic (18:1n-9), linoleic (18:2n-6), γ-linolenic (18:3n-6), α-linolenic, eiscoastrienoic (20:3n-3); arachidonic (20:4n-6); eicosapentaenoic (20:5n-3), docosapentaenoic (22:5n-3); elaidic (18:1), linolelaidic (18:2); and arachidic (20:0) acids.

D. PRODUCTION PROCESS

1. Description of the Production Strain

The subject of this GRAS Determination is manufactured from a non-genetically modified strain of *S. limacinum* (*S. limacinum* TKD-1) that was isolated from a decayed leaf in the East China Sea using pine pollen as bait. As summarized in GRN 913 (pages 9-11), *S. limacinum* is a thraustrochytrid, also known as *Aurantiochytrium limacinum*, and a member of the Chromista Kingdom (Table 1; https://www.catalogueoflife.org/data/taxon/67XWW, accessed on February 7, 2021). In 2007, taxonomic classification of the genus *Schizochytrium* was amended based on genetic and phenotypic analyses, and new genera such as *Aurantiochytrium* and *Oblongichytrium* were defined, resulting in the genera *Schizochytrium*, *Aurantiochytrium* and *Oblongichytrium* (Yokoyama and Honda, 2007; Fossier Marchan et al., 2018). As a consequence, some strains initially described as *Schizochytrium* now belong to *Aurantiochytrium* or *Oblongichytrium genus*, such as *S. limacinum* and *Schizochytrium mangrovei* which are now *Aurantiochytrium limacinum* and *Aurantiochytrium mangrovei*, respectively.

Schizochytrium limacinum is most often called a microalgae, although it is autotrophic and not photosynthetic, and known to produce large amounts of DHA, docosapentaenoic acid (DPA), and eicosapentanenoic acid (EPA) (Bindea et al., 2018; Du et al., 2019; Liang et al., 2011; Ye et al., 2015; Zhang et al., 2017). In the European Union, *S. limacinum* has been recommended for Qualified Presumption of Safety status by the European Food Safety Authority with qualification for production purposes only (EFSA Panel on Biological Hazards et al., 2020). Importantly, the strain used by TK Biohealth has been phenotypically and genotypically confirmed to be a member of *S. limacinum* and deposited in the China Center for Type Culture Collection with the deposition number M2020378 (see Appendix 1, Phenotypic and Genotypic Identity; see Appendix 2, Strain Preservation Certificate). TK Biohealth also maintains stocks of *S. limacinum* TKD-1 at -80°C at the production facility.

Table 1. Taxonomic Classification S*chizochytrium limacinum*

Class	Scientific Classification[1]
Kingdom	Chromista
Phylum	Bigyra
Class	Labyrinthula
Order	Thraustochytriida
Family	Thraustochytriaceae
Genus	S*chizochytrium*
Species	*limacinum*

[1]Obtained from https://www.catalogueoflife.org/data/taxon/67XWW (accessed on February 7, 2021).

a. Phenotypic Identity

Schizochytrium limacinum TKD-1 proliferates by schizogenesis in mask medium with sea water to a colony diameter of 3-5 mm. It is initially white, becomes light orange as it grows, and does not produce spores. The cell wall is thin, transparent, and spherical with a diameter of 7.0-12.2 μm.

b. Genotypic Identity

18s rRNA BLASTN alignments of upstream and downstream segments of the 18s rRNA sequence of *Schizochytrium limacinum* TKD-1 with other *Schizochytrium* strains that are GRAS, for use in infant formula and conventional foods, and supported by toxicology studies, shows that the strain has the highest percentage of identity to *Schizochytrium* sp. T18, also known as *Schizochytrium* sp. ONC-T18, which is the subject of GRN 677 and 862 (Tables 2 and 3; See Appendix 3, Part 1 18s rRNA Alignments; Appendix 4, Part 2 18s rRNA Alignments). The 18s rRNA sequences *S. limacinum* TKD-1 is also greater than 75 and 84 % identical to the *Schizochytrium* sp. strains PTA-9695 and ATCC 20888, which are the subjects of GRN 137 and 553. Additionally, molecular systematic analysis shows that the TK Biohealth strain is similar to *Aurantiochytrium limacinum* IFO 32693 and other strains of *Schizochytrium* (see Appendix 1, Phenotypic and Genotypic Identity), including *Schizochytrium* sp. ATCC 20888. Taken together, these data indicate that *S. limacinum* TKD-1 is a member of *Schizochytrium* and is genotypically equivalent to the strains used to produce DHA-rich oils that are GRAS for use in infant formula and conventional foods in the United States and undergone toxicology testing.

Table 2. Percent Identity Matrix of Part 1 of the *18s* rRNA Sequence of *Schizochytrium limacinum* TKD-1 with Other *Schizochytrium* sp. Strains that are GRAS for Use in Infant Formula and Conventional Foods

Schizochytrium strain	% Identity[a]			
	ATCC PTA-9695[b]	TKD-1	ATCC 20888[c]	ONC-T18[d]
ATCC PTA-9695[c]	100	74.76	83.19	82.25
TKD-1	74.76	100	84.47	86.41
ATCC 20888[b]	83.19	84.47	100	94.11
ONC-T18[d]	82.25	86.41	94.11	100

[a]Percent identity was determined using an upstream 202 nucleotide sequence of the *18s* rRNA sequence of *Schizochytrium limacinum* TKD-1 and the publicly available *18s* rRNA sequences of *Schizochytrium* sp. ATCC 20888, *Schizochytrium* sp. T18, *Schizochytrium* sp. FCC1324, and *Schizochytrium* sp. ATCC PTA-9695 using Clustal O Version 1.2.4. For alignments, see Appendix 3, Part 1 18s rRNA Alignments.
[b]*18s* rRNA Sequence obtained from the National Library of Medicine; Genebank Number DQ367050.1; strain is used to manufacture the subject of GRN 137.
[c]*18s* rRNA Sequence obtained from United States Patent # 10,362,794 B2; strain is used to manufacture the subject of GRN 553.
[d]*18s* rRNA Sequence obtained from United States Patent #10,435,725 B2; strain is used to manufacture the subject of GRNs 677 and 862.

Table 3. Percent Identity Matrix of Part 2 of the *18s* rRNA Sequence of *Schizochytrium limacinum* TKD-1 with Other *Schizochytrium* sp. Strains that are GRAS for Use in Infant Formula and Conventional Foods

Schizochytrium strain	% Identity[a]			
	ATCC PTA-9695[b]	TKD-1	ATCC 20888[c]	ONC-T18[d]
ATCC PTA-9695[c]	100	83.33	83.25	82.37
TKD-1	83.33	100	88.18	88.18
ATCC 20888[b]	83.25	88.18	100	94.11
ONC-T18[d]	82.37	88.18	94.11	100

[a]Percent identity was determined using a downstream 220 nucleotide sequence of the *18s* rRNA sequence of *Schizochytrium limacinum* TKD-1 and the publicly available *18s r*RNA sequences of *Schizochytrium* sp. ATCC 20888, *Schizochytrium* sp. T18, *Schizochytrium* sp. FCC1324 and *Schizochytrium* sp. ATCC PTA-9695 using Clustal O Version 1.2.4. For alignments, see Appendix 4, Part 2 18s rRNA Alignments.
[b]*18s* rRNA Sequence obtained from the National Library of Medicine; Genebank Number DQ367050.1; strain is used to manufacture the subject of GRN 137.
[c]*18s* rRNA Sequence obtained from United States Patent # 10,362,794 B2; strain is used to manufacture the subject of GRN 553.
[d]*18s* rRNA Sequence obtained from United States Patent #10,435,725 B2; strain is used to manufacture the subject of GRNs 677 and 862.

2. Manufacturing

The subject of this GRAS Notice is manufactured from a pure culture *S. limacinum* TKD-1 in a stainless-steel closed system and involves four steps: 1) fermentation; 2) isolation; 3) refining; and 4) formulation (Figure 1). All of the processes used by TK Biohealth are similar to those used in the production of other food oils, including *Schizochytrium* sp.-derived, DHA-containing oils that are GRAS for use in non-exempt term infant formulas in United States approved for use in the European Union.

a. Quality

The subject of this GRAS determination is manufactured by TK Biohealth according to the China Food Safety National Standard-GB 26400-2011 in a hazard analysis and critical control point (HACCP)-, Food Safety System Certification (FSSC) 22000-compliant facility that has also been determined to be Kosher- and Halal-compliant. Therefore, TK Biohealth manufactures the algal oil in accordance with current Good Manufacturing Practices (cGMP). All medium components and processing aids either comply with the conditions of use specified in Title 21 of the United States Code of Federal Regulations or are Food Chemicals Codex grade (Table 4). No solvents are used.

Table 4. Regulatory Compliance of Processing Aids

Processing aid	Regulatory status
Alkaline Protease	Food Chemical Codex Grade
Sodium Hydroxide	21 CFR 184.1763
Silicon dioxide	21 CFR 172.480
Activated clay (Bentonite)	21CFR 184.1155
Filter	21 CFR 177.1520
Sunflower Oil	Food Chemical Codex Grade
Tocopherols	21CFR 182.3890

b. Production

To produce Algal Oil from *S. limacinum* TKD-1, a frozen stock of *S. limacinum* TKD-1 is thawed and activated by inoculating a flask containing sterilized culture medium composed of water and food-grade ingredients, including yeast extract, glucose monohydrate, ammonium sulfate, sodium sulfate, calcium chloride dihydrate. The flask culture is then expanded in sterilized culture medium in a series of subsequent fermentations. When the final fermentation is complete, an alkaline protease derived from *Bacillus licheniformis* (IUB Number 3.4.21.62) is added to lyse the *S. limacinum* TKD-1 cells. The culture is then heated to inactivate the protease and centrifuged to separate the cellular debris and unlysed *S. limacinum* TKD-1 from the lysed *S. limacinum* TKD-1 and culture medium. The resulting supernatant, also known as the DHA-

containing crude oil, is collected in stainless steel tanks and overlaid with nitrogen. The tanks are then sealed and stored under ambient conditions for not more than 10 days before refining.

To refine the product, the DHA-containing crude oil is mixed with hot water to remove phospholipids, free fatty acids, monoglycerides, and diglycerides, and settled. The water phase is decanted, and the degummed oil is neutralized with sodium hydroxide. After another round of settling, the oil phase is collected and bleached with activated clay. The clay is removed using a stainless-steel plate filter and the bleached oil is deodorized with steam. The product is then cooled, tocopherols and sunflower oil are added to prevent oxidation and standardize DHA content, respectively, and filtered producing Algal Oil from *S. limacinum* TKD-1. The refined oil is placed in air-tight aluminum drums or barrels, sampled for product specification qualification, and blanketed with nitrogen. The oil-containing drums and barrels are then sealed and frozen at -18°C prior to release.

Critical process parameters that are monitored throughout the production process include adjusting the air ventilation, stirring velocity, culture pH, temperature, time, and culture density during fermentation to attain the target density. Additionally, the two critical points in the production of the Algal Oil from *S. limacinum* TKD-1 are the deodorization step and the final filtration step before the product is added to the aluminum barrels. During the final filtration step, visible impurities must not be seen at the beginning, middle and final stage of filtering.

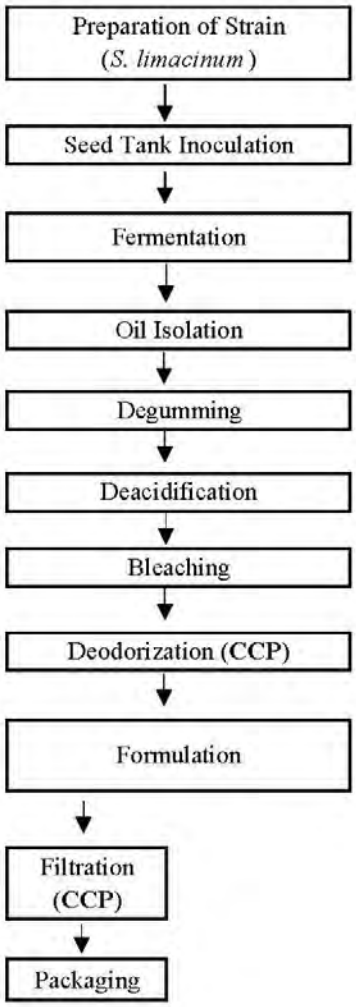


Figure 1. Production Process for the Algal Oil from *S. limacinum* TKD-1

Schizochytrium limacinum TKD-1 is expanded in series of fermentation steps to generate the biomass needed to produce the DHA-rich oil. The biomass is then hydrolysed and centrifuged to remove the cellular debris during an oil isolation step. The clarified oil is degummed, deacidified, bleached, and deodorized before food grade sunflower oil and tocopherols are added to adjust the DHA content and help prevent oxidation. The finished product is then filtered, aliquoted into aluminum barrels or drums, and overlayed with nitrogen. The barrels and drums are then sealed and frozen until release. The critical control points (CCPs) in the process are the deodorization and filtration steps prior to packaging.

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Product Specifications and Batch Data

Prior to releasing each batch of the Algal Oil from *S. limacinum* TKD-1, TK Biohealth evaluates the acid and peroxide values, and the moisture and volatiles, unsaponifiables, trans-fatty acids, free fatty acids, and DHA content on every batch of finished ingredient to ensure the quality of the finished product. Each batch must adhere to specific acceptance criteria that have been established for each parameter prior to release (Table 5). Data from five batches of Algal Oil from *S. limacinum* TKD-1 show that the manufacturing process produces a consistent finished product that meets the established product specifications.

Table 5. Product Specifications and Batch Data for Algal Oil from *S. limacinum* TKD-1

Parameter	Method	Specification	Batch Number				
			DD20191206	DD20191213	DD20191220	DD20200110	DD20200101
Physical Attributes							
Acid value[1]	AOCS Cd-3d-63	≤ 0.5 mg KOH/g	0.18	0.19	0.19	0.16	0.16
Peroxide value[1]	ISO 3960:2017	≤ 5.0 meq/kg oil	0.78	0.79	0.72	1.10	1.04
Moisture and volatiles[1]	ISO 662:2016	≤ 0.05 %	0.01	0.01	0.01	<0.01	<0.01
Unsaponifiables[1]	ISO 3596:2000	≤ 3.5 %	1	1	1	1.8	1.9
Chemical Attributes							
DHA content[1]	AOAC 996.06	≥ 35 %	56.44	53.34	56.07	59.59	61.16
Trans-fatty acids[1]	AOAC 996.06	≤ 2.0 %	0.05	0.1	0.07	0.05	0.04
Free fatty acids[1]	AOCS Ca 5a-40	≤ 0.4 %	0.09	0.09	0.09	0.09	0.09
Microbiologicals							
Total Plate Count[1]	ISO 4833-1:201	≤ 1000 CFU/g	< 10	< 10	< 10	< 10	< 10
Coliforms[1]	ISO 4831:2004	≤ 10 CFU/g	<0.3	<0.3	<0.3	<0.3	<0.3
Molds and Yeasts[1]	ISO 21527-1:2008	≤ 25 CFU/g	< 10	< 10	< 10	< 10	< 10
Salmonella[1]	ISO 6579-1:2017	(Negative/375 g)	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
Heavy Metals							
Lead [1]	GB 5009.268 ICP-OES	≤ 0.1 mg/kg	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
Arsenic[1]		≤ 0.1 mg/kg	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
Mercury[1]		≤ 0.04 mg/kg	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
Cadmium[1]		≤ 0.2 mg/kg	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
Copper[1]	GB 5009.13 ICO-PM	≤ 0.1 mg/kg	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
Iron[1]	AOAC 984.27 ICP-OES	≤ 0.1 mg/kg	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]

Abbreviations: LOQ – limit of quantitation.; ISO - International Organization for Standardization; AOCS - American Oil Chemists Society; meq – milliequivalents; KOH – potassium hydroxide; ND – not detected

[1]Determined by SGS-CSTC Standards Technical Services Co., Ltd.

[2]Denotes less than the limit of quantitation.

2. Other Quality Attributes

In addition to the product specifications, TK Biohealth has also evaluated the p-anisidine value, and fatty acid and sterol profiles of five batches of the Algal Oil from *S. limacinum* TKD-1. Because the subject of this determination is manufactured from *Schizochytrium*, which has a long history of use in the manufacture of DHA-rich oils intended for infant formula, in a closed system without solvents, environmental contaminants, such as polyaromatic hydrocarbons, polychlorinated biphenyls, pesticides, and marine biotoxins are not expected. Overall, the batch data on the additional quality attributes show that the TK Biohealth's manufacturing process and process controls produces a finished product with consistent p-anisidine values, and sterol and fatty acid profiles.

a. Physical Attributes

The color and p-anisidine values of five batches of the Algal Oil from *S. limacinum* TKD-1 were evaluated using an International Organization for Standardization (ISO) compendial methods by SGS-CSTC Standards Technical Service Co., Ltd., which is an ISO/IEX 17025: 2005-accredited lab (Table 6). The color of the five batches ranged from yellow 3.0, red 0.4 to yellow 3.0, red 5.0, white 0.6. The p-anisidine values of the five batches of the *S. limacinum* TKD-1 -derived oil ranged from 3.7 to 10.3 and comply with the p-anisidine limit established by the United States Pharmacopoeia for *Schizochytrium* oil (Ismail et al., 2016). Importantly, although the *S. limacinum* TKD-1-derived oil is light yellow to orange yellow, it is not intended to be used as a color additive.

Table 6. Physical Attributes of the *S. limacinum* TKD-1 -derived Oil[1]

Parameter	Method	Batch Number[2]				
		DD20191206	DD20191213	DD20191220	DD20200110	DD20200101
Color	ISO 15305:1998-	Yellow 3.0, Red 0.4, White 0.3	Yellow 3.0, Red 0.5, White 0.6	Yellow 3.0, Red 0.5, White 0.3	Yellow 3.0, Red 0.3	Yellow 3.0, Red 0.3
p-Anisidine value	ISO 6885:2016	3.7	8.6	6.8	10.1	10.3

Abbreviations: LOQ – limit of quantitation.; ISO - International Organization for Standardization
[1]Determined by SGS-CSTC Standards Technical Services Co., Ltd.

b. Fatty Acids

The fatty acid content of five batches of the Algal Oil from *S. limacinum* TKD-1 were determined using an AOAC compendial gas chromatographic method by SGS-CSTC Standards Technical Service Co., Ltd., which is an ISO/IEX 17025: 2005 accredited lab (Table 7). As expected, the most predominant fatty acid was DHA with levels ranging from 53 to 56 g/100 g. Other fatty acids were also present, including lauric (12:0), myristic (14:0), palmitic (16:0), margaric (17:0), stearic (18:0), behenic (22:0), palmitoleic (16:1n-7), oleic (18:1n-9), linoleic (18:2n-6), γ-linolenic (18:3n-6), α-linolenic, eiscoastrienoic (20:3n-3); arachidonic (20:4n-6); eicosapentaenoic (20:5n-3), docosapentaenoic (22:5n-3); elaidic (18:1), linolelaidic (18:2); and arachidic (20:0) acids.

Table 7. Fatty Acid Content of the Algal Oil from *S. limacinum* TKD-1[1]

Fatty Acid	Method	LOQ (g/100 g)	Batch Number[2]				
			DD20191206	DD20191213	DD20191220	DD20200110	DD20200101
C4:0 butanoic/ butyric	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C6:0 hexanoic/caproic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C8:0 octanoic/caprylic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C12:0 dodecanoic/ lauric	AOAC 996.06	0.01	0.06	0.06	0.06	0.06	0.06
C13:0 tridecanoic/tridecanoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C14:0 tetradecanoic/myristic	AOAC 996.06	0.01	0.54	0.55	0.55	0.50	0.50
C15:0 pentadecanoic	AOAC 996.06	0.01	0.08	0.08	0.08	0.06	0.06
C16:0 hexadecanoic/palmitic	AOAC 996.06	0.01	18.36	18.09	18.33	16.22	16.28
C17:0 heptadecanoic/margaric	AOAC 996.06	0.01	0.07	0.08	0.08	0.07	0.07
C18:0 octadecanoic/stearic	AOAC 996.06	0.01	1.02	1.1	1.06	0.98	0.98
C21:0 heneicosanoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C22:0 docosanoic/behenic	AOAC 996.06	0.01	0.15	0.2	0.19	0.15	0.14
C23:0 tricosanoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C24:0 lignoceric	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C14:1-9c cis-9-tetradecenoic/myristoleic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C10:0 decanoic/ capric	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C15:1-10c 10-pentadecenoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C16:1-9c cis-9-hexadecenoic/palmitoleic	AOAC 996.06	0.01	0.22	0.23	0.23	0.23	0.23
C17:1-10c 10-heptadecenoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C18:1-9c cis-9-octadecenoic/ oleic	AOAC 996.06	0.01	0.28	2.37	1.15	1.11	1.12
C20:1-11c cis-11-eicosenoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C22:1-13c cis-13-docosenoic/ erucic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C24:1-15c cis-15-tetracosenoic/nervonic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C18:2 -9c,12c cis-9,12-octadecadienoic/linoleic	AOAC 996.06	0.01	0.04	3.69	1.57	1.99	2.01
C18:3 (GLA)-6c,9c,12c cis-6,9,12-octadecatrienoic/r-linolenic	AOAC 996.06	0.01	0.1	0.09	0.1	0.09	0.09
C18:3 (ALA)-9c,12c,15c cis-9,12,15-octadecatrienoic/ linolenic	AOAC 996.06	0.01	0.16	0.19	0.18	0.18	0.18
C20:2-11c,14c cis-11,14-eicosadienoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]

Table 7. Fatty Acid Content of the Algal Oil from *S. limacinum* TKD-1[1]

Fatty Acid	Method	LOQ (g/100 g)	Batch Number[2]				
			DD20191206	DD20191213	DD20191220	DD20200110	DD20200101
C20:3-8c,11c,14c cis-8,11,14-eicosatrienoic (dihomo-gamma-linolenic)	AOAC 996.06	0.01	0.26	0.23	0.25	0.23	0.24
C20:4-5c,8c,11c,14c cis-5,8,11,14-eicosatetraenoic (arachidonic)	AOAC 996.06	0.01	0.14	0.14	0.15	0.15	0.15
C20:3-11c,14c,17c cis-11,14,17-eicosatrienoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C20:5-5c,8c,11c,14c,17c cis-5,8,11,14,17-eicosapentaenoic (EPA)	AOAC 996.06	0.01	0.47	0.45	0.48	0.54	0.55
C22:2-13c,16c cis-13,16-docosadienoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C22:4-7c,10c,13c,16c cis-7,10,13,16-docosatetraenoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C22:3-13c,16c,19c cis-13,16,19-docosatrienoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C22:5-4c,7c,10c,13c,16c cis-4,7,10,13,16-docosapentaenoic	AOAC 996.06	0.01	14.51	13.11	14.22	12.98	13.15
C22:5-7c,10c,13c,16c,19c cis-7,10,13,16,19-docosapentaenoic (DPA)	AOAC 996.06	0.01	0.13	0.14	0.15	0.14	0.15
C22:6 -4c,7c,10c,13c,16c,19c cis-4,7,10,13,16,19-docosahexaenoic (DHA)	AOAC 996.06	0.01	56.44	53.34	56.07	59.59	61.16
C14:1-9t trans-9-tetradecenoic/trans-myristelaidic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C16:1-9t trans-9-hexadecenoic/Trans-palmitelaidic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C18:1t trans-elaidic	AOAC 996.06	0.01	0.02	0.03	0.02	ND[2]	ND[2]
C18:2t trans linolelaidic	AOAC 996.06	0.01	0.03	0.07	0.05	0.05	0.04
C20:1-11t trans-11-eicosenoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
C20:0 eicosanoic/arachidic	AOAC 996.06	0.01	0.21	0.24	0.23	0.12	0.23
C11:0 henedecanoic	AOAC 996.06	0.01	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
Fatty Acid by Type							
Saturated fat	AOAC 996.06	0.01	20.49	20.4	20.58	18.49	18.54
Unsaturated fat	AOAC 996.06	0.01	72.76	73.98	74.55	77.14	79.03
Mono-unsaturated fat	AOAC 996.06	0.01	0.5	2.6	1.38	1.34	1.35
Multi-unsaturated fat	AOAC 996.06	0.01	72.26	71.38	73.17	75.08	77.68
Trans fat	AOAC 996.06	0.01	0.05	0.1	0.07	0.05	0.04
n-3 fat	AOAC 996.06	0.01	57.2	54.12	56.88	60.45	62.04
n-6 fat	AOAC 996.06	0.01	15.05	17.26	16.29	15.35	15.64

Table 7. Fatty Acid Content of the Algal Oil from *S. limacinum* TKD-1[1]

Fatty Acid	Method	LOQ (g/100 g)	Batch Number[2]				
			DD20191206	DD20191213	DD20191220	DD20200110	DD20200101
n-9 fat	AOAC 996.06	0.01	0.28	2.37	1.15	1.11	1.12
Free Fatty Acids	AOCS Ca 5a-40	0.01	0.09	0.09	0.09	0.09	0.09

Abbreviations: LOQ – limit of quantitation; AOCS - American Oil Chemists Society; ND – not detected
[1]Determined by SGS-CSTC Standards Technical Services Co., Ltd.
[2]Denotes less than the limit of quantitation.

c. Sterols

The sterol levels in five batches of the Algal Oil from *S. limacinum* TKD-1 were determined using the compendial gas chromatographic method NMKL 198:2014 by Eurofins, which is an ISO/IEX 17025: 2005-accredited lab (Table 8). All batches contained quantifiable sterols and the levels did not vary by more than an order of magnitude between the five batches. Total sterol levels in the five batches ranged from 260 to 444 mg/100 g oil, which remains within the natural variation commonly observed in different types of oils and is similar to the sterol content of other DHA-rich oils derived from *Schizochytrium* (Yang et al., 2019; EFSA Panel on Nutrition et al., 2020).

Table 8. Sterol Composition of the Algal Oil from *S. limacinum* TKD-1[1]

Sterol	Method	LOD	LOQ	Batch Number[2]									
				DD20191206		DD20191213		DD20191220		DD20200110		DD20200101	
				mg/ 100 g	wt/wt %[3]	mg/ 100 g	wt/wt %	mg/ 100 g	wt/wt %	mg/ 100 g	wt/wt %	mg/ 100 g	wt/wt %
Brassicasterol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	12	0.012	7	0.007	10	0.01	10	0.01	7	0.007
Cholesterol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	284	0.284	163	0.163	250	0.25	220	0.22	160	0.16
Campesterol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	1	0.001	4	0.004	2	0.002	3	0.003	11	0.011
Campestanol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	3	0.003	1	0.001	2	0.002	ND[4]	NA	ND[4]	NA
Stigmasterol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	22	0.022	13	0.013	19	0.019	16	0.016	16	0.016
Sitosterol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	52	0.052	38	0.038	47	0.047	34	0.034	74	0.074
Sitostanol+delta -5-avenasterol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	18	0.018	5	0.005	14	0.014	6	0.006	6	0.006
Delta-5, 24-stigmastadienol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	15	0.015	4	0.004	12	0.012	8	0.008	6	0.006
Delta-7-Stigmastenol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	32	0.032	23	0.023	29	0.029	28	0.028	31	0.031
Delta-7-Avenasterol	NMKL 198:2014	0.3 mg/100 g	1 mg/100 g	5	0.005	2	0.002	4	0.004	3	0.003	5	0.005
			Total (mg /100 g)[3]	444	-	260	-	389	-	328	-	316	-

Abbreviations: LOQ – limit of quantitation; NA – not applicable; ND – not detected.
[1]Determined by Eurofins Technology Service (Suzhou) Co., Ltd.
[2]w/w%: percentage sterol per weight of DHA-rich oil. Determined by dividing the mg/100 g values by 1000 to change units to g/100 g (%).
[3]Equals the sum of sterols quantified in each batch of Algal Oil from *S. limacinum* TKD-1.
[4]Denotes that value is less than the LOQ.

d. Glycidyl, 2-Monochloro-propanol-1,2-diol, and 3-Monochloro-propanol-1,2-diol Fatty Acid Esters

Esters of 2-monochloro-propanol-1,2-diol (2-MCPD) and 3-monochloro-propanol-1,2-diol (3-MCPD) and glycidol are contaminants of processed vegetable oils that are formed during oil refining when the oils are treated with hydrochloric acid at high temperatures and pressure. Although TK Biohealth does not used hydrochloric acid in the manufacturing process, glycidyl, 2-MCPD, and 3-MCPD fatty acid esters were quantified in five batches of Algal Oil from *S. limacinum* TKD-1 for due diligence purposes by SGS-CSTC Standards Technical Services Co., Ltd, which is an ISO/IEX 17025: 2005-accredited lab (see Annex 2 – Accred-Tela for certificate of accreditation) using a gas chromatography-mass spectrometry-based compendial method (Table 9). Glycidyl and 2-MCPD fatty acid esters were below the limit of quantitation in all five batches. 3-Monochloro-propanol-1,2-diol fatty acid esters were detected in all batches with mean concentration of 0.23 +/- 0.03 mg/kg and a maximum of 0.28 mg/kg. Although there are no limits for glycidyl, 2-MCPD, and 3-MCPD fatty acid esters in the United States, glycidyl and 3-MCPD fatty acid esters must not exceed the maximum levels of 6 and 15 μg/kg, respectively, for liquid infant and follow-on formula in the European Union (Commission Regulation (EU) 2018/290; EFSA Panel on Nutrition, 2020). There is no limit for 2-MCPD.

Per 21 CFR 107.100, infant formulas must contain a maximum of 6.0 g fat/100 kcal. Assuming that term infant formulas contain approximately 670 kcal/L (Martinez and Ballew, 2011), the target DHA concentration in term infant formulas is 0.5 % total fat, and Algal Oil from *S. limacinum* TKD-1contains not less than 35% DHA and a maximum amount of 0.28 mg/kg 3-MCPD fatty acid esters, the resulting amount of 3-MCPD fatty acid esters in infant formula will be approximately 0.15 μg/kg formula, which is orders of magnitude below the maximum level established in the European Union. Additionally, if glycidyl fatty acid esters were present at the limit of quantitation for the assay, the resulting level in infant formula will be 0.057 μg/kg formula, which is also orders of magnitude below the limit established in the European Union.

Table 9. Glycidyl, 2-Monochloro-propanol-1,2-diol (2-MCPD) and 3-Monochloro-propanol-1,2-diol (3-MCPD) Fatty Acid Esters in Algal Oil from *S. limacinum* TKD-1[1]

Toxin	Method	LOQ	Batch Number[2]				
			DD20191206	DD20191213	DD20191220	DD20200101	DD20200110
Sum of Free 3-MCPD, 3-MCPD-ester (determined as free 3-MCPD)	AOCS Official Method cd 29b-13 GC-MS	0.1 mg/kg	0.20	0.23	0.28	0.2	0.23
Sum of Free 2-MCPD, 2-MCPD-ester (determined as free 3-MCPD)	AOCS Official Method cd 29b-13 GC-MS	0.1 mg/kg	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]
Sum of Free glycidol, glycidol-ester (determined as free glycidol)	AOCS Official Method cd 29b-13 GC-MS	0.1 mg/kg	ND[2]	ND[2]	ND[2]	ND[2]	ND[2]

Abbreviations: LOQ: Limit of Quantitation; GC-MS – gas chromatography with mass spectroscopy; ND – not detected
[1]Determined by SGS-CSTC Standards Technical Services Co., Ltd.
[2]ND denotes that value is less than the LOQ.

3. Stability

The stability the Algal Oil from *S. limacinum* TKD-1 has been evaluated at -18, 4, and 25 ± 2°C by quantifying the amount of DHA, peroxide and p-anisidine values over the course of 24, 18, and 4 months, respectively. All batches used in these studies were blanketed with nitrogen and stored in sealed aluminum containers, which are the same containers used to store the finished ingredient. At -18°C, the DHA content, peroxide and p-anisidine values of the two batches were relatively constant over the course of 24 months, ranging from 55.3 to 56.5%, <1, and 3.3 to 5.1, respectively (Table 10). Similar results were observed in the two batches stored at 4°C over the course of 18 months (Table 11). At 25 ± 2°C, DHA content was relatively stable, ranging from 56.3 to 55.8, 53.4 to 54.1, and 56.1 to 55.6% in the three batches, respectively, whereas the peroxide values increased from <1 to <2 after two months of storage (Table 12). The p-anisidine levels also increased after processing (Table 12). Importantly, the DHA content and peroxide values at all time points and in all batches tested comply with the product specifications. Additionally, although p-anisidine is not a specification, the highest levels found in Algal Oil from *S. limacinum* TKD-1 batches fall below the p-anisidine limits for *Schizochytrium* oil established by the United States Pharmacopoeia (Ismail et al., 2016). Therefore, based on these data, the proposed shelf-life of the subject of this GRAS Determination is 1 year at 4-5°C and 2 years at -18°C.

Table 10. Stability of the Algal Oil from *S. limacinum* TKD-1 at -18°C

Batch Number	Parameter	Specification	Time (months)				
			0	6	12	18	24
DD20180313	DHA (%)	≥ 35 %	55.3	56.5	54.9	55.6	56.3
	Peroxide Value	≤ 5.0 meq/kg	<1	<1	<1	<1	<1
	P-Anisidine Value	NS	4.8	4.5	5.1	4.8	3.4
DD20181128	DHA (%)	≥ 35 %	56.0	55.6	55.8	54.7	56.2
	Peroxide Value	≤ 5.0 meq/kg	<1	<1	<1	<1	<1
	P-Anisidine Value	NS	3.3	4.8	4.1	4.5	4.2
Average	DHA (%)	NA	55.7	56.1	55.4	55.2	56.3
	Peroxide Value	NA	<1	<1	<1	<1	<1
	P-Anisidine Value	NA	4.1	4.7	4.6	4.7	3.8

Abbreviations: NS – not specified; NA – not applicable

Table 11. Stability of the Algal Oil from *S. limacinum* TKD-1 at 4°C

Batch Number	Parameter	Specification[1]	Time (months)			
			0	6	12	18
DD20180313	DHA (%)	≥ 35 %	55.3	56.1	55.4	55.3
	Peroxide Value	≤ 5.0 meq/kg	<1	<1	<1	<1
	P-Anisidine Value	NS	4.8	4.2	5.2	3.8
DD20181128	DHA (%)	≥ 35 %	56.0	54.5	55.3	55.6
	Peroxide Value	≤ 5.0 meq/kg	<1	<1	<1	<1
	P-Anisidine Value	NS	3.3	2.8	4.1	3.5
Average	DHA (%)	NA	55.7	55.3	55.4	55.5
	Peroxide Value	NA	<1	<1	<1	<1
	P-Anisidine Value	NA	4.1	3.5	4.7	3.7

Abbreviations: NS – not specified; NA – not applicable

Table 12. Stability of the Algal Oil from *S. limacinum* TKD-1 at 25 ± 2°C

Batch Number	Parameter	Specification[1]	Time (months)				
			0	1	2	3	4
DD20191206	DHA (%)	≥ 35 %	56.3	56.8	55.9	55.3	55.8
	Peroxide Value	≤ 5.0 meq/kg	<1	<1	<1	<2	<2
	P-Anisidine Value	NS	3.4	3.8	4.3	5	5.9
DD20191213	DHA (%)	≥ 35 %	53.4	54.9	53.3	55	54.1
	Peroxide Value	≤ 5.0 meq/kg	<1	<1	<1	<2	<2
	P-Anisidine Value	NS	8.4	7.2	8.3	9.3	9.8
DD20191220	DHA (%)	≥ 35 %	56.1	54.8	56.4	55.8	55.6
	Peroxide Value	≤ 5.0 meq/kg	<1	<1	<1	<2	<2
	P-Anisidine Value	NS	6.3	7.8	7.3	8.5	8.4
Average +/- Standard Deviation	DHA (%)	NA	55.3 ± 1.6	55.5 ± 1.1	55.2 ± 1.6	55.4 ± 0.4	55.2 ± 0.9
	Peroxide Value	NA	<1	<1	<1	<2	<2
	P-Anisidine Value	NA	6.0 ± 2.5	6.3 ± 2.2	6.6 ± 2.1	7.6 ± 2.3	8.0 ± 2.0

Abbreviations: NS – not specified; NA – not applicable

III. DIETARY EXPOSURE

The subject of this GRAS Notice is isolated from a strain of *S. limacinum*, which is a related species to those that are used to manufacture the DHA-rich oils that are the subjects of GRNs 137, 553, 677, 776 and 843. The subject of this GRAS Notice is also intended to be used as a substitute for other DHA-rich oils that are GRAS for use in exempt and non-exempt infant formulas for preterm and term infants, and conventional foods. Although the strains are not identical, the product specifications for the *S. limacinum* TKD-1-derived oil are similar to those for the DHA-rich oils that are the subjects of GRNs 137, 553, 677, 776, and 843. Therefore, the subject of this GRAS Notice is equivalent to the DHA-rich oils that are GRAS for use in exempt and non-exempt infant formulas for preterm and term infants, and conventional foods, and the resulting dietary exposures to this product will be the same as the dietary exposures for the subjects of GRNs 137, 553, 677, 776 and 843, which are incorporated by reference and summarized below for convenience.

A. INTENDED EFFECT

Algal Oil from *S. limacinum* TKD-1 is intended to be used as a source of DHA, which is a naturally occurring long chain polyunsaturated fatty acid (PUFA) present in human milk known to play a role in infant development, especially brain and retinal development (Duttaroy et al., 2016). In 2007, Brenna et al. conducted a meta-analysis of the ARA and DHA concentrations in mature human milk reported in 65 published studies spanning 1986 to 2006 and involving 2,474 women worldwide. The mean and standard deviation of DHA concentration as a percentage of total fatty acids was 0.32 ± 0.22% (range: 0.06-1.4%), with the highest concentrations occurring in coastal regions, possibly due to the ingestion of marine-rich diets. Importantly, Brenna et al. (2007) revealed that DHA levels in human milk range widely across the world, providing some perspective on the range of infant exposures to DHA and a guide for the levels of DHA supplementation in infant formulas.

Based on scientific consensus and current knowledge regarding the importance of PUFAs in the infant diet and their presence in human milk, supplementation of infant formula with DHA together with ARA is recommended (Koletzko et al., 2014; Koletzko et al., 2020). For term and preterm infants, the recommended intakes of DHA and ARA per day are 18 to 60 mg/kg body weight and 18 to 45 mg/kg body weight, respectively, in a ratio of 1:1 to 1:2.

B. HISTORY OF USE

Docosahexanaeoic acid-rich polyunsaturated oils derived from *Schizochytrium* sp. in infant formula and general foods have a long history of safe use around the world. In the United States, *Schizochytrium* sp.-derived, DHA-rich oils have been GRAS for use in foods and/or infant formula since 2003 (GRN 137; GRN 553; GRN 677; GRN 731; GRN 732; GRN 776; GRN 777; GRN 836). Additionally, DHA-rich oils derived from other sources, such as *Crypthecodinium cohnii*, *Ulkenia* sp. SAM2179, *Chlorella protothecoides* strain S106, *Prototheca moriformis* strain S2532, tuna and other fish are GRAS for use in infant formula and general foods since 2002 (GRN 41; GRN 105; GRN 137; GRN 138; GRN 193; GRN 319;

GRN 384; GRN 469; GRN 527; GRN 553; GRN 677; GRN 731; GRN 732; GRN 776; GRN 777; GRN 836; GRN 843; GRN 844; GRN 862; GRN 913; GRN 933). Algal oils produced from *Schizochytrium* sp. have also been approved for direct use in foods by Health Canada, the United Kingdom, the European Union, the Food Standards Agency of Australia and New Zealand (FSANZ), China's Ministry of Health, and Brazil's National Health Surveillance Agency (ANVISA) since the early 2000s.

C. INTENDED USE

The intended use of the subject of this Notice is to be a substitute for other *Schizochytrium* sp.-derived, DHA-rich oils in cow's milk and soy-based exempt and non-exempt preterm and term infant formulas at a level that is consistent with the level of DHA in human milk, and the same food categories specified in GRN 137 (Table 13). The subject of this GRAS Notice will be used in infant formulas with a safe and suitable source of ARA.

Table 13. Maximum Intended Use Levels of Algal Oil from *S. limacinum* TKD-1

Category of Food	Maximum Intended Use Level (%)
Cookies, crackers	1.45
Breads, rolls	0.29
Fruit pies, custard pies	2.03
Cakes	2.9
Baked goods and baking mixes	1.45
Cereals	1.16
Fats and oils (not including infant formula)	5.8
Yogurt	1.16
Frozen dairy products	1.45
Condiments	1.45
Soup mixes	0.87
Snack foods	1.45
Nut Products	1.45
Gravies and sauces	1.45
Soy protein bars	1.45
Plant protein products	1.45
Processed vegetable drinks	0.29
Hard candy	2.9
Soft candy	1.16
Non-dairy and powdered cream substitutes	1.45
Jams and jellies	2.03
Milk-based meal replacements	0.29
Non-dairy milk, imitation and soy milk	0.3
Dairy product analogs	1.45
Nonalcoholic beverages	0.15
Pastas	0.58
Processed Fruit Juices	0.29
White granulated sugar	1.16
Sugar substitutes	2.9
Chewing gum	0.87
Gelatins and puddings	0.29
Confections and frostings	1.45
Sweet sauces, toppings, and syrups	1.45
As described in GRN 137, stamped pages 27-28.	

D. ESTIMATED DAILY INTAKE

1. Infant Formula

TK Biohealth estimated the intake of DHA from infant formula using the same rationale presented and discussed in GRN 776 (pg. 16), which is the same rationale presented in GRAS Notices GRN 553 and GRN 677. The estimated intake of DHA from its addition to exempt and non-exempt infant formulas for preterm and term infants is based on a target DHA concentration of 0.5% of total fat. Assuming infants consume approximately 100 to 120 kcal/kg body weight/day, of which fat comprises approximately 50%, an infant will consume approximately 5.6 to 6.7 g of fat/kg body weight/day (1 g fat = 9 kcal by convention), corresponding to 27 to 33 mg DHA/kg body weight/day at the target DHA concentration of 0.5% of total fat and approximately 43 to 62 mg Algal oil from *S. limacinum* TKD-1/kg body weight/day, assuming the oil contains 53 to 62 % DHA. Importantly, these DHA exposures are consistent with current recommendations for DHA consumption by term and preterm infants of 18 to 60 mg/kg bw/day (Koletzko et al., 2014).

2. General Foods

For general foods, the subject of this GRAS Notice will be added to the same food categories as those currently listed in 21 CFR 184.1472(a)(3) (menhaden oil) at the maximum use levels, with the exception of egg, meat, poultry, and fish products, and is intended to be the sole source of DHA in any given food category. Therefore, the proposed use levels of Algal Oil from *S. limacinum* TKD-1 are expected to result in a maximum dietary exposure of less than 1.5 grams of DHA/day.

IV. SELF-LIMITING LEVELS OF USE

This part does not apply.

V. COMMON USE IN FOOD BEFORE 1958

This part does not apply.

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

The GRAS status of the subject of this GRAS Notice is based on the following: the identity of the strain used to produce the subject of this GRAS Notice; the quantitative and qualitative equivalence of the subject of this GRAS Notice with the *Schizochytrium* sp.-derived oil that is the subject of GRN 677; the published toxicology studies conducted on the subject of GRN 677; the numerous toxicology and clinical studies showing that DHA-rich oils derived from other strains of *Schizochytrium* sp. are non-genotoxic, non-toxigenic, and well-tolerated in preterm and term infants, children, and adults; and the GRAS status of *Schizochytrium* sp.-derived oils for use in preterm and term exempt and non-exempt infant formulas and general foods. TK Biohealth therefore concludes that there is reasonable certainty of no harm to consumers of the subject of this GRAS Notice per the intended uses and use levels. Algal Oil from *S. limacinum* TKD-1 is therefore GRAS as an ingredient in preterm and term exempt and non-exempt infant formulas and general foods at the intended use levels.

A. EQUIVALENCE OF THE SUBJECT OF THIS NOTICE TO THE SUBJECT OF GRN 677

Eleven *Schizochytrium sp*.-derived, DHA-rich oils are currently GRAS for use in infant formulas and general foods in the United States. The safe use of all of these oils are supported by 15 GRAS Notices (Table 14; GRN 137; GRN 553; GRN 677; GRN 731; GRN 732; GRN 776; GRN 777; GRN 836; GRN 843; GRN 844; GRN 860; GRN 862; GRN 913; GRN 933; GRN 934). Some these GRAS Notices support the safe use of DHA-rich oils derived from the same strain of *Schizochytrium* sp., but for different uses. Specifically, GRNs 677 and 862 support the safe use of an oil derived from *Schizochytrium* sp. ONC-T18, GRNs 731 and 732 support the safe use of an oil derived from *Schizochytrium* sp. LU310, GRNs 776 and 843 support the safe use of *Schizochytrium* sp. FCC-1324, and GRNs 777 and 844 support the safe use of *Schizochytrium* sp. FCC-3204. Additionally, ten of the fifteen GRAS Notices rely on toxicology studies conducted with *Schizochytrium* sp. biomass or *Schizochytrium* sp.-derived oils manufactured from other strains that are GRAS for use in infant formula and general foods. Specifically, the subjects of GRNs 731, 732 and 836 bridge to all toxicology studies conducted with *Schizochytrium* sp. whereas the subjects of GRNs 776, 777, 834, 844, 860, 913, and 934 bridge to the toxicology studies that have been conducted with *Schizochytrium* sp. strain ONC-T18. The GRAS status of the subjects of GRNs 776, 777, 834, 844, 860, 913, and 934 are supported by all of the toxicology studies conducted with oils derived other strains *Schizochytrium* sp., including those used by Lewis et al. (2016) and Falk et al. (2017), which have not been the subjects of GRAS Notices.

Table 14. *Schizochytrium* sp. Strains and Toxicology Studies Used to Support the GRAS Status of *Schizochytrium* sp.-derived Oils

GRN	Oil Manufacturer	Source Strain Used to Manufacture the Subject of the GRAS Notice	Source Strain Used to Manufacture the Oil Used in the Supporting Toxicology Studies	Published Studies Cited in the GRAS Notice to Support the Safety of the Ingredient	Additional Toxicology Studies that Support the GRAS Status of the Ingredient
GRN 137	Martek	*Schizochytrium* sp. ATCC 20888	*Schizochytrium* sp. ATCC 20888	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b	None
GRN 553	DSM Nutritional Products	*Schizochytrium* sp. PTA-9695	*Schizochytrium* sp. PTA-9695	Fedorova-Dahms et al., 2014	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014
GRN 677	Mara Renewables Corporation	Schizochytrium sp. ONC-T18	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017
GRN 731	Linyi Youkang Biology Co, Ltd.	*Schizochytrium* sp. LU310	All *Schizochytrium* sp. strains	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al., 2014; Schmitt et al., 2012a; Schmitt et al., 2012b; Lewis et al., 2016; Falk et al., 2017	NA

Table 14. *Schizochytrium* sp. Strains and Toxicology Studies Used to Support the GRAS Status of *Schizochytrium* sp.-derived Oils

GRN	Oil Manufacturer	Source Strain Used to Manufacture the Subject of the GRAS Notice	Source Strain Used to Manufacture the Oil Used in the Supporting Toxicology Studies	Published Studies Cited in the GRAS Notice to Support the Safety of the Ingredient	Additional Toxicology Studies that Support the GRAS Status of the Ingredient
GRN 732	Linyi Youkang Biology Co, Ltd.	*Schizochytrium* sp. LU310	All *Schizochytrium* sp. strains	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al., 2014; Schmitt et al., 2012a; Schmitt et al., 2012b; Lewis et al., 2016; Falk et al., 2017	NA
GRN 776	Fermentalg	*Schizochytrium* sp. FCC-1324	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017
GRN 777	Fermentalg	*Schizochytrium* sp. FCC-3204	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017
GRN 836	Xiamen Huison Biotech Co., LTD	*Schizochytrium* sp. HS01	All *Schizochytrium* sp. strains	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al., 2014; Schmitt et al., 2012a; Schmitt et al., 2012b; Lewis et al., 2016; Falk et al., 2017	NA

Table 14. *Schizochytrium* sp. Strains and Toxicology Studies Used to Support the GRAS Status of *Schizochytrium* sp.-derived Oils

GRN	Oil Manufacturer	Source Strain Used to Manufacture the Subject of the GRAS Notice	Source Strain Used to Manufacture the Oil Used in the Supporting Toxicology Studies	Published Studies Cited in the GRAS Notice to Support the Safety of the Ingredient	Additional Toxicology Studies that Support the GRAS Status of the Ingredient
GRN 843	Fermentalg	*Schizochytrium* sp. FCC-1324	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017
GRN 844	Fermentalg	*Schizochytrium* sp. FCC-3204	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017
GRN 860 (Ceased)	Hubei Fuxing BioTechnology, Co., Ltd.	*Schizochytrium* sp. DHF	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017
GRN 862	BASF Corporation	*Schizochytrium* sp. ONC-T18	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017

Table 14. *Schizochytrium* sp. Strains and Toxicology Studies Used to Support the GRAS Status of *Schizochytrium* sp.-derived Oils

GRN	Oil Manufacturer	Source Strain Used to Manufacture the Subject of the GRAS Notice	Source Strain Used to Manufacture the Oil Used in the Supporting Toxicology Studies	Published Studies Cited in the GRAS Notice to Support the Safety of the Ingredient	Additional Toxicology Studies that Support the GRAS Status of the Ingredient
GRN 913	Mara Renewables Corporation	*Schizochytrium limacinum* G3	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017
GRN 933	Hubei Fuxing BioTechnology, Co., Ltd.	*Schizochytrium* sp. DHF	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017
GRN 934 (pending)	Cabio	*Schizochytrium* sp. CABIO-A-2	*Schizochytrium* sp. ONC-T18	Schmitt et al., 2012a; Schmitt et al., 2012b	Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Hammond et al., 2002; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al. 2014; Lewis et al., 2016; Falk et al., 2017

NA – not applicable

As summarized in Chapter 2, Section D.1.b, the subject of this GRAS Notice is most similar to *Schizochytrium* sp. ONC-T18 based on 18s rRNA sequence percent identity (approximately 87%). For reference, *Schizochytrium* sp. ONC-T18 is used to manufacture the subject of GRNs 677 and 862. Additionally, both the subject of this GRAS Notice and the subject of GRN 677 are manufactured using similar processes: the microalgae is expanded in a series of fermentation steps, hydrolyzed enzymatically, and centrifuged to obtain the crude algal oil; and the crude oil is then degummed, bleached, deodorized, and mixed with antioxidants generating the refined oil. The product specifications of the subject of this GRAS Notice and those described for the subject of GRN 677 are also comparable: they have the same specifications for the physical parameters (acid value, peroxide value, moisture, and unsaponifiable matter), DHA, arsenic, copper, and lead (Table 15); the limits for trans fatty acids, iron and mercury for the subject of this GRAS Notice are lower than those for the subject of GRN 677; and the subject of this GRAS Notice has additional specifications for free fatty acids, cadmium and microbiological parameters (total plate count, coliforms, molds and yeasts, and Salmonella). Thus, not only is *S. limacinum* TKD-1 genotypically similar to *Schizochytrium* sp. ONC-T18, but Algal Oil from *S. limacinum* TKD-1 meets the quality of the oil that is the subject of GRN 677.

Table 15. Specifications for *Schizochytrium* sp.-derived DHA-rich Oils that are GRAS for Use in Infant Formulas and General Foods

Parameter	Specifications	
	TK Biohealth	GRN 677
Physical Parameters		
Acid Value (mg KOH/g)	≤ 0.5	< 0.5
Peroxide Value (meq/kg)	≤ 5.0	< 5.0
Moisture (%)	≤ 0.05	<0.05
Unsaponifiable Matter (%)	≤ 3.5	< 3.5
Fatty Acids		
Docosahexaenoic Acid (DHA) (%)	≥ 35	> 35
Trans Fatty Acid (%)	≤ 1.0	< 2.0
Free Fatty Acids (%)	≤ 0.4	-
Heavy Metals		
Total Arsenic (ppm)	≤ 0.1	< 0.1
Cadmium (ppm)	< 0.2	-
Copper (ppm)	≤ 0.1	< 0.1
Iron (ppm)	≤ 0.1	< 0.2
Mercury (ppm)	≤ 0.04	< 0.1
Lead (ppm)	≤ 0.1	< 0.1
Microbiologicals		
Total Plate Count (CFU/g)	≤ 1000	-
Coliforms (CFU/g)	≤ 10	-
Molds and Yeasts (CFU/g)	≤ 25	-
Salmonella	Negative/375g	-
"-" denotes parameter not included in specifications		

Additionally, although the fatty acid and sterol profiles of the subject of this GRAS Notice are not identical to those of the subject of GRN 677 (Table 16 and 17), the amount of the fatty acids other than DHA and sterols will either approximate or be less than those from the subject of this GRN 677 in the marketed products. Specifically, the DHA content of the subject of this GRAS Notice is approximately 1.3-fold higher than the subject of GRN 677, and the percentage of the other fatty acids and sterols are similar to those in the subject of GRN 677. They also fall within the ranges reported for the *Schizochytrium sp*.-derived oils that are the subjects GRN 137 and 553 (Table 16 and 17). Moreover, the sterols present in the subject of this GRAS Notice, as well as the subjects of GRN 137, 553 and 677 are ubiquitous in food, commonly used sources of essential fatty acids in infant formula, including corn, palm, safflower, soybean, and sunflower oil, and do not pose safety concerns. Therefore, because infant formulas and general foods will be formulated to attain a specific level of DHA and the inclusion rate of the subject of this GRAS Notice in the infant formula and general foods will be less than the subject of GRN 677, the resulting exposure to the other fatty acids and sterols will be similar to those from the subject of GRN 677.

Taken together, the similarities in the genetic identity of the *Schizochytrium sp.* TKD-1 and *Schizochytrium sp.* ONC-T18, and the production processes, product specifications, and fatty acid and sterol levels of the oils derived therefrom indicate that the subject of this GRAS Notice as delivered will be quantitatively and qualitatively equivalent to the subject of GRN 677.

Table 16. Comparison of the Fatty Acid Profiles of *Schizochytrium*-derived, DHA-rich Oils That Are GRAS

Lipid Number	Common Name	Average Content (%)[1]			
		TK Biohealth (n=3)	GRN 677 (n=6)	GRN 553 (n=5)	GRN 137 (n=5)
12:0	Dodecanoic/Lauric Acid	0.06 ± 0.00	0.91 ± 0.12	0.06 ± 0.01	0.4 ± <0.1
14:0	Tetradecanoic/Myristic Acid	0.55 ± 0.01	11.87 ± 2.09	1.05 ± 0.13	10.11 ± 0.86
14:3n3	Tetradecatrienoate	-	-	-	tr-0.45
15:0	Pentadecanoic Acid	0.08 ± 0.00	0.52 ± 0.09	0.22 ± 0.017	-
15:1n10	10-Pentadecenoic Acid	ND	ND	ND	-
16:0	Hexadecanoic/Palmitic Acid	18.26 ± 0.15	25.43 ± 3.86	12.72 ± 0.64	23.68 ± 0.94
16:1n9	*cis*-9-Hexadecenoic/Palmitoleic Acid	0.23 ± 0.01	3.42 ± 1.60	ND	1.76 ± 0.99
16:3	Hexadecatrienoic Acid	-	ND	-	tr-5.0
17:0	Heptadecanoic/Margaric Acid	0.08 ± 0.01	0.13 ± 0.022	ND	-
18:0	Octadecanoic/Stearic Acid	1.06 ± 0.04	0.82 ± 0.036	1.51 ± 0.073	0.45 ± 0.05
18:1n7	Vaccenic Acid	-	-	0.28 ± 0.09	tr-13.6
18:1n9	*cis*-9-Octadecenoic/ Oleic Acid	1.27 ± 1.05	-	23.20 ± 2.04	-
18:1	*trans*-Elaidic Acid	0.02 ± 0.01	-	-	-
18:1n9 + 18:1n7	Oleic + Vaccenic Acid	-	4.77 ± 3.11	-	-
18:2n6	*cis*-9,12-Octadecadienoic/Linoleic Acid	1.77 ± 1.83	0.56 ± 0.22	1.80 ± 0.12	-
18:2	*trans*-Linolelaidic Acid	0.05	-	-	-
18:3	Linolenic Acid	-	0.23 ± 0.13	-	-
18:3n3	*cis*-9,12,15-Octadecatrienoic/α-Linolenic Acid	0.18 ± 0.02	-	0.06 ± 0.01	-
18:3n6	*cis* -6,9,12-Octadecatrienoic/γ-Linolenic Acid	0.10 ± 0.01	-	ND	-
18:4n3	Stearidonic Acid	-	0.25 ± 0.049	ND	tr-8.5
20:0	Eicosanoic/Arachidic Acid	0.23 ± 0.02	ND	0.30 ± 0.007	-
20:1	Eicosanoic Acid	-	ND	0.07 ± 0.008	-
20:2n6	*cis*-11,14-Eicosadienoic Acid	ND	ND	0.09 ± 0.011	-
20:3n3	*cis*-11,14,17-Eicosatrienoic Acid	ND	0.15	ND	2.21 ± 0.24
20:3n6	*cis*-8,11,14-Eicosatrienoic Acid	0.25 ± 0.02	-	ND	-
20:4n3	Eicosatetraenoic Acid	-	-	-	0.87 ± 0.04
20:4n6	*cis*-5,8,11,14-Eicosatetraenoic/Arachidonic Acid	0.14 ± 0.01	0.70 ± 0.061	0.62 ± 0.05	0.91 ± 0.17
20:5n3	*cis*-5,8,11,14,17-Eicosapentaenoic Acid (EPA)	0.47 ± 0.02	1.18 ± 0.30	5.63 ± 0.23	2.63 ± 0.64
22:0	Docosanoic/Behenic Acid	0.18 ± 0.03	ND	0.32 ± 0.022	-
22:2n6	*cis*-13,16-Docosadienoic Acid	ND	-	0.46 ±0.030	-
22:4n6	*cis*-7,10,13,16-Docosatetraenoic Acid	ND	ND	-	0.54 ± 0.13
22:5n6	*cis*-4,7,10,13,16-Docosapentaenoic Acid	13.95 ± 0.74	7.81 ± 0.40	2.29 ± 0.22	13.5 ± 1.5
22:5n3	*cis*-7,10,13,16,19-Docosapentaenoic Acid (DPA)	0.14 ± 0.01	-	0.67 ± 0.17	-

Table 16. Comparison of the Fatty Acid Profiles of *Schizochytrium*-derived, DHA-rich Oils That Are GRAS

Lipid Number	Common Name	Average Content (%)[1]			
		TK Biohealth (n=3)	GRN 677 (n=6)	GRN 553 (n=5)	GRN 137 (n=5)
22:6n3	*cis*-4,7,10,13,16,19-Docosahexaenoic Acid (DHA)	55.28 ± 1.69	40.22 ± 1.93	41.08 ± 2.32	35 ± 2.46
24:0	Lignoceric Acid	ND	ND	0.12 ± 0.011	-
24:1	*cis*-15-Tetracosenoic/Nervonic Acid	ND	0.41 ± 0.0	ND	-
Total fat		94.33 ± 0.96	99.38	92.54	92.06

Abbreviations: ND, not detected; "-" indicates that fatty acid was not measured; n = the number of batches
[1]Only fatty acids that were detected in at least one of the subjects of the GRAS Notices are shown.

Table 17. Comparison of the % Total Sterols of *Schizochytrium*-derived DHA-rich Oils That Are GRAS

Sterol	% Total Sterols			
	TK Biohealth	**GRN 677**	**GRN 553**	**GRN 137**
24-methylene cholesterol	-	4.25 ± 1.92	1.5 ± 0.42	-
Brassicasterol	2.65 ± 0.30	5.80 ± 1.04	1.28 ± 0.33	15 ± 3
Campestanol	0.52 ± 0.15	ND	0.1 ± 0.0	-
Campesterol	1.33 ± 1.30	2.32 ± 1.11	1.84 ± 0.27	-
Cholesterol and/or fucosterol	-	12.72 ± 5.26	1.6 ± 0.0	25 ± 3
Cholesterol	61.73 ± 6.40	23.03 ± 8.56	12.30 ± 1.81	-
Delta-5, 23-Stigmastadienol	-	5.52 ± 2.03	0.84 ± 0.089	-
Delta-5, 24-stigmastadienol	2.47 ± 0.77	5.68 ± 1.35	0.44 ± 0.05	-
Delta-5-Avenasterol	-	3.02 ± 1.98	1.78 ± 0.72	-
Delta-7-Avenasterol	1.08 ± 0.31	4.28 ± 2.86	0.90 ± 1.29	-
Delta-7-campesterol	-	5.70 ± 2.00	0.44 ± 0.089	-
Delta-7-Stigmastenol	8.37 ± 1.06	17.03 ± 7.99	1.92 ± 0.36	-
Ergosta-7,22-dien-3-ol	-	-	-	<5 - 7
Ergosta-7,24-dien-3-ol	-	-	-	<5 - 6
Sitostanol+delta-5-avenasterol	2.66 ± 1.08	0.5 ± 0.0	0.52 ± 0.045	-
Sitosterol	14.44 ± 5.25	12.37 ± 1.96	11.32 ±1.94	-
Stigmasterol	4.96 ± 0.08	16.28 ± 8.44	63.20 ± 2.37	19 ± 2
Unidentified sterols	-	-	-	8 ± 1
Total (mg/100 g)	347.40 ± 70.82	150.02 ± 64.01	536.0 ± 24.08	31

Abbreviations: ND, not detected
"-" denotes that the sterol was not quantified in the GRAS notice.

B. SAFETY

Because the identity of the *Schizochytrium sp*. TKD-1 and the composition of the resulting DHA-rich oil are quantitatively and qualitatively equivalent to *Schizochytrium* sp. ONC-T18 and oil that is the subject of GRN 677, respectively, the toxicology studies conducted on the subject of GRN 677 support the safe use of the subject of this GRAS Notice in exempt and non-exempt infant formulas for preterm and term infants, and general foods (Schmitt et al., 2012a; Schmitt et al., 2012b). Therefore, the extensive summaries of the toxicology studies conducted by Schmitt et al. (2012a) and Schmitt et al. (2012b) in GRN 677 are incorporated by reference. The safety of the subject of this GRAS Notice is also supported by the numerous published toxicology and clinical studies conducted on DHA-rich oils derived from other strains for *Schizochytrium* and *Schizochytrium* sp. biomass. These additional studies have also been extensively reviewed in other GRAS Notices and therefore, their summaries are also incorporated by reference.

Additionally, to obtain a current, thorough, and comprehensive understanding of publicly available studies that support the safe use of *Schizochytrium* sp. and *Schizochytrium* sp.-derived, DHA-rich oils in infant formula and general foods, literature searches were conducted using both PubMed and GoogleScholar to identify new toxicology and clinical studies that have been published since the filing date of the last GRAS Notice that received a "no questions" letter for a *Schizochytrium* sp.-derived, DHA-rich oil (GRN 933, March 20, 2020). The search terms "docosahexaenoic acid AND toxicity AND schizochytrium",

"docosahexaenoic acid AND subchronic AND schizochytrium", and "docosahexaenoic acid AND safety AND schizochytrium" were used to identify recently published toxicology studies conducted with *Schizochytrium* sp. and oils derived therefrom. The search term "docosahexaenoic acid" was used to identify newly published clinical studies conducted with *Schizochytrium* sp. and oils derived therefrom. Peer-reviewed studies that were published in English from January 1, 2020, which precedes the filing date of GRN 933, to March 2, 2021, conducted in toxicologically relevant model organisms, with *Schizochytrium* sp.- or *Schizochytrium* sp. oil-supplemented infant formulas or reported safety parameters such as anthropometric measures, clinical chemistry, hematology, and adverse events in the abstract were considered relevant. If new studies were identified, they were then retrieved and reviewed to determine if *Schizochytrium* sp. and/or oils derived therefrom were administered. Only the studies administering *Schizochytrium* sp. and/or oils derived therefrom were subsequently summarized. If no clinical studies administering *Schizochytrium* sp. and/or oils derived therefrom have been conducted, studies administering DHA-rich oils derived from other sources were considered relevant because DHA-rich oils are generally accepted to be equivalent.

1. Absorption, Distribution, Metabolism, and Excretion

DHA is mainly found in the form of triglycerides, although they also occur in phospholipids in breast milk, comprising of 0.32% of the total fatty acids (Martin et al., 1993; Brenna et al., 2007). Dietary triglycerides, which can contain DHA, undergo enzymatic hydrolysis in the upper intestine to free fatty acids and 2-monoglycerides, which are then integrated into bile acid micelles for diffusion into the interior of the intestinal epithelial cells for subsequent incorporation into new or reconstituted triglycerides (Kroes et al., 2003). These reconstructed triglycerides enter the lymph in the form of chylomicrons for transport to the blood, which allows distribution and incorporation into plasma lipids, erythrocyte membranes, platelets, and adipose tissue. The chylomicron-contained triglycerides are then hydrolyzed by lipoprotein lipase during passage through the capillaries of adipose tissue and the liver to release free fatty acids to the tissues for metabolism or for cellular uptake, with subsequent re-esterification into triglycerides and phospholipids for storage as energy or as structural components of cell membranes. Once inside cells, the fatty acids are transported across the mitochondrial membrane the form of acylcarnitine into the mitochondria where they metabolized predominantly via beta-oxidation, a process that involves a shortening of the fatty acid carbon chain and the production of acetic acid and acetyl CoA. Acetyl CoA then combines with oxaloacetic acid and enters the citric acid cycle for energy production. The degree of transport of fatty acids across the mitochondrial membrane is contingent upon the length of the carbon chain; fatty acids of 20 carbons or more are transported into the mitochondria to a lesser degree than shorter chain fatty acids. Therefore, long chain fatty acids, such as DHA, may not undergo mitochondrial beta-oxidation to the same extent (Kroes et al., 2003). Instead, they are preferentially channeled into the phospholipid pool where they are rapidly incorporated into the cell membranes of the developing brain, retina, and other tissues. These fatty acids may be

conditionally essential depending on the essential fatty acid availability. Importantly, it is widely accepted that the DHA-rich oils derived from *Schizochytrium* sp. and other sources, such as *C. cohnii*, are equivalent (Fedorova et al., 2014).

2. Toxicology

a. Genotoxicity Studies

Numerous genotoxicology studies have been conducted with *Schizochytrium* sp. biomass and DHA-rich oils derived from *Schizochytrium* sp. (Table 18). As first summarized on page 35 of GRN 677, the genotoxicity of the DHA-rich oil derived from *Schizochytrium* ONC-T18 was evaluated in OECD-compliant bacterial reverse mutation, *in vivo* rat bone marrow micronucleus, and chromosomal aberration assays (Schmitt et al., 2012a). The DHA-rich oil derived from *Schizochytrium* ONC-T18 was not mutagenic, clastogenic or aneugenic. As first summarized in GRN 137, 553, and 731, the battery of OECD-compliant genotoxicology studies conducted with *Schizochytrium* sp. biomass and DHA-rich oils derived from other strains of *Schizochytrium* sp. also show that biomass and oils derived from other *Schizochytrium* strains are also not mutagenic, clastogenic or aneugenic (Fedorova-Dahms et al., 2011a, Fedorova-Dahms et al., 2011b, Lewis et al., 2016, and Hammond et al., 2002). Additionally, since January 1, 2020, no new genotoxicology studies have been published. Thus, based on the weight of the evidence, there is general consensus that *Schizochytrium* sp.-derived, DHA-rich oils are not genotoxic or mutagenic and there is reasonable certainty that the subject of this GRAS Notice is also not genotoxic or mutagenic.

Table 18. Summary of Genotoxicology Studies Performed using DHA-rich Oils from *Schizochytrium*

Reference	Test Substance	Study Type	NOAEL of DHA-rich oil	Summarized in
Studies on Schizochytrium sp.-derived oils				
Fedorova-Dahms et al., 2011a	Algal Oil from *Schizochytrium* sp. (37% DHA and 16% EPA) Source strain: *Schizochytrium* sp. ATCC 20888	OECD-compliant bacterial reverse mutation assay	The DHA-containing oil was not mutagenic	GRN 553 (pages 34-49)
		OECD-compliant micronucleus assay in erythrocytes	The DHA-containing oil was not clastogenic or aneugenic	
		OECD-compliant chromosomal aberration assay in human lymphocytes	The DHA-containing oil was not clastogenic or aneugenic	
Fedorova-Dahms et al., 2011b	DHA-rich algal oil from *Schizochytrium* sp. 43% DHA and 8% EPA) Source strain: *Schizochytrium* sp. ATCC 20888	OECD-compliant bacterial reverse mutation assay	The DHA-containing oil was not mutagenic	GRN 553 (pages 34-49)
		OECD-compliant micronucleus assay in erythrocytes	The DHA-containing oil was not clastogenic or aneugenic	
		OECD-compliant chromosomal aberration assay in human lymphocytes	The DHA-containing oil was not clastogenic or aneugenic	
Schmitt et al., 2012a	DHA-rich algal oil (39-42% DHA) Source strain: *Schizochytrium* sp. ONC-T18	OECD-compliant bacterial reverse mutation assay	The DHA-containing oil was not mutagenic	GRN 677 (page 35)
		OECD-compliant in vivo rat bone marrow micronucleus assay	The DHA-containing oil was not clastogenic or aneugenic	
		OECD-compliant chromosomal aberration assay in human peripheral blood lymphocytes	The DHA-containing oil was not clastogenic or aneugenic	
Lewis et al., 2016	DHA-rich algal oil from *Schizochytrium* sp. (41.37% DHA) Source strain: Unknown	OECD-compliant bacterial reverse mutation assay	The DHA-containing oil was not mutagenic	GRN 731 (pages 28 and 29)
		OECD-compliant micronucleus assay in erythrocytes	The DHA-containing oil was not clastogenic or aneugenic	
		OECD-compliant chromosomal aberration assay	The DHA-containing oil was not clastogenic or aneugenic	

Table 18. Summary of Genotoxicology Studies Performed using DHA-rich Oils from *Schizochytrium*

Reference	Test Substance	Study Type	NOAEL of DHA-rich oil	Summarized in
Studies on Schizochytrium sp. Biomass				
Hammond et al., 2002	Homogenized *Schizochytrium* sp. Source strain: *Schizochytrium* sp. ATCC 20888	OECD-compliant Ames/Salmonella Reverse Mutation Assay	Homogenized *Schizochytrium* sp. was not mutagenic	GRN 137 (page 12)
		OECD-compliant CHO AS52/XPRT Gene Mutation Assay	Homogenized *Schizochytrium* sp. was not mutagenic	
	Intact *Schizochytrium* sp. Source strain: *Schizochytrium* sp. ATCC 20888	OECD-compliant In Vitro Mammalian Cytogenetic Test	*Schizochytrium* sp. not clastogenic or aneugenic	
		OECD-compliant Mouse Bone Marrow Micronucleus Assay	*Schizochytrium* sp. not clastogenic or aneugenic	

Abbreviations: DHA: docosahexaenoic acid; GRN: GRAS Notice; NOAEL: no observed adverse event level; ppm: parts per million; LD_{50}: 50% of the lethal dose; ARA: arachidonic acid.

b. Toxicology Studies

Numerous acute, subchronic, and developmental and reproductive toxicology studies have been conducted on *Schizochytrium* sp. biomass and DHA-rich oils-derived from *Schizochytrium* sp. (Table 19). As summarized on pages 33-35 of GRN 677, OECD-compliant acute, subchronic, and developmental toxicity studies have been conducted with an oil derived from *Schizochytrium* sp. ONC-T18 (Schmitt et al., 2012a; Schmitt et al., 2012b). The studies collectively show that the oil derived from *Schizochytrium* ONC-T18 has an acute oral LD_{50} of greater than 5000 mg/kg body weight (bw) in rats, a subchronic toxicity no observed adverse effect level (NOAEL) of 50,000 ppm (equivalent to 3,305 and 3,679 mg/kg/bw/day in male and female rats, respectively), a maternal toxicity and embryo/fetal development NOAEL of 2000 mg/kg/day, and a reproductive toxicity NOAEL of 50,000 ppm for F_0 male and female rats and F_1 male rats, and 25,000 ppm for F_1 female rats. Except for the reproductive toxicity NOAEL in F_1 female rats, which was established at 25,000 ppm based on higher mean body weight, body weight gain, and food consumption, all of the NOAELs established in these studies were at the highest dose tested.

As summarized in GRN 137, 553, and 731, additional published acute, subchronic, developmental, and reproductive toxicology studies have been conducted using DHA-rich oils and biomass derived from other strains *Schizochytrium* sp. (Table 19; Hammond et al., 2001a; Hammond et al., 2001b; Hammond et al., 2001c; Abril et al., 2003; Fedorova-Dahms et al., 2011a; Fedorova-Dahms et al., 2011b; Fedorova-Dahms et al., 2014; Lewis et al., 2016; Falk et al., 2017). Consistent with the results obtained in the toxicology studies conducted with the oil derived from *Schizochytrium* sp. ONC-T18, no toxicologically significant treatment-related effects were reported for the DHA-rich oils and biomass derived from other strains *Schizochytrium* sp. and all NOAELs are equivalent to, if not greater than, those established for the oil derived from *Schizochytrium* sp. ONC-T18. Additionally, since January 1, 2020 no new toxicology studies on *Schizochytrium* sp.-derived, DHA-rich oils or biomass have been published.

Taken together, all of the publicly available toxicology studies show that DHA-rich oils derived from *Schizochytrium* sp. are not toxigenic. Therefore, there is a general consensus and reasonable certainty that the subject of this GRAS Notice is also not toxigenic for its intended uses.

Table 19. Summary of Animal Toxicology Studies Performed using *Schizochytrium*

Reference	Species	Dose	DHA%	Study Type	NOAEL of DHA-rich oil	Summarized in
Studies on Schizochytrium sp.-derived oils						
Fedorova-Dahms et al., 2011a	Male and female Sprague-Dawley rats	0.5% (312 mg/kg/day), 1.5% (965 mg/kg/day), 5% (3246 mg/kg/day) Source strain: *Schizochytrium* sp. ATCC 20888	37% DHA	90-day subchronic toxicity Study	• 5% of the diet (equivalent to 3149 mg/kg/day for males and 3343 mg/kg/day for females)	GRN 553 (pages 34-49)
Fedorova-Dahms et al., 2011b	Male and female Sprague-Dawley rats Source strain: *Schizochytrium* sp. ATCC 20888	0.5% (5000 ppm), 1.5% (15000 ppm), 5% (50000 ppm) Source strain: *Schizochytrium* sp. ATCC 20888	42.6% DHA	90-day subchronic toxicity study with 28-day in utero exposure, and a 30-day recovery	• 5% of the diet (equivalent to 4122 mg/kg/day for males and 4399 mg/kg/day for females)	GRN 553 (pages 34-49)
Schmitt et al., 2012a	Male and female Sprague-Dawley rats	Control (tuna oil): 50000 ppm Algal Oil: 0, 10000, 25000, 50000 ppm Source strain: *Schizochytrium* sp. ONC-T18	39-42% DHA	OECD-compliant acute toxicity	• Not applicable, LD_{50} was greater than 5000 mg/kg.	GRN 677 (pages 33-35)
				OECD-compliant subchronic toxicity study with 28-day recovery period	• 50000 ppm (equivalent to 3305 mg/kg/day for males and 3679 mg/kg/day for females)	
Schmitt et al., 2012b	Male and female rats (Sprague-Dawley)	DHA fish oil: 0, 50000 ppm	26 – 27% DHA	OECD-compliant 90-day subchronic toxicity study with 28 day in utero exposure	• F_0 male and females: 50000 ppm • F_1 males: 50000 ppm (equivalent to 3421and 2339 mg/kg/day for F_0 males, premating and after mating, respectively; 3558, 3117 and 7464 mg/kg/day for F_0 females during pre-mating, gestation and lactation, respectively and 3526 and 4138 mg/kg/day for F_1 males and females, respectively)	GRN 677 (pages 33-35)
		Algal oil: 10000 ppm, 25000 ppm, 50000 ppm Source strain: *Schizochytrium* sp. ONC-T18	42% DHA			

Table 19. Summary of Animal Toxicology Studies Performed using *Schizochytrium*

Reference	Species	Dose	DHA%	Study Type	NOAEL of DHA-rich oil	Summarized in
					• F_1 females: 25000 ppm (higher body weight and food consumption, intake on mg/kg basis not reported)	
		Algal oil: 400, 1000, 2000 mg/kg/day Source strain: *Schizochytrium* sp. ONC-T18	42% DHA	OECD-compliant developmental toxicity study days 6 - 19 of gestation	• NOAEL for maternal and embryofetal toxicology of 2,000 mg/kg/day	
Fedorova-Dahms et al., 2014	Domestic Yorkshire Crossbred Piglets	0.32 % and 0.96% DHA as % of total fatty acids (dose volume of formula 500 ml/kg/day) Source strain: *Schizochytrium* sp. PTA-9695	41.5% DHA (in combination with ARA oil)	21-day repeat dose toxicity, oral (diet)	• Well-tolerated at up to 0.96% DHA (equivalent to 700 mg DHA/L).	GRN 553 (page 17-41)
Lewis et al., 2016	Female Wistar rats	5000 mg/kg/day Source strain: Unknown	41.37% DHA (in combination with ARA)	Acute toxicity	• Not applicable, LD_{50} was greater than 5000 mg/kg.	GRN 731 (page 31)
	Male and female Wistar rats	0 mg/kg/day, 1000 mg/kg/day, 2500 mg/kg/day, 5000 mg/kg/day Source strain: Unknown		28-day repeat dose toxicity	• Not applicable, no treatment related adverse effects at any dose.	
				90-day subchronic toxicity study with 28-day recovery period	• 5000 mg/kg/day	
Falk et al., 2017	Male and female Wistar rats	Vehicle control (corn oil), 1000 mg/kg/day, 2500 mg/kg/day, 5000 mg/kg/day Source strain: Unknown	41.37% DHA (in combination with ARA)	Developmental toxicity study days 6 - 20 of gestation	• 5000 mg/kg/day	GRN 731 (page 31)
				Reproductive toxicology study; administration through mating, pregnancy, nursing and lactation	• 5000 mg/kg/day	

Table 19. Summary of Animal Toxicology Studies Performed using *Schizochytrium*

Reference	Species	Dose	DHA%	Study Type	NOAEL of DHA-rich oil	Summarized in
Studies on Schizochytrium sp. Biomass						
Hammond et al., 2001a	Male and female Sprague Dawley rats	0, 400, 1500, 4000 mg/kg/day Source strain: *Schizochytrium* sp. ATCC 20888	Dried *Schizochytrium* sp. whole cell biomass (35% DHA)	90-day subchronic toxicity study	• 4000 mg/kg/day (of dried *Schizochytrium* sp. whole cell biomass)	GRN 137 (page 10 and 11)
Hammond et al., 2001b	Male and female Sprague Dawley rats	0.6, 6, 30% Source strain: *Schizochytrium* sp. ATCC 20888	Dried *Schizochytrium* sp. whole cell biomass (35% DHA)	Developmental toxicity	• 30% (equivalent to 22000 mg/kg dried *Schizochytrium* sp. whole cell biomass for maternal and developmental toxicity)	GRN 137 (page 11 and 12)
	Male and female New Zealand White rabbits	180, 600, 1800 Source strain: *Schizochytrium* sp. ATCC 20888		Developmental toxicity	• 600 mg/kg/day for maternal toxicity (reductions seen in food consumption and body weight) • 1800 mg/kg/day for developmental toxicity of dried *Schizochytrium* sp. whole cell biomass	
Hammond et al., 2001c	Male and female Sprague Dawley rats	0, 0.6, 6, 30% Source strain: *Schizochytrium* sp. ATCC 20888	Dried *Schizochytrium* sp. whole cell biomass (35% DHA)	One-generation reproductive toxicity	• 30% (equivalent to 17,800 and 20,700 mg/kg/day for F_0 males and females, respectively) of dried *Schizochytrium* sp. whole cell biomass	GRN 137 (page 12)
Abril et al., 2003	Mixed commercial breeds of piglets (Land Race & Large White)	1.10 % (throughout study; 114 mg DHA/kg bw/day) Finisher - 1.10 % (day 79-106); 0.39 % (day 107-120) (261 g DHA/pig) Finisher – 3.30% (day 79-106); 1.17 % (day 107-120)(756 g DHA/pig) Finisher – 5.51% (day 79-106); 1.94 % (day 107-120) (1281g g DHA/pig) Source strain: *Schizochytrium* sp. ATCC 20888	Dried *Schizochytrium* sp. whole cell biomass (22.3% DHA)	120-day repeat dose toxicity, oral (diet)	• Well-tolerated at up to 5.51% DHA	GRN 137 (page 15)

Abbreviations: DHA: docosahexaenoic acid; GRN: GRAS Notice; NOAEL: no observed adverse event level; ppm: parts per million; LD_{50}: 50% of the lethal dose; ARA: arachidonic acid.

C. CLINICAL STUDIES

1. Clinical Studies in Preterm Infants

Although no clinical studies conducted in preterm infants with infant formulas supplemented with DHA-rich oils derived from *Schizochytrium* sp. have been published, numerous studies have been conducted in preterm infants with infant formulas supplemented with DHA-rich oils derived from a variety of other sources, including fish, algae, and fungi (Table 20). Because DHA-rich oils are generally accepted to be equivalent, these studies are therefore relevant to understanding the tolerability of *Schizochytrium* sp.-derived oils in preterm infants. A majority of these studies are extensively summarized in GRNs 326, 553 and, 677, which support the safe use of DHA-rich oils derived from tuna and *Schizochytrium* sp. in preterm infant formulas (Koletzko et al., 1989; Ryan et al., 1999; O'Connor et al., 2001; Fewtrell et al., 2004; Clandinin et al., 2005; van de Lagemaat et al., 2011; Pittaluga et al., 2011; Sauerwald et al., 2012; Kitamura et al., 2016). Studies conducted with DHA-rich oil-supplemented infant formulas in preterm infants have also been summarized in GRN 326, which supports the safe use of arachidonic acid (ARA)-rich oils in infant formula. Because ARA-rich oils are used in combination with DHA-rich oils in infant formulas following the recommendations of Koletzko et al. (2020), these additional studies are also relevant to understanding the safe use of *Schizochytrium* sp.-derived oils in preterm infants (Fang et al., 2005; Groh-Wargo et al., 2005; Carnelli et al., 2007). Lastly, a recent literature search conducted using the strategy discussed in Chapter 6, Section B revealed an additional study involving preterm infants not previously reviewed in the GRNs for *Schizochytrium* sp.-derived oils, Alshweki et al. (2015). All of the summaries of these studies from the previous GRNs are incorporated by reference and are summarized, along with the study conducted by Alshweki et al. (2015), in tabular format below. Importantly, these studies show that the ingestion of infant formulas containing up to 0.6% DHA (approximately 42.8 mg/kg bw/day) by preterm infants for up to 2 years are generally well-tolerated and do not adversely affect growth. Therefore, adverse effects resulting from the ingestion of the subject of this GRAS Notice per the intended use level are not expected.

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Koletzko et al., 1989	Randomized, controlled study in premature infants with a birth weight of ≥1,300g	Infant formula (control); n=10 Infant formula supplemented with 0.1% DHA (approx. 6.7 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.2% ARA; n=8* Human milk; n=11 *DHA and ARA were obtained from fish oil. All subjects consumed the formulas from day 4 to 21 of life.	• The 3 groups of infants did not differ significantly for birthweight, gestational age and other clinical characteristics such as Apgar scores or weight gain during the study period. • All infants tolerated the feeds well, and no side effects of the DHA- and ARA-supplemented formula were noted.	GRN 379, pg. 17
Ryan et al., 1999	Randomized, blinded, controlled study in low-birth-weight infants weighting 750–2250 g	Preterm infant formulas • Control: Preterm infant formula; n=44 • Preterm infant formula w/ DHA and from fish oil (ratio of DHA to EPA of 5:1; 0.2% DHA (approx. 13.4 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n=46 Term infant formulas • Control: Term infant formula • Term infant formula w/ DHA and from fish oil (ratio of DHA to EPA of 2.8:1; 0.2% DHA (approx. 13.4 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]) Infants were fed the assigned preterm formula from 7–10 days prior to hospital discharge and for an additional 8 weeks (to 43 weeks premenstrual age (PMA) following hospital discharge. Then, from 43–59 weeks PMA, infants were fed the assigned term infant formula.	• Sixty-three infants completed the study (control, n=32; DHA, n=31). o There was a significant difference in the incidence of sudden infant death syndrome (SIDS) (control, n=0; DHA, n=4; $P < 0.05$) ▪ One SIDS death occurred one week after removal from the study following an episode of bronchiolitis ▪ The other three SIDS deaths were attributed to severe immaturity. ▪ A Safety Review Committee considered each death to be unrelated to study participation and/or the formula feeding. o Four infants (control, n=1; DHA, n=3) were removed from study participation because of reported formula intolerance (i.e., loose, watery stools, or diarrhea). o Fourteen infants (control, n=9; DHA, n=5) were withdrawn because of poor compliance with the study procedures and 4 infants exited early (control, 2; DHA, 2) because of an illness/condition that compromised study participation o The attending physicians reviewed the cases for the infants that were removed or withdrawn and reported that the illnesses and/or conditions were not associated with the formula • Males had significantly ($P < 0.05$) smaller gains in weight, length, and head circumference between study enrollment to 59 weeks PMA than those fed the control formula.	GRN 379, pg. 18-19

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
			• For all males, neither fat free mass (FFM) nor total body fat (TBF), when expressed as a percentage of total body weight, differed significantly between feeding groups. • Among females, there were no significant differences between the feeding groups in measures of growth, or body composition. • All formulas were well-tolerated and tolerance did not differ significantly between males and females.	
O'Connor et al., 2001	Randomized, masked, controlled trial in preterm infants with <33 weeks' gestational age and birth weights of 750 to 1805 g	<u>In-Hospital Infant Formulas</u> • Control: Preterm infant formula (control); n=144 • Preterm infant formula w/ 0.26% DHA (approx. 17.4 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.42% ARA of fatty acids derived from fish and a fungus (fish/fungal); n=140* • Preterm infant formula w/ 0.26% DHA (approx. 17.4 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.42% ARA of fatty acids derived from egg triglycerides and fish (egg-TG/fish); n=143 • Human milk; n=43 <u>Post-Discharge Preterm Infant Formulas</u> • Control: Preterm infant formula • Preterm infant formula w/ 0.16% DHA (approx. 10.7 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.41% ARA of fatty acids derived from fish and a fungus (fish/fungal)* • Preterm infant formula w/ 0.16% DHA (approx. 10.7 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]	• Three hundred seventy-six of the 470 infants enrolled completed the study to 12 months CA. o Of the 144 infants in the preterm infant formula group, 126 and 91 remained on study feeding at term and 12 months CA. o Of the 140 infants in the preterm infant formula fish/fungal group, 120 and 89 remained on study feeding at term and 12 months CA. o Of the 143 infants in the preterm infant formula egg-TG/fish group, 126 and 91 remained on study feeding at term and 12 months CA. o Nineteen (13%), 20 (14%), 11 (8%), and 1 (2%) of infants in the control, fish/fungal, egg-TG/fish, and human milk groups, respectively, discontinued study feeding because of symptoms typically associated with feeding intolerance. o Six, 3, 6, and 0 infants in the control, fish/fungal, egg-TG/fish, and human milk groups, respectively, died. o No infant deaths were related to study feedings as judged by the investigator at each site. o No statistically significant differences existed among the formula groups with respect to the exit outcomes. • There were no consistent differences in weight, length, head circumference, or anthropometric gains among the groups. • The percentage of infants who had at least one SAE did not differ among study formula groups. • The number of infants experiencing at least one hospital readmission was similar among the study formula groups.	GRN 379, pg. 18

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
		and 0.41% ARA of fatty acids derived from egg triglycerides and fish (egg-TG/fish) • Human milk *the source of DHA was not specified. After randomization, participants were fed human milk and/or the assigned in hospital preterm formula with or without AA- and DHA-enriched oils until term corrected age (CA). At term CA, infants were transitioned to an assigned postdischarge preterm formula with and without the same sources of AA and DHA and/or human milk to 12 months CA.	• The number of SAEs and hospital readmissions did not differ when comparisons among feeding groups were made within each birth weight stratum.	
Fewtrell et al., 2004	Randomized, controlled trial in preterm infants (<35 weeks, ≤2000 g birth weight)	• Unsupplemented infant formula (control); n=116 • Supplemented infant formula containing 0.5% DHA (approx. 33.5 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day), 0.04% ARA, and 0.1% EPA; n=122* *The source of DHA was tuna oil. Subjects consumed the formulas to 9 months after term.	• 25 infants in the unsupplemented formula group and nine infants in the supplemented formula group stopped using the trial formula by 9 months. o In two of the infants in the supplemented formula group, the change from the trial formula was temporary, lasting 4 and 5 weeks. o In 18 and all 7 infants in the unsupplemented and supplemented groups, respectively, the parents initiated stopping the trial formula. o In the remaining infants in the unsupplemented group, stopping the trial formula was initiated by either health advisor (n=3), family practitioner (n=1), or pediatrician (n=3). o Two infants were withdrawn from the study; one control infant with abdominal distension at the parents' request, and one in the supplemented group developed NEC and was withdrawn by the attending pediatrician. o One infant in the unsupplemented group died of bronchopulmonary dysplasia at 25 days of age. • The incidence of intraventricular hemorrhage, periventricular leukomalacia, patent ductus arteriosus requiring treatment,	GRN 379, pg. 19-21

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
			retinopathy of prematurity, and pulmonary hemorrhage did not differ between groups. • Although the infants fed the supplemented formula required ventilation and supplemental oxygen for significantly longer than did control infants and had umbilical catheters in situ for longer periods, suggesting they may have been sicker during the early neonatal period, there were no significant differences in the proportion of infants requiring ventilation or therapy with >30% oxygen. o In the majority of cases, these events preceded random assignment onto the trial formula: two infants (one in the unsupplemented group and one in the supplemented group) had an umbilical catheter in situ, and 8 infants (three infants in the unsupplemented group and five in the supplemented group) required supplemental oxygen at the time of randomization. o Six (5%) control infants and 10 (8%) supplemented infants required respiratory assistance (either ventilation, continuous positive airway pressure, or supplemental oxygen) at 36 weeks of gestation (p = .4). o The proportion of infants who had skin infections and suspected or proven systemic infections was similar in the two groups. • The infants receiving the supplemented formula showed significantly greater weight gain (difference, 310 g; 95% CI, 30 to 590 g; P = .03) and length gain (difference, 1.0 cm; 95% CI, 0.02 to 1.9; P = .05) between birth and 9 months.	
Clandinin et al., 2005	Prospective, randomized double-blind study in preterm infants with a post-menstrual age (PMA) of ≤ 35 weeks and that received <10 day of enteral feedings	• Control: Infant formula; n = 119 • Test group 1: Infant formula with 0.33% algal-DHA (approx. 22.1 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.67% ARA; n = 112* • Test group 2: Infant formula with 0.33% algal-DHA (approx. 22.1 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.67% ARA; n = 130	• Fifty-six infants (21 in the control, 17 in the algal-DHA, and 18 in the fish-DHA groups) discontinued before 40-week PMA. o The most common reasons for discontinuation were formula intolerance (n = 15), medical complications unrelated to the study (n = 13), and parental request (n = 11). There were no differences among groups in discontinuation rates or distribution of reasons for discontinuation. • Sixty preterm infants (15 control, 23 algal-DHA, 22 fish- DHA) completing the first phase were not enrolled in the second phase.	GRN 379, pg. 21 - 23

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
		• Reference Group: term infants breast-fed for ⩾ 4 months; n=105 *The source of the algal DHA oil was not specified. Each study group was provided with premature (24 kcal/ oz), discharge (22 kcal/oz), and term (20 kcal/oz) ready-to-use formulas, with the only differences being the polyunsaturated fatty acid profiles due to absence of DHA and ARA in control formulas and the sources of DHA in the supplemented formulas. The protocol recommended feeding premature formula ≥14 days until or near hospital discharge, discharge formula to 53 weeks PMA (3 months after term), and term formula to 92 weeks PMA (12 months after term). A follow up was conducted at 118 weeks PMA.	o The reasons were: <80% of enteral feedings during hospitalization or <100% at 40 weeks PMA from study formula (n = 27); birth weight >1500 g (n = 19); formula intolerance (n = 6); parent (n = 4) or physician (n = 3) elected withdrawal; and >7 consecutive days off study formula (n = 1). • Two hundred forty-five preterm infants (83 control, 72 algal-DHA, 90 fish-DHA) and 105 breast-fed term infants were enrolled in the second phase. o A total of 179 preterm (62 control, 52 algal-DHA, 65 fish-DHA) and 76 term infants completed the second phase. o Discontinuation rates did not differ among study groups. • Results showed that weight of the infant group given ARA together with DHA was significantly ($p<0.05$) greater than the control group from 66 to 118 weeks PMA, but did not differ from infants in the reference group at 118 weeks PMA. • Bayley mental (MDI) and psychomotor development (PDI) scores at 118 weeks PMA (18 months after term) were higher in infants given ARA/DHA supplemented formula compared to the control group. The MDI and PDI scores for the infants in the breast-fed term reference group were near the reference norm and significantly higher than the preterm groups. • Mean weight, length and head circumference and respective growth rates did not differ among the preterm groups. • Analysis of clinical data including severity of medical conditions relating to prematurity, serum chemistry and hematology found no safety issues related to the supplemented formulas. There were no increases in morbidity or adverse events in the groups given supplemented formulas relative to the control.	

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Fang et al., 2005	Double-blind, randomized, controlled study in preterm infants with a gestational age between 30 and 37 weeks	• Control: Infant formula; n=11 • Infant formula supplemented with 0.05% DHA (approx. 3.35 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.15% ARA; n=16* *The sources of DHA and ARA were not specified. Infants were to consume the formulas for 6 months.	• Twenty-eight infants were enrolled in the study. One infant was excluded later because of retinopathy of prematurity grade III found at 7 weeks. • At 1 year, one infant could not return to the hospital to complete the Bayley scale examination because of an outbreak of SARS. One infant was lost to follow-up and another infant could not complete the PDI examination because of neurodevelopmental delay. • At 6 months, the Bayley physical development index (PDI) examination could not be completed in two infants, because of an accidental fracture in one and a neurodevelopmental delay in another. • PDI and mental developmental index (MDI) scores were significantly higher in the group receiving the infant formula containing DHA and ARA. • There were no differences in visual acuity, physical examination variables or vital signs between these two groups. • No obvious adverse effects were observed during the study period.	GRN 326, pg. 61
Groh-Wargo et al., 2005	Controlled, double blind study conducted preterm infants with birth weights from 750 to 1800 g and gestational age at birth <33 weeks (n=60)	<u>In-Hospital Infant Formulas</u> • Control: Preterm infant formula; n=22 • Preterm infant formula w/0.26% DHA (approx. 17.4 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.42% ARA of fatty acids derived from fish and a fungus (fish/fungal); n=20* • Preterm infant formula w/0.26% DHA (approx. 17.4 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.42% ARA of fatty acids derived from egg triglycerides and fish (egg-TG/fish); n=18	• Of the 60 infants that were randomized to the different treatment groups, o One infant who received a diagnosis of a rare neurologic disorder and two infants who were breast-fed exclusively throughout the study were excluded from the analyses. o Sixteen infants dropped out of the study between study day 1 and the 12-mo CA visit. The reasons were: switching to a non-study formula per physician recommendation [control, n= 7; fish/fungal, n = 0; egg-TG/fish, n = 1], voluntary withdrawal by parent or investigator [control, n = 0; fish/fungal, n = 0; egg-TG/fish, n= 2], noncompliance with study visits [control, n = 0; fish/fungal, n = 3; egg-TG/fish, n = 2], and death unrelated to study participation [control, n = 0; fish/fungal, n = 1; egg-TG/fish, n = 0]	GRN 326, pg. 62

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils				
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**	**Summarized In**
		Post-Discharge Preterm Infant Formulas • Control: Preterm infant formula • Preterm infant formula w/ 0.16% DHA (approx. 10.7 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.41% ARA of fatty acids derived from fish and a fungus (fish/fungal) * • Preterm infant formula w/ 0.16% DHA (approx. 10.7 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1] and 0.41% ARA of fatty acids derived from egg triglycerides and fish (egg-TG/fish) *The source of DHA was not specified. After randomization, participants were fed human milk and/or the assigned in hospital preterm formula with or without AA- and DHA-enriched oils until term corrected age (CA). At term CA, infants were transitioned to an assigned post-discharge preterm formula with and without the same sources of AA and DHA and/or human milk to 12 months CA.	• No significant differences were seen among the three groups were seen at any time point in weight, length, or head circumference. • Bone mineral content and bone mineral density did not differ among groups. At 12 mo. Term corrected age (TCA) infants who were fed ARA/DHA-supplemented formulas had significantly greater lean body mass and significantly less fat mass than infants who were fed the non-supplemented control formula. • The ARA/DHA-supplemented formulas supported normal growth and bone mineralization in premature infants who were born at < 33 weeks gestation. No differences among the groups were seen in the percentage of infants with adverse clinical complications. • At 12 months TCA, preterm infants that were fed the ARA/DHA supplemented formula had increased lean body mass and significantly less fat mass by one year of age than infants fed non-supplemented formula. • The authors concluded supplementation of infant formula with ARA and DHA had a beneficial effect on growth and lean body mass.	

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Carnelli et al., 2007	Randomized, placebo-controlled study in preterm infants with gestational ages of approximately 31 weeks	• Control: Infant formula; n = 11 • Infant formula supplemented with fungus-derived ARA (0.84%) and fish oil-derived DHA (0.64%; approx. 42.8 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n = 11 Formulas were administered from birth to 7 months	• All infants grew normally during the trial (7 months) and no significant difference between groups was found in weight gain. • The authors did not report adverse events.	GRN 326, pg. 82
van de Lagemaat et al., 2011	Randomized, controlled trial in pre-term infants born at gestational ages of ≤ 32 and birth weights of ≤ 1500 g	• Control: human milk (n = 46) • Test group 1: Post-discharge infant formula (0.4% ARA, 0.4% DHA; approx. 26.8 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n = 52 • Test group 2: Term infant formula (0.2% ARA, 0.2% DHA; approx. 26.8 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1;] n = 41* *the source of DHA was not specified. Formulas were administered from birth to 6 months corrected age.	• There were no significant differences in weight, length, or head circumference between any of the groups. • The authors did not report adverse events.	GRN 553, pg. 50

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Pittaluga et al., 2011	Prospective study in preterm infants with a birth weight <1500 or gestational age <32 weeks	Hospital Formula Group • Term infant formula I (DHA content not available) • Modified cow's milk + energy supplement (DHA content not available) Postdischarge Formula Group • Preterm infant formula (0.15 – 0.25% DHA of total fatty acids; approx. 10.1 to 16.7 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]* • Term infant formula II (0.12 % DHA of total fatty acids; approx. 8.04 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]* *Source of DHA not provided. All preterm infants received preterm infant formula for up to 40 weeks corrected age or a body weight of 3.5 kg, whichever came first. Infants then continued on term infant formula up to 3 month corrected age and thereafter modified cow's milk up to 12 months corrected age.	• Five hundred and sixty preterm infants were recruited for the postdischarge formula group; 95 infants received a dual X-ray adsorptiometry scan and were evaluated in the current study. • Five hundred twenty-nine preterm infants were recruited for the hospital formula group; 87 infants received a dual X-ray adsorptiometry scan and were evaluated in the current study. • There were no between-group differences in morbidity in the first 2 years of life. • By the time of discharge from the neonatology unit, the infants in the postdischarge formula group weighed more and were longer than those in the hospital formula group. • Bone mineral density, content, and lean mass were not different in the two groups at 1 and 2 years. • Total fat mass and fast insulin were lower in the post-discharge formula groups in the 1st and 2nd year.	GRN 553, pg. 51

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Sauerwald et al., 2012	Randomized, double-blind in preterm infants with birth weights between 1000 and 2200 g	• Low DHA infant formula (0.04% DHA; approx. 2.7 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n=14* • Medium DHA infant formula (0.33% DHA; approx. 22.1 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n=13* • High DHA infant formula (0.52% DHA; approx. 34.8 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n=15* • Human milk (contained 0.38% DHA; approx. 25.4 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n=24 *The source of DHA was not specified. All infants received the study formulas until the postconceptional age of 48 weeks.	• Of the 66 infants that were enrolled: o Nine infants had to be excluded from the human milk group during the 1st 28 days because formula feeding exceeded 20% of their energy intake. o One formula-fed infant (Low DHA infant formula) was excluded during the 1st 28 days because they were switched to another formula due to diarrhea. o Four formula-fed infants were excluded because they met exclusion criteria. o Therefore, 52 infants remained in the protocol until day 28 (15 in the human milk group, 12 in the low DHA infant formula group, 12 in the medium DHA infant formula group, and 9 in the high infant formula group). • There were no indications of formula-related adverse events. • DHA content in the plasma phospholipids increased with the increase in DHA content in the formulas.	GRN 553, pg. 50

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Alshweki et al., 2015	Randomized, double-blind trial in preterm infants <1500 g and/or <32 weeks of gestational age	• Control: breast milk (n = 25) • Test group 1: Infant formula containing 2:1 ARA:DHA (0.62 – 0.72% ARA and 0.31 – 0.36% DHA; approx. 20.7 - 24.1 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n = 30* • Test group 2: Infant formula containing 1:1 ARA:DHA (0.30 – 0.37% ARA and 0.30 – 0.37% DHA; approx. 20.1 - 24.8 mg DHA/kg/day assuming that infants consume 6.7 g fat/kg bw/day)[1]; n = 30* *The source of DHA was not specified Formulas were administered from birth to 14 months corrected age.	• At 3 months, 28 infants remained in test group 1 and 26 infants remained in test group 3 o In test group 1, one infant was withdrawn due to intraventricular hemorrhage and one infant was lost to follow-up. o In test group 2, two infants were withdrawn due to intraventricular hemorrhages, one infant was lost to follow-up, and one infant died. • At 6 months, 24 infants remained in test group 1 and 21 infants remained in test group 3 o In test group 1, four infants switched to exclusive breast feeding. o In test group 2, five infants switched to exclusive breast feeding • There were no significant differences in the number withdrawals among the test groups. • There were no significant differences between to the two test groups in weight, length, or head circumference. • Psychomotor development scores were higher in the group receiving 2:1 ARA:DHA than the 1:1 ARA:DHA group, similar to the control. • ARA was significantly higher in the test group receiving 2:1 ARA:DHA than the test group receiving 1:1 ARA:DHA.	Current GRAS Notice

Table 20. Preterm Infant Clinical Studies with DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Kitamura et al., 2016	Randomized, double-blind trial in low or very low birth weight infants with body weight of >1000 g	• Control: Infant formula supplemented with 1 mg ARA and 9.1 mg DHA/100 ml (approx. 16.3 mg DHA/kg/day assuming that the formulas contain 67 kcal/100 ml and infants consume 120 kcal/kg bw/day)[1]; n = 16 • Test group: Infant formula with 4.6 mg ARA and 9.1 mg DHA/100 ml (approx. 16.3 mg DHA/kg/day assuming that the formulas contain 67 kcal/100 ml and infants consume 120 kcal/kg bw/day)[1]; n = 19 Intervention started at after discharge from intensive care unit and lasted for 1 month; breast milk feeding was prioritized during the test period, and the infant formula was given alone or in combination with breast milk when breast milk was insufficient.	• No subjects dropped out and all subjects completed the study • No serious adverse event or reaction due to the test formula, such as milk allergy, allergy-associated diarrhea, bloody stools, and anaphylaxis, was observed in any of the subjects. • No difference was found in body weight gain, height gain and head circumference gain development. • The ARA content in red blood cells was higher in the test group than the control.	GRN 677, pg. 30

Abbreviations: ARA: arachidonic acid; DHA: docosahexaenoic acid.
[1]See Chapter 3, Section D.1 for information relating to the intake calculations

2. Clinical Studies in Term Infants

Currently, two studies have been conducted in term infants with infant formulas supplemented with *Schizochytrium* sp. derived, DHA-rich oils, Chase et al. (2015) and Yeiser et al. (2016) (Table 21). Because both of these studies are extensively summarized on page 35 of GRNs 731 and page 25 of GRN 776, respectively, their summaries are incorporated by reference and briefly summarized in tabular format below. Additionally, no new studies were found using the search strategy discussed in Chapter 6, Section B. In the studies conducted by Chase et al. (2015) and Yeiser et al. (2016), term infants consumed *Schizochytrium* sp. oil-supplemented infant formulas containing up to 61.2 mg DHA/kg bw/day for up to 5 months. The oils were well-tolerated and, moreover, the amount of DHA consumed by the participants was within the range recommended by Koletzko et al. (2020). Therefore, adverse effects resulting from the ingestion of the subject of this GRAS Notice per the intended use level are not expected.

Table 21. Term Infant Clinical Studies in *Schizochytrium* sp.-Derived DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized in
Chase et al., 2015	Multicenter, two-arm, randomized, double-blind pilot trial in the first 5 months after birth (57 infants)	• Control group: 3.4 mg DHA/ounce infant formula (approx. 20.4 mg DHA/kg body weight (bw)/day, assuming that infant formulas contain 20 kcal/ounce and infants consume 120 kcal/kg bw/day) • Test group: 10.2 mg DHA/ounce infant formula (approx. 61.2 mg DHA/kg bw/day, assuming that infant formulas contain 20 kcal/ounce and infants consume 120 kcal/kg bw/day) • At 12 months, all infants received 400 mg DHA/day (derived from microalgae) or corn/soy oil until 36 months (approx. 40.9 mg DHA/kg bw/day, assuming that the average weight of an infant is 9.76 kg[1]).	• Infants that received DHA supplementation had a 20% increase in DHA levels in red blood cells. • The were no adverse events related to the study.	GRN 731 (pg. 35)
Yeiser et al., 2016	Multicenter, double blind, randomized controlled parallel trial for 106 days in healthy term infants	• Control group: cow milk-based formula with 17 mg DHA/100 kcal (DHA obtained from an *Crypthecodinium cohnii* oil; approx. 20.4 mg DHA/kg bw/day assuming infants consume 120 kcal/kg bw/day) • Test group: cow milk-based formula with 17 mg DHA/100 kcal (DHA obtained from *Schizochytrium* sp. oil; approx. 20.4 mg DHA/kg bw/day assuming infants consume 120 kcal/kg bw/day) • Both control and test formula included ARA, galactooligosaccharides, and a prebiotic blend of polydextrose	• No statistically significant group differences were detected for study discontinuation or discontinuation related to study formula • No significant differences in adverse events reported between the different groups. • Although serious adverse events were reported, the study site physicians determined them to be unrelated to the study formulas.	GRN 776 (pg. 25)

Abbreviations: GRN: GRAS Notice Number; ARA: arachidonic acid; DHA: docosahexaenoic acid.
[1]The average weight of an infant was determined by averaging the median weights of boys and girls 11.5 months-old (10.16 and 9.37 kg, respectively), which were obtained from the CDC growth tables (https://www.cdc.gov/growthcharts/clinical_charts.htm; accessed on 3-23-21).

1

3. Clinical Studies in Children and Adults

Six clinical studies, resulting in nine publications, have been conducted in children and adults with *Schizochytrium* sp.-derived oils, Sanders et al. (2006), Singhal et al. (2013); Maki et al. (2014), Keim et al. (2018), Ingol et al. (2019), Boone et al. (2019), Boone et al. (2020), Dams et al. (2020), and Marc et al. (2020). Tabular summaries of these studies are provided in Table 22. The studies conducted by Sanders et al. (2006), Singhal et al. (2013), and Maki et al. (2014) have been previously summarized in GRAS Notices for other *Schizochytrium* sp.-derived, DHA-rich oils and are incorporated by reference (Table 22). Since January 1, 2020, which precedes the filing date of the last GRAS Notice for a *Schizochytrium* sp. derived oil that received a "no questions" letter from FDA (GRN 933), three new studies have been published. Keim et al. (2018), Ingol et al. (2019), Boone et al. (2019), and Boone et al. (2020) reported developmental, growth and adiposity, sleep, and socioemotional outcomes from the Omega Tots study, respectively, whereas Dams et al. (2020) and Marc et al. (2020) conducted stand-alone studies. The amount of DHA consumed in these studies ranged from approximately 3.5 to 26 mg/kg body weight/day and none of the studies reported adverse events attributable to the ingestion of the *Schizochytrium* sp.-derived, DHA-containing oils. Collectively, these data indicate that the ingestion of *Schizochytrium* sp.-derived, DHA-containing oils and, moreover, the subject of this GRAS Notice, are well-tolerated in children and adults.

Table 22. Clinical Studies in Children and Adults with *Schizochytrium* sp.-Derived DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Sanders et al., 2006	Double-blind randomized placebo-controlled parallel-design trial in healthy men and women.	• Control: 4 g/day refined olive oil • Test group: 4 g/day refined DHA-rich triacylglycerol derived from *Schizochytrium* sp. (1.5 g DHA/day (approx. 23 mg DHA/kg bw/day assuming a body weight of 65 kg))	• Treatment was well tolerated and did not adversely affect cardiovascular risk • No significant differences in hematology, liver function tests, or self-reported adverse events.	GRN 836 (pg. 46)
Singhal et al., 2013	Double-blind, parallel group, placebo controlled randomized trial in healthy adults aged 18 – 37 years.	• Control: 4 g/day olive oil • Test group: 1.6 g DHA/day with 2.4 g/d carrier oil (approx. 24 mg DHA/kg bw/day assuming a body weight of 65 kg)	• DHA supplementation had no effect on endothelial function. • There were no serious adverse events in either group, both diets were well tolerated. No participant dropped out due to adverse effects of the study.	GRN 934 (pg. 54 and 55)
Maki et al., 2014	Double-blind, parallel trial in 93 healthy adults with hypertriglyceridemia.	• Control: 4 g Corn oil/soy oil/day with meals for 14 weeks • Test group 1: 2.5 g/day 2.7:1 ratio of marine algal derived DHA and EPA delivering 1.7 g DHA/day 0.6 g EPA/day (approx. 26 mg DHA/kg bw/day assuming a body weight of 65 kg) • Test group 2: 2 g/day 0.7:1 fish oil derived DHA and EPA delivering 0.82 g DHA/day and 1.16 g EPA/day (approx. 13 mg DHA/kg bw/day assuming a body weight of 65 kg)	• No significant differences in systolic and diastolic blood pressures, heart rate, or body weight changes. • No safety issues arose from routine screening of serum chemistry and hematology • The frequencies of any treatment-emergent adverse events were not significantly different among treatment groups. • Ingestion of algal-derived DHA and EPA lowered triacylglycerol levels to a similar degree as the fish oil derived product.	GRN 934 (pg. 54 and 55)

Table 22. Clinical Studies in Children and Adults with *Schizochytrium* sp.-Derived DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Keim et al., 2018 Boone et al., 2019 (sleep) Ingol et al., 2019 (growth and adiposity) Boone et al., 2020 (socioemotional outcomes)	Single-site, 180-day, randomized (1:1), double-blinded, placebo-controlled trial in toddlers aged 10-16 months born at less than 35 weeks gestation (Omega Tots trial) (n=377).	Either placebo or test article was provided as a powder to be dissolved in milk or food • Placebo control: 0.4 g corn oil (n=188) • Test group: 0.2 g DHA/day (from *Schizochytrium* sp.-algal oil; approx. 22.9 mg DHA/kg bw/day assuming that the average weight of the toddlers was 8.7 kg[1]) + 0.2 g ARA/day (from *Mortierella alpine* oil); n=189	• In the placebo-treated group, 16 subjects were lost to follow-up and one discontinued the intervention due to no interest in continuing the trial. • In the DHA+ARA-treated group, 14 subjects were lost to follow-up and five discontinued intervention due to lack of time and no interest in continuing the trial. • Two hundred and fifty-six subjects experienced at least one adverse event, totaling 683 adverse events (1.7/subject in test group, 2.0/subject in placebo group, not statistically significant). • The most common reported adverse events were minor gastrointestinal illness and respiratory tract infections. None were judged to be serious or test article-related. • The proportion of participants reporting adverse events was similar between groups (1.7 per child in the DHA+ARA group; 2.0 per child in the placebo group; difference = 20.37 [95% confidence interval (CI): 20.07 to 0.80]; P = .10). • Daily supplementation with ARA + DHA resulted in no improvement in cognitive development and early measures of executive function compared to placebo. • Subjects with lower birth weight in the ARA+DHA group had a small to medium negative effect on Bayley-III language scores compared to placebo • No statistically significant differences in sleep parameters were observed in between the placebo and test groups. A statistically significant improvement in sleep was observed in the test group compared to the	Current GRAS Notice

Table 22. Clinical Studies in Children and Adults with *Schizochytrium* sp.-Derived DHA-Rich Oils				
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**	**Summarized In**
			placebo for male toddlers or toddlers whose caregivers have depression. • Daily supplementation of DHA+ARA in toddlers born preterm resulted in no short-term differences in growth or adiposity compared to placebo. • Differences between DHA+ARA and placebo on Brief Infant-Toddler Social and Emotional Assessment scores were of small magnitude and not statistically significant. • Children in the DHA+ARA group had a decreased risk of scoring at-risk for Autism Spectrum Disorder on the Pervasive Developmental Disorders Screening Test-II, Stage 2 than the children in the placebo group.	

Table 22. Clinical Studies in Children and Adults with *Schizochytrium* sp.-Derived DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Dams et al., 2020	Randomized, controlled, open-labelled, parallel-grouped clinical trial in adults aged 20-65 years with low dietary intake of omega-3 fatty acids. Subjects were asked to adhere to a 4-week washout period prior to randomization to limit the effects of food supplements taken in their normal diet. Subjects were randomized to one of four groups and had 3 study visits: at baseline, 8 weeks and 16 weeks of supplementation.	• Control, habitual diet with no supplementation (n=15, 8 males, 7 females) • 2 capsules/day of *Schizochytrium* sp.-derived fatty acid blend (0.225 g DHA (approx. 3.5 mg DHA/kg bw/day assuming a body weight of 65 kg) and 0.19 g EPA/day; n=17 (9 males, 8 females)) • 4 capsules/day of *Schizochytrium* sp.-derived fatty acid blend (0.45 g DHA (approx. 6.9 mg DHA/kg bw/day assuming a body weight of 65 kg) and 0.38 g EPA/day; n=18 (9 males, 9 females) • 2 capsules/day of *Schizochytrium* sp.-derived fatty acid blend (0.225 g DHA (approx. 3.5 mg DHA/kg bw/day assuming a body weight of 65 kg) and 0.19 g EPA/day) + 6 FVB capsules/day juice powder concentrate; n=18 (9 males, 9 females)	• There were three dropouts in the control-treated group (2 subjects did not appear to the 3rd study visit without giving a reason; one subject dropped out due to an illness not related to the intervention). • In the 2 capsules/day of *Schizochytrium* sp.-derived fatty acid blend-treated group, one subject dropped out due to an illness not related to the intervention. • No differences were observed in weight, body mass index (BMI), cholesterol, HDL, LDL, triglycerides, fasting blood glucose, ferritin, iron, or vitamin D3 between baseline and either the 8- or 16-week follow up. • Supplementation had no effect on markers or hepatic (alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transferase), renal (creatinine, uric acid, urea) or thyroid function (thyroid stimulating hormone). • Intake of *Schizochytrium* sp.-derived fatty acid blend increased omega-3-indices significantly after 8 and 16 weeks of supplementation compared to baseline and to the control group. A greater effect was observed with the addition of juice powder concentrate providing additional vitamins and carotenoids.	Current GRAS Notice

Table 22. Clinical Studies in Children and Adults with *Schizochytrium* sp.-Derived DHA-Rich Oils

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters	Summarized In
Marc et al., 2020	Randomized, placebo-controlled trial in women who gave birth to infants with less than 29 weeks of gestation to assess if DHA supplementation would affect bronchopulmonary dysplasia in preterm infants	• Subjects took either placebo or DHA capsules daily during the neonatal period (for a total of 8-13 weeks) until the infants reached 36 weeks postmenstrual age • Control group: Soy and corn oil placebo, identical capsule to the test group; n=229 mothers and their 255 infants • Test group: women supplemented with 1.2 g/day *Schizochytrium* sp.-derived oil containing 40% DHA (0.48 g DHA/d; approx. 7.45 mg/kg bw/day assuming a body weight of 65 kg); n=232 mothers and their 273 infants	• In the control group, 41 subjects discontinued the intervention (18 subjects stopped breastfeeding, took pills or both; 12 lost interest; 7 discontinued based on medical advice or due to an adverse event; 2 decided to take an omega-3 supplement outside the protocol; 2 were lost to follow-up). • In the test group, 44 subjects discontinued the intervention (21 subjects stopped breastfeeding, took pills or both; 15 lost interest; 5 discontinued based on medical advice or due to an adverse event; 3 decided to take an omega-3 supplement outside the protocol; 1 were lost to follow-up). • At the interim analysis, the proportion of infants who survived free of bronchopulmonary dysplasia was 57.8% (119/206 infants) in the DHA group vs. 62.4% (121/194 infants) in the placebo group. Although this finding was not statistically significant, it agreed with the findings of a concurrent trial and the trial was prematurely terminated. • No neonatal adverse events or serious adverse events were reported to be related to DHA supplementation in mothers. • Headache (6.9% in the DHA group v. 6.1% in the placebo group) and gastrointestinal disorders (6.9% in the DHA group v. 5.7% in the placebo group) were the most frequently reported adverse events.	Current GRAS Notice

Abbreviations: HDL: high density lipoprotein; LDL: low density lipoprotein; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid
[1]The average weight of the toddlers was determined by averaging the median weights of boys and girls 8.5 months-old (9.08 and 8.31 kg, respectively), which were obtained from the CDC growth tables (https://www.cdc.gov/growthcharts/clinical_charts.htm; accessed on 3-23-21).

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D. ALLERGENICITY

The subject of this GRAS Notice is a highly refined oil that contains less than 0.1% protein (See Table 5). Although food allergenic reactions to carbohydrates such as galactooligosaccharides have been reported, food allergic responses occur almost exclusively to protein (Crevel et al., 2000). Therefore, to understand whether proteins derived from *Schizochytrium* sp. could be allergenic, a literature search performed on February 24, 2021 using PubMed and the search terms “schizochytrium AND allergy”. No reports were found. Additionally, there were no reports of allergic responses to any member of the family Chromista, including the thraustochytrids. A literature search using the search terms “docosahexaenoic acid AND allergy” revealed one study, which evaluated the effect of DHA supplementation in pre-term infants on the incidence of allergy seven years later (Gunaratne et al., 2019). DHA supplementation had no effect the incidence of allergy. Thus, allergenic reactions resulting from the ingestion of Algal Oil from *S. limacinum* TKD-1 are not expected.

E. REGULATORY APPROVALS ACROSS THE WORLD

In the United States, DHA-rich oils derived from *Schizochytrium* sp. are GRAS for use in infant formulas and general foods. Currently, there are thirteen GRAS Notices that document the GRAS status DHA-rich oils derived from seven different strains *Schizochytrium* sp.

Schizochytrium sp.-derived oils are also approved for use in infant and follow-on formulas and general foods in the European Union, Health Canada, the United Kingdom, Australia and New Zealand, China and Brazil.

VII. REFERENCES

A. REFERENCES

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd):* _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd):* _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Bean Wei	International Business Manager

Organization *(if applicable)*: TK Biohealth Co., Ltd.

Mailing Address *(number and street)*: 109 Husong Road, Shitan Industrial Park, Shizi Town, Quanjiao County

City	State or Province	Zip Code/Postal Code	Country
Chuzhou City	China	239514	China

Telephone Number	Fax Number	E-Mail Address
+86 198 5500 2923		bean.wei@ahtk-health.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Dietrich B. Conze, PhD	Managing Partner

Organization *(if applicable)*: Spherix Consulting Group, Inc.

Mailing Address *(number and street)*: 751 Rockville Pike, Unit 30-B

City	State or Province	Zip Code/Postal Code	Country
Rockville	Maryland	20852	United States of America

Telephone Number	Fax Number	E-Mail Address
240-367-6089		dconze@spherixgroup.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Algal oil from Schizochytrium limacinum TKD-1

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN GRN137

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☒ e) Other or Additional *(describe or enter information as above)* GRNs 326, 379, 533, 677, 731, 776, 843, and 934

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

The intended use of the subject of this Notice is to be a substitute for other *Schizochytrium* sp.-derived, DHA-rich oils in cow's milk and soy-based exempt and non-exempt preterm and term infant formulas at a level that is consistent with the level of DHA in human milk, and the same food categories specified in GRN 137 (Table 13). The subject of this GRAS Notice will be used in infant formulas with a safe and suitable source of ARA.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that TK Biohealth Co., Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Algal oil from Schizochytrium limacinum TKD-1
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. TK Biohealth Co., Ltd. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

109 Husong Road, Shitan Industrial Park, Shizi Town, Quanjiao County, Chuzhou City, China
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	**Printed Name and Title**	**Date (mm/dd/yyyy)**
Dietrich B. Conze, PhD Digitally signed by Dietrich B. Conze, PhD Date: 2021.05.07 16:28:39 -04'00'	Dietrich B. Conze, Managing Partner	05/07/2021

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	TK Biohealth GRAS for Algal Oil from Schizochytrium limacinum 5-7-21	Submission
	References	Submission

From:	kbrailer@spherixgroup.com
To:	Morissette, Rachel
Cc:	"Dietrich Conze"; ckruger@spherixgroup.com; "Jennifer Symonds"
Subject:	[EXTERNAL] Response to Questions on GRN 001008
Date:	Wednesday, November 3, 2021 4:10:37 PM
Attachments:	image001.png image002.png image003.png image004.png image005.png image006.png Appendix 1 - Revised Chapter I for GRN1008 - ATK Biotech.pdf ATK Response to FDA on GRN1008 11-3-21.pdf

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Dear Rachel,

Attached please find our response to your request for additional information regarding GRN 001008. Please confirm receipt and let us know if anything else is needed.

Best regards,

Kathy Brailer
Director of Administrative Services
Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852
+1-301-557-0375
kbrailer@spherixgroup.com
www.spherixgroup.com

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Tuesday, October 12, 2021 7:19 AM
To: Dietrich Conze <dconze@spherixgroup.com>
Cc: Claire Kruger <ckruger@spherixgroup.com>; Kathy Brailer <kbrailer@spherixgroup.com>
Subject: RE: [EXTERNAL] Re: questions for GRN 001008

Hi Dietz,

November 3 will be fine.

Thanks,

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

From: Dietrich Conze <dconze@spherixgroup.com>
Sent: Monday, October 11, 2021 1:48 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Claire Kruger <ckruger@spherixgroup.com>; Kathy Brailer <kbrailer@spherixgroup.com>
Subject: [EXTERNAL] Re: questions for GRN 001008

Hi Rachel,

We've reviewed your questions and should be able to address them all. Would it be possible to extend the due date for our responses by 7 days to November 3, 2021? Our client is located in China and the extra time is needed to give us with some flexibility in corresponding with them and obtaining the necessary data. Of course, if we are able to complete our responses before November 3, we will submit them to you as soon as possible.

Regards.
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
11821 Parklawn Drive, Suite 310
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

On Oct 8, 2021, at 3:41 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dr. Conze,

Please see attached our questions for GRN 001008. Let me know if you have any questions at this time,

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

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<image007.gif>

<2021-10-08 Questions for notifier GRN 001008.pdf>

November 3, 2021

Rachel Morissette, Ph.D.
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 001008

Dear Dr. Morissette:

Below are our responses to your requests for additional information regarding GRN 001008 as stated in your email on October 8, 2021. Your requests are in italicized text and our responses are below in plain text:

Regulatory:

1. *Part 1 of the submitted notice contains the old company name of the notifier. Please provide an updated Part 1 of the notice that reflects the new company name of the notifier with appropriate signature.*

   Part 1 of the GRAS Notice has been updated and now includes the new company name of the notifier and appropriate signature. Please see Appendix 1.

2. *Please provide a statement that when algal oil (≥35% DHA) is used as an ingredient as the sole added source of DHA in any given food category, or if blended with a source of eicosapentaenoic acid (EPA), the total dietary exposure to DHA will be no more than 1.5 g/person (p)/day (d) and no more than 3.0 g/p/d of DHA and EPA combined.*

   When the subject of this Notice (≥35% DHA) is used as an ingredient, as the sole added source of DHA in any given food category, or if blended with a source of EPA, the total dietary exposure to DHA will be not more than 1.5 g/p/d and not more than 3.0 g/p/d of DHA and EPA combined.

Chemistry:

3. *The intended use includes exempt infant formula; however, the use is not clearly limited to pre-term infant formula. Please confirm that the intended use in exempt infant formula refers to formulas for pre-term infants only and does not include use in other exempt formulas (e.g., hypoallergenic formulas, formulas for inborn errors of metabolism).*

   The use in exempt infant formulas is for formulas for pre-term infants only.

4. *On p. 3 of the notice under #11, the estimated dietary exposure is based on a target DHA concentration of 0.5% fat for formulas for term and pre-term infants, but there is no maximum use level indicated. Please clarify the maximum intended use level of algal oil (≥35% DHA) in formulas for term and pre-term infants.*

   The maximum intended use level of algal oil (≥35% DHA) in formulas for term and pre-term infants is 0.5% fat.

5. *In an update on July 27, 2021, ATK Biotech clarified that the use levels of algal oil (≥35% DHA) were the same as those for the subject of GRN 000137 (29% of the use levels specified for Menhaden oil per 21 CFR 184.1472) based on the assumption that the level of DHA in both algal oils is the same (i.e., ≥35%). However, Table 10 on p. 21 of the notice indicates a specification of ≥35% DHA, while elsewhere in the notice higher levels of DHA (53-62%) are indicated based on the results of batch analyses (see Table 5 on p. 14 and the statement under D.1. Infant Formula on p. 25).*

   a. *Please clarify the level of DHA in ATK Biotech's algal oil (≥35% DHA) after standardization with sunflower oil.*

      The batch data presented in Table 5 in GRN 001008 was determined on the oil prior to standardization with sunflower oil. The DHA content in the unstandardized oil is approximately 57%. As stated in the Chapter II. Section C. of GRN 001008, sunflower oil is added to standardize the DHA content to not less than 35%.

   b. *If the level of DHA in ATK Biotech's algal oil (≥35% DHA) is higher than that of the oil described in GRN 000137, please adjust the use levels in foods accordingly to maintain an estimated dietary exposure to DHA below 1.5 g/p/d for the U.S. population 2 years of age and older.*

      Because the oil is standardized to not less than 35% with food-grade sunflower oil, which is the same level of DHA in the subject of GRN 000137, the estimated dietary exposure to DHA will be the same as those specified in GRN 000137 and will prevent the estimated dietary exposure to DHA from exceeding 1.5 g/p/d for the U.S. population 2 years of age and older.

6. *Please provide specifications for the DHA and DPA n-6 content of algal oil (≥35% DHA) that includes the upper limit for these fatty acids as a percent of total fatty acids or total fat in algal oil (≥35% DHA).*

   ATK Biotech has put in place additional specifications for DHA and DPA content on a percent of total fatty acid basis of 34 to 70 % and 5 to 20 %, respectively.

7. *On p. 10 of the notice under 2. Manufacturing, ATK Biotech states: "All of the processes used by TK Biohealth are similar to those used in the production of other food oils, including Schizochytrium sp.-derived, DHA-containing oils that are GRAS for use in non-exempt term infant formulas in United States approved for use in the European Union." Please clarify if the processes are similar for infant formulas for term and pre-term infants in the US.*

   The processes used by ATK Biotech are similar to those used in the production of other food oils, including *Schizochytrium* sp.-derived, DHA-containing oils that are GRAS for use in both exempt and non-exempt preterm and term infant formulas, respectively, in the United States.

8. *We request that ATK Biotech provides a comparison of the specifications for its algal oil (≥35% DHA) to those in the 12th edition of the Food Chemicals Codex (FCC 12 (2021)) monograph for DHA from algal (Schizochytrium sp.) oil and provide the additional supporting information:*

   A comparison of the specifications for the subject of this GRAS Notice and the those listed in the FCC monograph for Algal (*Schizochytrium* sp.) Oil is provided in Table 1. The specifications in the FCC monograph include ranges for dihomo-gamma-linolenic acid (DGLA), arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) on an area percent basis, as well as limits for DHA, free fatty acids, heavy metals, unsaponifiables, anisidine value, peroxide value, and total oxidation value. ATK Biotech's specifications include limits for DHA, free fatty acids, heavy metals, unsaponifiables, peroxide value, as well as acid value, moisture and volatiles, trans-fatty acids, and microbes. ATK Biotech's specifications do not include ranges for DGLA, ARA, EPA, DPA, and DHA on an area percent basis, or limits for anisidine value or total oxidation value. Importantly, the highest anisidine, peroxide, and total oxidation values ((2 x peroxide value) + anisidine value as specified in the FCC monograph) for the algal oil that is the subject of this Notice are approximately 10.3, 1.10, and 12.38, respectively, which comply with the specifications for the anisidine, peroxide and total oxidation values in the FCC monograph. Additionally, the amount of DLGA, ARA, EPA, and DPA in the subject of this Notice on an area percent basis fall below the range specified in the FCC monograph, whereas the amount of DHA exceeds the DHA specification in the FCC monograph. Importantly, because the subject of this Notice is intended to be used as a source of DHA, consumers will be exposed to less DLGA, ARA, EPA, and DPA when the subject of this GRAS Notice is used to attain the same target DHA level as algal oils that comply with the specifications listed in the FCC monograph.

Table 1. Comparison of FCC and ATK Biotech Specifications		
Parameter	**FCC Specification**	**ATK Biotech Specification**
Dihomo-gamma-linolenic acid (DGLA; area %)	1.7 – 2.8	NS
Arachidonic acid (ARA; area %)	0.6 – 1.3	NS
Eicosapentaenoic acid (EPA; area %)	1.3 – 3.9	NS
Docosapentaenoic acid (DPA; area %)	10.5 – 16.5	NS
Docosahexaenoic acid (DHA; area %)	30.0 – 40.0	NS
DHA content (%)	≥ 30	≥ 35
Arsenic (mg/kg)	≤ 0.1	≤ 0.1
Lead (mg/kg)	≤ 0.1	≤ 0.1
Mercury (mg/kg)	≤ 0.1	≤ 0.04
Anisidine Value	≤ 20	NS
Free fatty acids (%)	≤ 0.4	≤ 0.4
Peroxide value (meq/kg oil)	≤ 5.0	≤ 5.0
Total Oxidation Value	≤ 26	NS
Unsaponifiables (%)	≤ 4.5 %	≤ 3.5
Acid value (mg KOH/g)	NS	≤ 0.5
Moisture and volatiles (%)	NS	≤ 0.05
Trans-fatty acids (%)	NS	≤ 2.0
Total Plate Count (CFU/g)	NS	≤ 1000
Coliforms (CFU/g)	NS	≤ 10
Molds and Yeasts (CFU/g)	NS	≤ 25
Salmonella (/375 g)	NS	Negative
Cadmium (mg/kg)	NS	≤ 0.2
Copper (mg/kg)	NS	≤ 0.1
Iron (mg/kg)	NS	≤ 0.1
Abbreviations: meq – milliequivalents; KOH – potassium hydroxide; NS – no specification; CFU – colony forming units		

a. *Given that the anisidine value is included in the specifications listed in the FCC 12 (2021) monograph (which are cited in the notice) and in the discussion of the stability of algal oil (≥35% DHA) in the notice, we request that ATK Biotech provides a specification for anisidine value for its algal oil (≥35% DHA).*

ATK Biotech has added a specification for anisidine value of not more that 20 to be consistent with the anisidine value specification listed in the FCC 12 (2021) monograph for DHA from Algal (*Schizochytrium*) Oil.

b. *Please compare the fatty acid composition of algal oil (≥35% DHA) to the fatty acid profile in the FCC 12 (2021) monograph. Please provide a discussion of any differences and how ATK Biotech concluded that any differences did not affect the safety conclusion.*

On an area percent basis, the subject of this Notice contains approximately 0.27, 0.16, 0.50, 0.17, and 56.18 % DGLA, ARA, EPA, DPA, and DHA, respectively (Table 2). Compared to the specifications listed in the FCC monograph for algal oil, the levels of DGLA, ARA, EPA, and DPA in *S. limacinum* TKD-1-derived oil

fall below the lower limits of the ranges for the corresponding fatty acid listed in the FCC monograph for Algal (*Schizochytrium* sp.) Oil. In contrast, the level of DHA in the subject of this Notice exceeds the upper limit specified in the FCC monograph. Importantly, because the subject of this GRAS Notice is intended to be a source of DHA and the level of DHA is higher than the specification listed in the FCC monograph, less of the oil will be needed to attain the same targeted DHA concentration in the finished products compared to oils that comply with the FCC monograph. Additionally, because less *S. limacinum* TKD-1-derived oil will be needed to attain the targeted concentration of DHA, the resulting exposure to DGLA, ARA, EPA, and DPA in the finished product will be less than oils that comply with the FCC specifications. Therefore, the differences in the DGLA, ARA, EPA, DPA, and DHA content in the *S. limacinum* TKD-1-derived oil compared to FCC grade algal oil do not affect the ATK Biotech's conclusion that the subject of this Notice is safe for use in pre-term and term infant formulas and conventional foods.

Table 2. Comparison of Long Chain Polyunsaturated Fatty Acid Composition in the *Schizochytrium limacinum* TKD-1-Derived Oil with the Long Chain Polyunsaturated Fatty Acid Ranges Specified in the FCC Monograph for Algal (*Schizochytrium* sp.) Oil

Fatty Acid	FCC Specification	Lot Number					Average ± Standard Deviation
		DD20191206	DD20191213	DD20191220	DD20200101	DD20200110	
Dihomo-gamma-linolenic acid (DGLA; area %)	1.7 – 2.8	0.29	0.26	0.28	0.27	0.27	0.27 ± 0.1
Arachidonic acid (ARA; area %)	0.6 – 1.3	0.15	0.15	0.16	0.17	0.17	0.16 ± 0.01
Eicosapentaenoic acid (EPA; area %)	1.3 – 3.9	0.49	0.46	0.49	0.53	0.53	0.50 ± 0.03
Docosapentaenoic acid (DPA; area %)	10.5 – 16.5	0.16	0.16	0.17	0.19	0.18	0.17 ± 0.01
Docosahexaenoic acid (DHA; area %)	30.0 – 40.0	57.30	53.02	55.63	57.67	57.27	56.18 ± 1.93
Abbreviations: meq – milliequivalents; KOH – potassium hydroxide; NS – no specification							

c. Please address whether the difference in fatty acid composition, including higher levels of long chain polyunsaturated fatty acids, affects the oil's stability or the necessary quantity of antioxidants added to the oil to maintain stability.

As summarized in Chapter 1, Section E.3, the subject of this GRAS Notice is stable for up to 1 year at 4-5°C and 2 years at -18°C. Although the product contains higher levels of DHA and lower levels of DGLA, ARA, EPA, and DPA than those specified in the FCC monograph, the oil contains a similar amount of long chain polyunsaturated fatty acids as whole compared to oils that comply with the FCC specifications (approximately 57% compared to a maximum of 64.5 % on an area percent basis). Therefore, the difference in the fatty acid composition is not expected to affect the oil's stability or the amount of antioxidants added to the oil to maintain stability.

9. In section D.1.a. Phenotypic Identity on p. 8 of the notice, please clarify what is meant by the term "mask medium."

The term "Mask medium" was used to describe the medium that is used to culture *S. limacinum* TKD-1 on a plate. The medium contains glucose, yeast extract, corn flour, agar, penicillin, and streptomycin.

10. ATK Biotech provide the results of analyses for unsaponifiables and sterols in Table 5 (p. 14) and Table 8 (p. 19) of the notice, respectively.

a. The level of unsaponifiables in the analyzed batches ranges from 0.26-0.44 g/100 g (i.e., 0.26-0.44%) per Table 8, while the level of unsaponifiables per Table 5 ranges from 1-1.9%. Considering that both tables report the results for the same batches, please address the difference in the ranges, including a discussion of other non-saponifiable components that comprise the bulk of the unsaponifiable fraction. In other words, why are sterols (total) such a small portion of all unsaponifiables?

The methods used to quantify the amount of unsaponifiables in Table 5 and Table 8 are different. The method used to quantify the amount of unsaponifiables in Table 5 is diethyl ether extraction whereas the total amount of sterols in Table 8 is the sum of the individual sterols quantified by gas chromatography coupled with a flame ionization detector (GC-FID). Similar methods were used to quantify the amount of the unsaponifiables and sterols in the subject of GRN 000677. Due to methodological differences, it is therefore not possible to accurately compare the total amount of sterols determined using GC-FID with the amount of unsaponifiables determined using diethyl ether extraction. Additionally, the specification for unsaponifiables for the subject of this Notice of not more than 3.5% is consistent with, if not more restrictive than, the specifications for other *Schizochytrium* sp.-derived, DHA-rich oils that are GRAS for use in infant formula and general foods (US FDA, GRN 000137, 2003; US FDA, GRN 000553, 2014; US FDA, GRN 000677, 2017; US FDA, GRN 000776, 2018; US FDA, GRN 000777, 2018; US FDA, GRN 000836, 2019; US FDA, GRN 000843, 2019; US FDA, GRN 000844, 2019; US FDA, GRN 000862, 2020; US FDA, GRN 000913, 2020). The total sterol fraction of these other *Schizochytrium* sp.-derived, DHA-rich oils also accounts for comparable portions of the unsaponifiable fractions. Specifically, unsaponifiable fraction batch data is not provided for the subject of GRN 000137. whereas it ranges from 0.78 to 0.97%, and 0.3 to 2.97% for the subjects of GRNs 000553 and 000677, respectively. The total sterols in the subjects of GRNs 000137, 000553, and 000677 are approximately 3.1%, 0.51 to 0.54 wt%, and 0.09 to 0.23% (900 – 2310 mg/kg), respectively. Importantly, as stated on page 9 of GRN 000137, the unsaponifiable fraction of DHA-rich, algal oils derived from *Schizochytrium* sp. consists primarily of sterols, squalene, and carotenoids. Squalene is found in the body, human milk at approximately 0.011 mmol/L (4.5 mg/L)), and edible vegetable oils such as olive, corn, sunflower, palm, and peanut oil (Kallio et al., 1989; Popa

et al., 2014). Carotenoids are also components of human milk and the diet, and their levels in human milk likely depend on the maternal diet (Zielinska et al., 2019). The most predominant carotenoids in human milk are β-carotene, lutein, and zeaxanthin, lycopene, α-carotene, and β-cryptoxanthin, and their concentrations can vary greatly depending on the stage of lactation, and the diet/geographical location of mother. Therefore, considering the *S. limacinum* TKD-1 is genotypically similar to strains used to manufacture the subjects of GRNs 000137, 000553, and 000677, and the subject of this Notice is manufactured using similar processes, ATK Biotech does not expect significant differences in the other components of the unsaponifiables compared to those in the unsaponifiables of other *Schizochytrium* sp.-derived, DHA-rich oils that are GRAS for use in infant formula and conventional foods.

11. *For the sterol analysis provided in Table 17 (p. 37), ATK Biotech states that "–" "denotes that the sterol was not quantified in the GRAS notice." Please clarify:*

    a. *If the values ATK Biotech presented for these sterols are below the limit of detection or if the method used was not able to resolve and measure these sterols.*

    b. *If total unidentified sterols denoted as "–" means unidentified peaks were observed, but not quantified, or if the method did not result in any unidentified peaks.*

    The statement "denotes that the sterol was not quantified in the GRAS notice," for describing the "-" in the footnote Table 17 is vague. In addition to addressing the errors outlined in Question 13, we have now corrected Table 17 (see below) and specify the analytical data that was "not detected" and "not reported" for the subject of this Notice, as well as for the subjects of GRNs 000137, 000553, and 000677.

12. *We note that Table 17 (p. 37) indicates certain sterols present in the DHA- containing oils described in GRNs 000553 and 000677 but are designated as not quantified in this notice: 24-methylene cholesterol, clerosterol, delta-5,23- stigmastadienol, delta-5-avenasterol, and delta-7-campesterol.[2] The method used in this notice to measure sterols is described as "compendial gas chromatographic method NMKL 198:2014." From the literature, it appears that this method measures specific plant sterols and stanols, and does not measure certain algal sterols present in Schizochytrium sp. oil.[3] Please address if the method used in GRN 001008 is suited for measuring algal phytosterols or if it is*

[2] *The GRN 000677 method "JK073-sterol profile and content" (internal method) yielded results for these sterols.*

[3] *Laakso, 2014. JAOAC Intl. 97(4):1097-1108 and https://www.nmkl.org/index.php/en/publications/item/vaxtstanoler-och-vaxtsteroler-bestamning-av-fytosterolberikade-livsmedel-med-gaskromatografi-gc-fid-nmkl-198-2014.*

limited to plant sterols. If different methods were used for GRNs 000677 and 001008, please briefly address why omitting certain sterols does not impact ATK Biotech's safety conclusion.

As discussed in our response for Question 11, the statement in the footnote of Table 17, "denotes that the sterol was not quantified in the GRAS notice," for describing the "-" is vague. We have now corrected Table 17 (see below) and specify the analytical data that was either "not detected" or "not reported" for the subject of this Notice, as well as for the subjects of GRNs 000137, 000553, and 000677. As noted, NMKL 198:2014 is a compendial method for quantifying plant sterols and stanols. It is also capable of quantitating phytosterols in sterol-enriched foods (Laakso, 2014). Importantly, there are no compendial methods for identifying and quantifying algal sterols. As a result, the notifiers of other *Schizochytrium* sp.-derived, DHA-rich oils that are GRAS have resorted to using a variety of methods to determine sterol content, including NMKL 198:2014, as well as non-compendial methods (US FDA, GRN 000137, 2003; US FDA, GRN 000553, 2014; US FDA, GRN 000677, 2017; US FDA, GRN 000731, 2018; US FDA, GRN 000732, 2018; US FDA, GRN 000776, 2018; US FDA, GRN 000777, 2018; US FDA, GRN 000836, 2019; US FDA, GRN 000843, 2019; US FDA, GRN 000844, 2019; US FDA, GRN 000862, 2020; US FDA, GRN 000913, 2020; US FDA, GRN 000933, 2020; US FDA, GRN 000934, 2021). The actual techniques used and the validation status of the non-compendial methods, however, are not publicly available.

As discussed in our response to Question 13, ATK Biotech quantified the sterols in the subject of this Notice using NMKL 198:2014. Cholesterol and the unidentified sterols fraction represent the bulk of the sterols (>70%) in the subject of this Notice. With the exception of 24-methylenecycloartenol, cycloartenol, and citrostadienol, all the remaining sterols are present in the oil that is the subject of GRN 000677, although at lower levels. Additionally, NMKL 198:2014 defines the unidentified sterols as those that elute from the gas chromatography column between stigmasterol and sitosterol, which are likely to be delta-7-campesterol, delta-5,23-stigmastadienol, clerosterol, and delta-4-sitosterol. Delta-7-campesterol, delta-5,23-stigmastadienol, and clerosterol have all been quantified in other *Schizochytrium* sp.-derived, DHA-rich oils that are GRAS and received "no questions" letters from the Agency, including GRN 000677 (US FDA, GRN 000553; US FDA, GRN 000862; US FDA, GRN 000913). Delta-4-sitosterol does not occur naturally as described in NMKL-198:2014 and is not present in the subject of this Notice. Importantly, all of the sterols found in the subject of this Notice, including 24-methylenecycloartenol, cycloartenol, and citrostadienol, as well as the subject of GRN 000677 and other *Schizochytrium* sp.-derived, DHA-rich oils that are GRAS, are found in human milk, infant formula, edible oils, edible oils used to formulate infant formula and/or other foods (see page 23 of GRN 000553; Mo et al., 2013, Lipids, 48, 949-56; Yang et al., 2019; Xu et al., 2018a). Therefore, although NMKL 198:2014 is not able to directly quantify all of the sterols that are present in subject of this Notice compared to the subject of GRN 000677, because the unidentified fraction likely contains delta-7-campesterol, delta-5,23-stigmastadienol, clerosterol, and 24-methylenecycloartenol,

cycloartenol, and citrostadienol are also present in other edible oils, the results from the analysis do not change ATK Biotech's conclusion of safety for this DHA-rich oil. .

13. We note a number of errors in Table 17 (p. 37) that should be corrected:

a. In the 5th row of Table 17 (p. 37), the term "cholesterol and/or fucosterol" should be changed to clerosterol (or chlerosterol) and the GRN 000137 value of 25±3 % is for cholesterol and should be moved to the 6th row.

b. Values for delta-5-avenasterol and sitostanol should not be combined for GRNs 000553 and 000677.

c. The total sterols units (mg/100 g) do not apply to GRN 000137, which contains 31 mg/g or 3100 mg/100 g (3.1%) total sterols.

The data that was presented in Table 17 was derived from Table 8 in the Notice, which did not include the non-4-desmethyl sterols (cycloartenol, 24-methylenecycloartenol, and citrostadienol) that were also quantified in *S. limacinum* TKD-1-derived oil using NMKL 198.2014. Therefore, to accurately compare the subject of this Notice with the subjects of GRNs 000137, 000677, 000553 in Table 17, the complete sterol profiles of three of the same batches presented in Table 8 are provided below (Table 3). Cholesterol and the unidentified sterols fraction represent the bulk of the sterols (>70%) in each of the batches. Of the remaining sterols, stigmasterol, sitosterol, and delta-7 stigmasterol are the most prominent. As summarized in our response to Question 12, NMKL 198.2014 defines the unidentified sterols as those that elute from the gas chromatography column between stigmasterol and sitosterol, which are likely the 4-desmethyl sterols delta-7-campesterol, delta-5,23-stigmastadienol, clerosterol, and delta-4-sitosterol. Because it is difficult to identify these peaks unambiguously, they are summed and reported as unidentified 4-desmethyl sterols and included in the total plant sterol and phytosterol content. Importantly, delta-7-campesterol, delta-5,23-stigmastadienol, and clerosterol have all been quantified in other *Schizochytrium* sp.-derived, DHA-rich oils that are GRAS and have received "no questions" letters from the Agency (US FDA, GRN 000553, 2014; US FDA, GRN 000677, 2017; US FDA, GRN 000862, 2020; US FDA, GRN 000913, 2020). As described in NMKL 198.2014, delta-4-sitosterol does not occur naturally. It has also not been reported or detected in any of the DHA-rich oils that are the subjects of GRAS Notices that received "no questions" letters.

Table 3. Sterol Composition of the *S. limacinum* TKD-1-derived Oil[1]

Sterol (mg/100 oil)	Lot Number		
	DD20191206	DD20191213	DD20191220
Brassicasterol	12	6	10
Cholesterol	278	170	247
Campesterol	6	4	5
Campestanol	2	ND[2]	1
Stigmasterol	22	14	19
Sitosterol	52	39	48
Sitostanol+delta-5-avenasterol	17	6	14
Delta-5, 24-stigmastadienol	15	7	13
Delta-7-Stigmastenol	31	25	30
Delta-7-Avenasterol	5	3	5
Cycloartenol	9	9	10
24-Methylenecycloartenol	3	2	2
Citrostadienol	ND[2]	3	1
Unidentified Sterols	266	135	225
Total Plant Sterols and Stanols[3]	429	239	370
Total Sterols[4]	718	423	630

Abbreviations: ND, not detected.
[1]Determined by Eurofins Technology Service (Suzhou) Co., Ltd using NMKL 198:2014. Limit of detection = 0.3 mg sterol/100 g oil; limit of quantitation = 1 mg sterol/100 g oil.
[2]Denotes that value is less than the LOQ.
[3]Does not include cholesterol or non-4-desmethyl sterols.
[4]Includes all sterols that were quantified.

Using the complete sterol data provided above in Table 3, we have now recalculated the % of Total Sterols and corrected the errors in Table 17 (see Table 17 below). We have also clarified the analytical data that was not reported or not detected in the subjects of GRNs 000137, 000553, and 000677 as requested in Question 11.

Table 17. Comparison of the Sterol Content of *Schizochytrium*-derived DHA-rich Oils That Are GRAS

Sterol	% Total Sterols			
	ATK Biotech[b]	GRN 000677	GRN 000553	GRN 000137
24-methylene cholesterol	NR	4.25 ± 1.92	1.5 ± 0.42	NR
24-methylenecycloartenol	0.4 ± 0.08	NR	NR	NR
Brassicasterol	1.56 ± 0.13	5.80 ± 1.04	1.28 ± 0.33	15 ± 3[a]
Campestanol	0.15 ± 0.14	ND	0.1 ± 0.0	NR
Campesterol	0.86 ± 0.08	2.32 ± 1.11	1.84 ± 0.27	NR
Clerosterol	NR	12.50 ± 5.48	1.6 ± 0.0	NR
Cholesterol	39.37 ± 0.75	23.03 ± 8.56	12.30 ± 1.81	25 ± 3[a]
Cycloartenol	1.66 ± 0.44	NR	NR	NR
Citrostadienol	0.05 ± 0.09	NR	NR	NR
Delta-5, 23-Stigmastadienol	NR	5.52 ± 2.03	0.84 ± 0.089	NR
Delta-5, 24-stigmastadienol	1.94 ± 0.24	5.68 ± 1.35	0.44 ± 0.05	NR
Delta-5-Avenasterol	NR	3.02 ± 1.98	1.78 ± 0.72	NR
Delta-7-Avenasterol	0.73 ± 0.05	4.28 ± 2.86	0.90 ± 1.29	NR
Delta-7-Campesterol	NR	5.70 ± 2.00	0.44 ± 0.089	NR
Delta-7-Stigmastenol	5.0 ± 0.82	17.03 ± 7.99	1.92 ± 0.36	NR
Ergosta-7,22-dien-3-ol	NR	NR	NR	<5 – 7[a]
Ergosta-7,24-dien-3-ol	NR	NR	NR	<5 – 6[a]
Sitostanol	NR	0.17 ± 0.16	0.52 ± 0.04	NR
Sitostanol + delta-5-avenasterol	2.0 ± 0.51	NR	NR	NR
Sitosterol	8.03 ± 1.05	12.37 ± 1.96	11.32 ±1.94	NR
Stigmasterol	3.13 ± 0.16	16.28 ± 8.44	63.20 ± 2.37	19 ± 2[a]
Stigmastadien-3-ol	NR	NR	NR	8 ± 1[a]
Other sterols	34.89 ± 2.66	NR	ND	NR
Total (mg/100 g oil)	590.33 ± 151.45[c]	150.02 ± 64.01[d]	533.8 ± 25.59[e]	3100[f]

Abbreviations: ND, not detected; NR, not reported.

[a]Presented on a % peak area basis.

[b]Calculated by diving the dividing the amount of each sterol (mg/100 g oil), including cholesterol and the non-4-desmethyl sterols cycloartenol, 24- methylene cycloartenol, and citrostadienol, by the total amount of sterols (mg/100 g oil) and multiplying the quotient by 100.

[c]Represents the average and standard deviation of total sterol content of the three batches presented in Table 3.

[d]Represents the average and standard deviation of the total sterol content of the subject of GRN 000677 as presented in the Certificates of Analysis.

[e]Represents the average and standard deviation of the total sterol content of the subject of GRN 000553 as presented in the Certificates of Analysis.

[f]Obtained from Table 4 from GRN 000137.

14. *The statement that "these DHA exposures are consistent with current recommendations for DHA consumption by term and preterm infants of 18 to 60 mg/kg bw/d (Koletzko et al., 2014)" is a statement we have seen before in previous GRNs. However, given that this statement is often repeated, we note that this is a simplification of the statements made in the cited reference (Koletzko et al., 2014),[4] and previous GRNs have not addressed dietary exposure of 60 mg/kg body weight/d DHA for pre-term infants. Please clarify the dietary exposure estimates for term and pre-term infants, indicating assumptions of maximum use levels and body weights for term and pre-term infants.*

The dietary exposure of extremely low, very low, and low birth weight premature infants to DHA from the ingestion of preterm infant formulas containing *S. limacinum* TKD-1-derived oil was calculated assuming the following: the maximum daily caloric requirement of 150 kcal/kg as defined by the American Academy of Pediatrics Committee on Nutrition (1985); the maximum amount of fat allowed in infant formula per 21 CFR 107.100 (6 g/100 kcal); the typical caloric content of infant formula (670 kcal/L; Martinez and Ballew, 2011); and the maximum use level of DHA (0.5% fat) (Table 4). Extremely low, very low, and low birth weight premature infants will be exposed to a maximum of 0.045 g DHA/kg body weight per day or 0.045, 0.0675, and 0.1125 g DHA/day, respectively, due to their corresponding maximum body weights.

Table 4. Docosahexaenoic Acid Exposure in Premature Infants

Category[a]	Maximum Weight (kg)[b]	Maximum Caloric Intake (kcal/kg/day)[c]	Maximum Exposure to DHA (g DHA/kg/day)[d]	Maximum Exposure to DHA (g DHA/day)[e]
Low birth weight premature infants	2.5	150	0.045	0.1125
Very low birth weight premature infants	1.5	150	0.045	0.0675
Extremely low birth weight premature infants	1	150	0.045	0.045

[a,b]As defined by Berkowitz et al. (1993).
[c]As defined by the American Academy of Pediatrics (1985).
[d]Calculated by multiplying the maximum amount of fat allowed in infant formula per 21 CFR 107.100 (6 g/100 kcal) by the maximum DHA use level of 0.5% fat (0.5 g/100g) and the maximum caloric intake/day (150 kcal/kg/day).
[e]Calculated by multiplying the maximum exposure to DHA in g DHA/kg body weight/day by the maximum weight of the infants in the category.

[4] *In Koletzko et al. (2014), "reasonable intakes" for very low birth weight (VLBW) infants are stated to be 18-60 mg/kg/d DHA and 18-45 mg/kg/d AA, while the authors note "higher intakes (55-60 mg/kg/day DHA, ~1% fatty acids; 35-45 mg/kg/day AA, ~0.6-0.75%) appear preferable." Recommendations for term infants of 100 mg/d for DHA are noted separately in this reference. Koletzko et al. (2014) Ann. Nutr. Metab. 65: 49-80.*

The dietary exposures of term infants to DHA from the ingestion of term infant formulas containing *S. limacinum* TKD-1-derived oil were calculated by dividing the mean and 90th percentile two-week caloric intakes determined by Fomon (1993) during the first six months of life by the typical caloric content of infant formula (670 kcal/L; Martinez and Ballew, 2011)(Table 5). The quotient was then multiplied by the maximum amount of DHA in formula on a liter basis (0.201 g DHA/L), which was determined by multiplying the maximum amount of fat allowed in infant formula per 21 CFR 107.100 (6 g fat/ 100 kcal) by the typical caloric content of infant formula (670 kcal/L) and assuming the maximum use level of DHA of 0.5% fat. The dietary exposure on a g DHA/day basis was then calculated by multiplying the DHA intake on a g/kg body weight/day basis by the median weights of males and females at the corresponding age, which are available at https://www.cdc.gov/growthcharts/html_charts/wtageinf.htm.

At the mean caloric intakes, males will consume 0.03 - 0.04 g DHA/kg body weight/day, which will result in intakes ranging from 0.12 to 0.22 g DHA/day as they age. At the 90th percentile, males will also consume 0.03 – 0.04 g DHA/day, resulting in intakes ranging from 0.14 to 0.27 g/day as they age. For females, the DHA intakes at the mean and 90th percentile will be slightly less than males due to their lower daily caloric intakes and median body weights. Importantly, these estimates are extremely conservative because they assume that infants are consuming the DHA-containing infant formula as their sole source of nutrition over the first six months of life. According to Grimes et al. (2015), who determined the dietary sources of total energy intake in infants and toddlers in the United States using the National Health and Nutrition Examination Survey 2005-2012 database, the actual total daily caloric intake from infant formula is 65% in infants from birth to 5.9 months-old and decreases to 47% in infants 6 to 11.9 months-old. Therefore, the actual exposures to DHA from the ingestion of DHA-containing formula during the first six months of life will be less than those calculated above and the exposures to DHA from the ingestion of DHA-containing formula from 7 to 12 months will be less than the exposures from 0 to 6 months.

Table 5. Docosahexaenoic Acid Exposure in Term Infants

Interval (days)	Median Weight (kg)	Mean			90th Percentile		
		Caloric Intake (kcal/kg/day)[a]	DHA Intake (g/kg/day)[b]	DHA Intake (g/day)[c]	Caloric Intake (kcal/kg/day)[a]	DHA Intake (g/kg/day)[b]	DHA Intake (g/day)[c]
Males							
0-13	3.53	113.8	0.03	0.12	136.70	0.04	0.14
14-27	4.00	121.1	0.04	0.15	141.30	0.04	0.17
28-41	4.00	117.9	0.04	0.14	136.90	0.04	0.16
42-55	4.88	110.5	0.03	0.16	129.00	0.04	0.19
56-83	5.67	101	0.03	0.17	115.60	0.03	0.20
84-111	6.39	94.7	0.03	0.18	106.10	0.03	0.20
112-139	7.04	94.4	0.03	0.20	112.10	0.03	0.24
140-167	7.63	95.4	0.03	0.22	113.10	0.03	0.26
168-195	8.16	91.5	0.03	0.22	108.50	0.03	0.27
Females							
0-13	3.40	111.9	0.03	0.11	135.50	0.04	0.14
14-27	3.80	117.8	0.04	0.13	138.90	0.04	0.16
28-41	3.80	115.2	0.03	0.13	136.80	0.04	0.16
42-55	4.54	108.8	0.03	0.15	127.40	0.04	0.17
56-83	5.23	101.1	0.03	0.16	114.40	0.03	0.18
84-111	5.86	95.7	0.03	0.17	106.80	0.03	0.19
112-139	6.44	96.6	0.03	0.19	113.10	0.03	0.22
140-167	6.97	94.7	0.03	0.20	113.30	0.03	0.24
168-195	7.45	91.2	0.03	0.20	107.90	0.03	0.24

[a]Adopted from Fomon (1993).
[b]Calculated by multiplying the maximum amount of fat allowed in infant formula per 21 CFR 107.100 (6 g/100 kcal) by the maximum DHA use level of 0.5% fat (0.5 g/100 g) and the maximum caloric intake/day/L infant formula, resulting in 0.201 g DHA/L formula. The caloric intake (kcal/kg/day) was then divided by the caloric content of infant formula (670 kcal/L) and then multiplied by the maximum amount of DHA/L infant formula.
[c]Calculated by multiplying the DHA intake on g/kg body weight/day basis by the median weight of males or females at the corresponding age; obtained from https://www.cdc.gov/growthcharts/html_charts/wtageinf.htm.

15. *Please confirm that the intended use of algal oil (≥35% DHA) in formulas for term and pre-term infants is in combination with a safe and suitable source of ARA and that the resulting ratio of ARA:DHA would be between 1:1 and 2:1. We note that although higher levels of DHA for pre-term infants may be supported in some references (Koletzko et al., 2014), recent reviews (e.g., Koletzko et al. 2020)[5] have still maintained the minimum ratio of 1:1 ARA:DHA.[6]*

The intended use of algal oil (≥35% DHA) in formulas for term and pre-term infants is in combination with a safe and suitable source of ARA and the resulting ratio of ARA:DHA will be between 1:1 and 2:1.

Toxicology:

16. *On p. 34 of the notice, ATK Biotech discusses the safety of the fatty acid and sterol profiles of algal oil (≥35% DHA) and states, "Additionally although the fatty acid and sterol profiles of the subject of this GRAS Notice are not identical to those of the subject of GRN 000677 (Table 16 and 17), the amount of the fatty acids other than DHA and sterols will either approximate or be less than those from the subject of this GRN 000677 in the marketed products." ATK Biotech also states that the DHA content in its algal oil (≥35% DHA) is 1.3-fold higher than the subject of GRN 000677. Given this information, please address the following questions.*

    a. *We note that the level of cis-4,7,10,13,16-docosapentaenoic acid in algal oil (≥35% DHA) is approximately 1.8-fold higher than the average of the levels reported in GRN 000677 (Table 16). Please discuss why this increase in cis-4,7,10,13,16-docosapentaenoic acid higher than the levels in GRN 000677 is not expected to be a safety concern. As part of this discussion, we suggest that a comparison of the final levels of this fatty acid in infant formula containing algal oil (≥35% DHA) to the levels reported in breast milk (e.g., Floris et al., 2020) may be helpful.*

    The 1.8-fold increase in the levels of cis- 4,7,10,13,16-docosapentaenoic acid (DPA) in the subject of this Notice compared the subject of GRN 000677 is not expected to be a safety concern because the levels of DPA in infant formulas formulated with either the subject of this Notice or the subject of GRN 000677

[5] *Koletzko et al. (2020) Am J. Clin. Nutr. 111: 10-16.*

[6] *We note that ratios of ARA:DHA below 1:1 have not been addressed in this or previous GRNs for DHA-containing oils intended for use in formulas for term and pre-term infants. To be consistent with previous GRAS conclusions for the use of DHA-containing oils in infant formula that have received "no questions" letters from FDA, DHA would be used with no less than an equal amount of ARA obtained from a safe and suitable source.*

fall within the range of DPA levels found in human milk. Assuming that human milk contains an average of 29.9 g fat/L, the highest amount of DPA in human milk is approximately 0.05 g/L (Sauerwald et al., 2012; Floris et al., 2020). If infant formula is formulated with the subject of this Notice, having a DHA content of approximately 57%, the resulting level in the infant formula will be approximately 0.05 g DPA/L. For reference, if infant formula is formulated with the subject of GRN 000677 with a DHA content of approximately 40%, the resulting levels in the infant formula will be approximately 0.04 g DPA/L. Therefore, assuming that infants consume infant formula and human milk to the same extent, the exposure to DPA will be similar in infants consuming an infant formula formulated with the subject of this Notice or GRN 000677, or those consuming human milk. Our calculations are provided below.

a. Calculation for the amount of DHA in 1 liter of infant formula.

(6.0 g fat/100 kcal formula)(670 kcal/L formula)(0.5 g DHA/100 g fat) = 0.201 g DHA/L formula

b. Calculation for the amount of the *S, limacinum* TKD-1-derived oil in 1 liter of infant formula, assuming a DHA content of 57%.

(0.201 g DHA/L formula)(0.1 kg oil/57 g DHA)=0.000353 kg oil/L formula

c. Calculation for the amount of DPA from *S. limacinum* TKD-1-derived oil in 1 liter of infant formula, assuming a DPA content of 13.95%.

(0.000353 kg oil/L formula)(13.95 g DPA/0.1 kg oil) = 0.049 g DPA/L formula

b. We note that the levels of cholesterol are higher (~2.7-fold) than the average of the levels reported in GRN 000677. Please discuss why this increase in cholesterol is not expected to be a safety concern. As part of this discussion, we suggest that a comparison of the final levels of cholesterol in infant formula containing algal oil (≥35% DHA) to either cholesterol levels reported in breast milk or in existing infant formulas (e.g., Claumarchirant et al., 2015) may be helpful.

Because the sterol data included in Table 8 did not include the non-4-desmethyl sterols (cycloartenol, 24-methylene cycloartenol, and citrostadienol), which are also quantified when using NMKL 198.2014, we have updated Table 8 with the complete sterol profiles of three of the same batches presented in the original Table 8 (see Table 3 in our response to Question 13). Using these new data, we have now rederived the % Total Sterols presented in the revised Table 17 (see Table 17 in our response to Question 13). There is now a 1.7-fold increase in the amount of cholesterol on the percent total sterols basis in the subject of this Notice relative to the amount in the subject of GRN 000677. Importantly, this 1.7-fold increase is not expected to be a safety concern.

When used as the source of DHA in infant formula at the maximum use level of 0.5 % fat, the subject of this Notice will account for a minor fraction of the overall

cholesterol content in the resulting infant formula. Specifically, human milk and infant formulas contain 43 to 292 mg and 3 to 258 mg cholesterol/L, respectively (Claumarchirant et al., 2015). If the maximum amount of fat allowed in infant formula is 6 grams/100 kcal per 21 CFR 107.100, infant formulas contain 670 kcal/L (Martinez and Ballew, 2011), the subject of this Notice contains an average of 57 % DHA and 231 mg cholesterol/100 g oil, and the oil is added to the infant formula to achieve a DHA level of 0.5 % total fat, then the amount of cholesterol derived from the subject of this Notice in the infant formula will be 0.817 mg/L, which is orders of magnitude below the levels found in human milk and infant formula. Therefore, the 1.7-fold increase in the levels of cholesterol derived from the subject of this Notice will not significantly increase the exposure of infants to cholesterol compared the subject of GRN 000677 and is not a safety concern. Our calculations for the amount of cholesterol from the subject of this Notice in infant formula are provided below.

a. Calculation for the amount of DHA in 1 liter of infant formula:

(6.0 g fat/100 kcal formula)(670 kcal/L formula)(0.5 g DHA/100 g fat) = 0.201 g DHA/L formula

b. Calculation for the amount of the *S. limacinum* TKD-1-derived oil in 1 liter of infant formula, assuming a DHA content of 57%:

(0.201 g DHA/L formula)(0.1 kg oil/57 g DHA)=0.000353 kg oil/L formula

c. Calculation for the amount of cholesterol from the *S. limacinum* TKD-1-derived oil in infant formula:

(0.000353 kg oil/L formula)(231 mg cholesterol/0.1 kg oil) = 0.817 mg cholesterol/L formula.

c. *We note that the levels of sitostanol and delta-5-avenasterol are reported separately in GRN 000677 (Table 7; page 21), while ATK Biotech reports the levels of these two sterols as a single value. Given ATK Biotech's reliance on the safety data and narrative from GRN 000677, please explain how an accurate comparison, and by extension equivalence and safety, of these two sterols can be achieved based on using the article of commerce in GRN 000677 as a comparator.*

Although the levels of sitostanol and delta-5-avenasterol are reported as a single value in this Notice, an accurate comparison and by extension equivalence and safety of these two sterols can be achieved using the article of commerce in GRN 000677 as a comparator as follows:

1. By comparing the total amount of these two sterols/stanols in infant formula and conventional foods from the use of the subjects of this Notice and GRN 000677 at the maximum use levels;

2. By comparing the relative amounts of these two sterols/stanols in the subject of this Notice, GRN 000677, and other sources of fat in infant formula;
3. By comparing the levels of these two sterols/stanols to those in other *Schizochytrium* sp.-derived oils that are GRAS for use in infant formulas and conventional foods.

The subject of this Notice contains approximately 12.3 mg sitostanol and delta-5-avenasterol/100 g oil whereas the subject of GRN 000677 contains approximately 4.8 mg sitostanol and delta-5-avenasterol/100 g oil (((3.02 % delta-5-avenasterol + 0.17 % sitostanol)/100) x 150 mg/100 g sterols); see Table 17 above). Because both oils are intended to be used in infant formula at the maximum use level of DHA of 0.5 % total fat, the total amount of delta-5-avenasterol and sitostanol added to infant formula from the subject of this Notice and the subject of GRN 000677 will be approximately 0.043 and 0.024 mg/L formula, respectively, assuming that the amount of fat in infant formula is 6.0 g fat/100 kcal per 21 CFR 107.10, infant formula contains 670 kcal/L (Martinez and Ballew, 2011), and the subject of this Notice and GRN 000677 contain 57% and 40% DHA, respectively. Our calculations for the amount of delta-5-avenasterol and sitostanol in infant formula from the subject of this Notice are below. Similar calculations were used to calculate the amount of delta-5-avenasterol and sitostanol from the subject of GRN 000677, although a DHA content of 40% was used instead of 57%.

a. Calculation for the amount of DHA in 1 liter of infant formula:

(6.0 g fat/100 kcal formula)(670 kcal/L formula)(0.5 g DHA/100 g fat) = 0.201 g DHA/L formula

b. Calculation for the amount of the *S. limacinum* TKD-1-derived oil in 1 liter of infant formula, assuming a DHA content of 57%:

(0.201 g DHA/L formula)(0.1 kg oil/57 g DHA)=0.000353 kg oil/L formula

c. Calculation for the amount of delta-5-avenasterol and sitostanol from the *S. limacinum* TKD-1-derived oil in infant formula:

(0.000353 kg oil/L formula)(12.3 mg 5-avenasterol + sitostanol /0.1 kg oil) = 0.043 mg delta-5-avenasterol + sitostanol/L formula.

Importantly, the vast majority (>99%) of fat and sterols in infant formulas are derived from blends of edible vegetable oils, such as rapeseed, sunflower, coconut, and palm oils. Moreover, the edible vegetable oils used in infant formulas contain a similar amount of sterols, have a similar proportion of fat to total sterols, and contain similar levels of sitostanol and delta-5-avenasterol as those in the subjects of this Notice and GRN 677 (Claumarchirant et al., 2015; Xu

et al., 2018a; Yang et al., 2019; Phillips et al., 2002). Because infant formulas must contain 6.0 g fat/100 kcal per 21 CFR 107.10 and 670 kcal/L (Martinez and Ballew, 2011), the maximum amount of fat allowed in infant formula is approximately 40.2 g fat/L. When the subject of this Notice or GRN 000677 are added to infant formula to achieve the maximum use level of DHA of 0.5% fat, the subject of this Notice or GRN 000677 will contribute 0.35 mg/L (0.9%) or 0.50 mg fat/L (1.3%) to the total fat content of the resulting infant formula, respectively. The subjects of this Notice and GRN 677 will therefore contribute approximately 1% to the total sitostanol and delta-5-avenasterol content of the infant formula, assuming the sterol content of all the oils used in the infant formula, including the subject of this Notice and GRN 000677, contain a similar amount of sterols and a similar proportion of fat to total sterols. Thus, the small difference in the sitostanol and delta-5-avenasterol levels in the subject of this Notice and the subject of GRN 000677 does not affect the equivalence of the subject of this Notice compared to that of GRN 000677 or the total sitostanol and delta-5-avenasterol content of the infant formula. Our calculations for the amount of fat contributed by the subject of this Notice are provided below. Similar calculations were be used to calculate the amount of fat contributed by the subject of GRN 000677, although a DHA content of 40% was used instead of 57%.

a. Calculation for the amount of fat in 1 liter of infant formula:

   (6.0 g fat/100 kcal formula)(670 kcal/L formula)= 40.2 g fat/L formula

b. Calculation for the amount of DHA in 1 liter of infant formula:

   (40.2 g fat/L formula)(0.5 g DHA/100 g fat)=0.201 g DHA/L formula

c. Calculation for the amount of fat from the *S. limacinum* TKD-1-derived oil in infant formula:

   (0.201 g DHA/L formula)(100 g fat/57 g DHA)=0.35 g S. limacinum TKD-1-derived oi/L formula

For conventional foods, consumers of the subject of this Notice and the subject of GRN 000677 are exposed to delta-5-avenasterol and sitostanol through diet as they are component of edible oils, fruits, vegetables, potatoes, nuts, and grains (Klingberg et al., 2008; Yang et al., 2019; Phillips et al., 2002; Xu et al., 2018a). Based on the 2011-2012 What We Eat in America Survey, males and females two years and over consume approximately 25 g of edible oils/day (https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/FPED/tables_1-4_FPED_1112.pdf; accessed on October 25, 2021). If the intended conventional foods are formulated to deliver 1.5 g DHA/day, consumers would be consuming 2.63 g of the subject of this Notice and 3.75 g of the subject of GRN 000677,

which is well below the mean intake of vegetable oils and by extension the sterols the contain in the United States.

Lastly, sitostanol and delta-5-avenasterol have also been reported as single values in GRN 000933 and 000944, which received "no questions" letters from the Agency. Although the subjects of these Notices were not directly tested in toxicology studies, the levels of sitostanol and delta-5-avenasterol in the subjects of GRNs 000933 and 000944 are comparable to those in the subject of this Notice: 7.2 mg/100 g vs 26 mg/100 g vs 9 mg/100 g, respectively.

Microbiology:

17. *Please confirm that A. limacinum TKD-1 is non-pathogenic and non-toxigenic.*

A. limacinum TKD-1 is non-pathogenic and non-toxigenic

18. *It is unclear what is meant by "Food Chemical Codex Grade" as the regulatory status of alkaline protease in Table 4 of the notice. Please confirm that the enzyme is food grade and provide the following information about the alkaline protease derived from Bacillus licheniformis:*

a. *Please confirm that the introduced protease is from a non-toxigenic and non-pathogenic source.*

The introduced protease is from a non-toxigenic and non-pathogenic source.

b. *Please confirm that the protease meets the specifications for an enzyme preparation in the FCC 12 (2021) and the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006).*

The enzyme meets the specifications laid down by the Joint FAO/WHO Expert Committee on Food Additives and the Food Chemicals Codex.

c. *Please indicate if the protease is expected to be present in the final product; if not, provide a narrative describing its removal.*

Due to the extreme temperatures used in refining the subject of this Notice, the protease will be completely denatured in the final product. To confirm that the *S. limacinum* TKD-1-derived oil is devoid of the alkaline protease, ATK Biotech determined the protease activity in five lots of finished product using the compendial method GB/T 23527-2009 at Eurofins, which is an ISO/IEC 17025:2005-accredited laboratory. The limit of detection is 1 Unit/g, which is defined as the production of 1 μg tyrosine in 1 minute. A sample of the *Bacillus licheniformis* alkaline protease used to produce the *S. limacinum* TKD-1-derived

oil was used as a positive control. 218,720 Units/ml were detected in the *Bacillus licheniformis* alkaline protease sample whereas no activity was detected in five lots of the *S. limacinum* TKD-1-derived oil. Therefore, the *S. limacinum* TKD-1-derived oil does not contain the *Bacillus licheniformis* alkaline protease.

19. Please provide a specification for Cronobacter sakazakii and data from at least three non-consecutive batch analyses to demonstrate that algal oil (≥35% DHA) can be manufactured to meet this specification.

Five lots of the *S. limacinum* TKD-1-derived oil were tested using the ISO 22964:2017 compendial method to confirm the absence of *Cronobacter spp.* in 10 g. *Cronobacter spp.* was not detected in any of the five lots that were tested (Table 6). Moving forward, ATK Biotech has added a product specification of negative in 10 g for *Cronobacter* spp.

Table 6. Absence of Cronobacter spp. In the *S. limacinum* TKD-1-derived Oil[1]

		Lot Number[2]				
Method	**LOQ**	**DOG20210515D**	**DOG20210518D**	**DOG20210604D**	**DOG20210606D**	**DOG20210710D**
ISO 22964:2017	0.01	Negative/10 g	Negative /10 g	Negative /10 g	Negative /10 g	Negative /10 g

20. We note that the strain deposition certificate in Appendix 2 is not in English; however, the deposition information for A. limacinum was provided in the notice so Appendix 2 was not needed.

Thank you for the guidance.

Should you need any additional information, please feel free to contact me at 240-367-6089 or dconze@spherixgroup.com.

Sincerely,

Dietrich B. Conze, Ph.D.
Managing Partner

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US FDA, GRN 000843 (2019). Algal oil (35% docosahexaenoic acid) from Schizochytrium sp. strain FCC-1324. Fermentalg.

US FDA, GRN 000844 (2019). Algal oil (55% docosahexaenoic acid) from Schizochytrium sp. strain FCC-3204. Fermentalg.

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US FDA, GRN 000913 (2020). Algal oil (minimum 35% DHA). Mara Renewables Corporation

US FDA, GRN 000933 (2020). Algal oil (≥36% docosahexaenoic acid) from *Schizochytrium* sp. strain DHF. Hubei Fuxing BioTechnology, Co., Ltd.

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I. SIGNED STATEMENT OF THE CONCLUSION OF GENERALLY RECOGNIZED AS SAFE (GRAS) AND CERTIFICATION OF CONFORMITY TO 21 CFR §170.205-170.260

A. SUBMISSION OF GRAS NOTICE

ATK Biotech Co., Ltd (formerly Jiangsu Tiankai Biotechnology Co., Ltd. and TK Biohealth Co., Ltd) is hereby submitting a GRAS notice in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

ATK Biotech Co., Ltd (formerly Jiangsu Tiankai Biotechnology Co., Ltd. and TK Biohealth Co., Ltd)
109 Husong Road
Shitan Industrial Park
Shizi Town, Quanjiao County
Chuzhou City, China

C. COMMON OR USUAL NAME

Algal oil from *Schizochytrium limacinum* TKD-1

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

This notification does not contain any trade secret or confidential information.

E. INTENDED USE

ATK Biotech Co., Ltd intends to use the algal oil from *Schizochytrium limacinum* TKD-1 as an ingredient in exempt and non-exempt cow's milk and soy-based infant formulas for preterm and term infants and as an ingredient in the food categories listed in 21 CFR 184.1472(a)(3).

F. BASIS FOR GRAS DETERMINATION

The use of Algal oil from *Schizochytrium limacinum* TKD-1 in exempt and non-exempt cow's milk and soy-based infant formulas for preterm and term infants, and general foods has been determined to be GRAS using scientific procedures, and generally available and accepted information specified under 21 CFR §170.30 (a) (b). The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part VII of this GRAS notice contains the citations for the published studies. These publicly available data and information fulfill the requirement of the GRAS standard for general availability of the scientific data, information, and methods relied on to establish the safety of DHA Algal Oil for its intended conditions of use. The peer-review of the published studies and lack of Letters to the Editor or other dissenting opinions provide ample evidence of general recognition among qualified experts that there is reasonable

certainty that consumption of Algal Oil for its intended use is not harmful. The general availability and acceptance of these scientific data, information, and methods satisfy the criterion of the GRAS standard that general recognition of safety requires common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food that there is reasonable certainty that the substance is not harmful under the conditions of its intended use.

As presented in this Notice, the scientific basis for GRAS is based on the following:

1. The subject of this GRAS Notice, Algal oil from *Schizochytrium limacinum* TKD-1, is derived from *S. limacinum* TKD-1, which is a strain of *S. limacinum.*

    a. *Schizochytrium limacinum* is a microalgae known to produce large amounts of DHA, as well as other omega-3 fatty acids.

    b. The 18s rRNA sequence of *S. limacinum* TKD-1 is 88% identical to *Schizochytrium* sp. ONC-T18, which is the subject of GRN 677.

2. The subject of this GRAS Notice is manufactured according to current Good Manufacturing Practices using processing aids, and food contact substances that conform to the conditions of use specified in Title 21 of the United States Code of Federal Regulations and/or Food Chemicals Codex specifications.

    a. Process procedures and product specifications are in place to control pivotal quality attributes that ensure a consistent, safe, food-grade finished ingredient.

    b. The available stability studies support a shelf-life of 1 year at 4-5°C and 2 years at -18°C.

3. There is a long history of safe use of *Schizochytrium* sp.-derived, DHA-rich oils in infant formulas and general foods worldwide.

4. The subject of this GRAS Notice is quantitatively and qualitatively equivalent with the subject of GRN 677. Therefore, the toxicology studies conducted by Schmitt et al. (2012a) and Schmitt et al. (2012b) with *Schizochytrium* sp. ONC-T18 support the safe use of the subject of this GRAS Notice in infant formula and general foods.

5. The published toxicology studies conducted by Schmitt et al. (2012a) and Schmitt et al. (2012b) demonstrate that the DHA-rich oil derived from *Schizochytrium* sp. ONC-T18 is not genotoxic and does not result in adverse effects at the highest levels tested (3305 and 3679 mg/kg body weight/day in male and female rats, respectively).

6. Numerous toxicology studies of *Schizochytrium* sp.-derived DHA-rich oils conducted over a period of more than a decade, include acute, subacute, and subchronic toxicity, a battery of genotoxicity studies, and developmental and reproductive toxicity studies. In these reports, no evidence of toxicity was noted at up to 5,000 mg/kg bw/day. Therefore, *Schizochytrium* sp.-derived, DHA-rich oils are not genotoxic or toxigenic.

7. DHA-rich oils from numerous sources including *Schizochytrium* sp., *Crypthecodinium cohnii*, *Ulkenia* sp. SAM2179, *Chlorella protothecoides* strain S106, *Prototheca moriformis* strain S2532, tuna and other fish are GRAS for use in general foods and exempt and non-exempt infant formulas for preterm and term infants (GRN 41; GRN 137; GRN 138; GRN 319; GRN 384; GRN 469; GRN 527; GRN 553; GRN 677; GRN 731; GRN 732; GRN 776; GRN 777; GRN 836; GRN 843; GRN 844; GRN 862; GRN 913; GRN 933).

8. The publicly available scientific literature on the consumption and the safety of DHA-rich oils in clinical studies with both term and pre-term infants is extensive and sufficient to support the safety and GRAS status of the proposed DHA-rich oil ingredients. Published clinical studies that show that *Schizochytrium* sp.-derived, DHA-rich oils are also safe and well-tolerated in adults.

9. Literature searches did not identify safety/toxicity concerns related to the *Schizochytrium* sp.-derived DHA-rich oil ingredient.

10. The use of Algal oil from *Schizochytrium limacinum* TKD-1 in exempt and non-exempt preterm and term infant formulas will be used in conjunction with a safe and suitable source of arachidonic acid (ARA).

11. The estimated exposure to the subject of the GRAS Notice from its addition to exempt and non-exempt infant formulas for preterm and term infants is based on a target DHA concentration of 0.5% of total fat. Assuming infants consume approximately 100 to 120 kcal/kg body weight/day of which fat comprises about 50%, the corresponding DHA intake will be 27 to 33 mg DHA/kg body weight/day at the target DHA concentration of 0.5 % of total fat and approximately 43 to 62 mg Algal oil from *Schizochytrium limacinum* TKD-1/kg body weight/day, assuming the oil contains 53 to 62 % DHA. This DHA intakes are 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).

12. The estimated exposure to the subject of the GRAS Notice from its addition to general foods will result in a maximum dietary exposure of less than 1.5 grams of DHA/day.

    a. The proposed uses of the subject of this GRAS Notice are identical to those for other *Schizochytrium* sp.-derived, DHA-rich oils.

In all the studies summarized in this Notification, there were no significant adverse effects/events or tolerance issues attributable to *Schizochytrium* sp.-derived DHA or DHA-rich oils. Because this safety evaluation was based on generally available and widely accepted data and information, it satisfies the so-called "common knowledge" element of a GRAS determination. In addition, the intended uses of DHA-rich oil have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination.

Algal oil from *S. limacinum* TKD-1 has been the subject of a thorough safety assessment as described above. The general availability and general acceptance, throughout the scientific community of qualified experts, of the data and information that establish the safety of DHA Algal Oil under its intended conditions of use establish the general recognition of this data and information. Together, the establishment of safety based on scientific procedures and its general recognition form the basis for ATK Biotech Co., Ltd conclusion of GRAS status of DHA Algal Oil for its intended uses.

Therefore, Algal oil from *S. limacinum* TKD-1 is safe and GRAS at the proposed levels of addition to exempt and non-exempt cow's milk and soy-based infant formulas for preterm and term infants, and general foods. The subject of this GRAS Notice is therefore excluded from the definition of a food additive and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

G. PREMARKET APPROVAL

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the substance is GRAS under the conditions of intended use.

H. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Dietrich Conze, PhD, Managing Partner, Spherix Consulting Group Inc., at 751 Rockville Pike, Unit 30-B, Rockville, MD 20852; Telephone: 240-367-6089; Email: dconze@spherixgroup.com; or be sent to FDA upon request.

I. FREEDOM OF INFORMATION ACT (FOIA)

Parts 2 through 7 of this notification do not contain data or information that is exempt from disclosure under the FOIA.

J. INFORMATION INCLUDED IN THE GRAS NOTIFICATION

To the best of our knowledge, the information contained in this GRAS notification is complete, representative and balanced. It contains both favorable and unfavorable information, known to ATK Biotech Co., Ltd and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

Signature of Authorized Representative of
ATK Biotech Co., Ltd

November 3, 2021
Date

From:	Dietrich Conze
To:	Morissette, Rachel
Cc:	Kathy Brailer; Claire Kruger
Subject:	Re: [EXTERNAL] clarifications on GRN 1008
Date:	Monday, January 3, 2022 3:45:38 PM
Attachments:	ATK Response to FDA on GRN1008 1-3-22.docx

Hi Rachel.
Hope you had a good break. Attached are our responses to your latest questions regarding GRN 001008.
If you have any additional questions, please let me know.
Regards.
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

> On Dec 21, 2021, at 8:00 AM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Sounds good. Thanks for the heads up. Just as an FYI, I'll be off starting Thursday this week and will return Mon Jan. 3. Have a great holiday!
>
> Best,
>
> *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

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From: Dietrich Conze <dconze@spherixgroup.com>
Sent: Monday, December 20, 2021 3:50 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Kathy Brailer <kbrailer@spherixgroup.com>; Claire Kruger <ckruger@spherixgroup.com>
Subject: [EXTERNAL] Re: clarifications on GRN 1008

Hi Rachel.
Just wanted to let you know that we're working on our responses. For Question 2C, our client had to go back to the lab to get the data because the FCC monograph specifies that the amount of DPA n-6 in the product needs to be reported on a % area basis. The lab should be sending the data to us by the end of the week, so we should have our responses to you shortly after that.

Regards.
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

> On Dec 15, 2021, at 8:51 AM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dietz,

We had a few minor clarification questions. Please see below.

1. In the Dietary Exposure section on p. 23 of the notice, ATK Biotech states:

> "Therefore, the subject of this GRAS Notice is equivalent to the DHA-rich oils that are GRAS for use in exempt and non-exempt infant formulas for preterm and term infants, and conventional foods, and the resulting dietary exposures to this product will be the same as the dietary exposures for the subjects of GRNs 137, 553, 677, 776 and 843, which are incorporated by reference and summarized below for convenience."

   We note that GRNs 000553, 000677, and 000776 refer to infant formula uses only. Excluding infant formula-only notices, the above statement indicates that the use of algal oil (≥35% DHA) is substitutional for algal oils in GRNs 000137 and 000843. However, we note that these notices included uses in meat and poultry, while ATK Biotech excluded uses in meat and poultry products under the purview of USDA in this notice. ATK Biotech excluded USDA-regulated foods in Form 3667 of this submission and on p. 25 of the notice stating that algal oil (≥35% DHA) will be added at the maximum use levels "with the exception of egg, meat, poultry, and fish products." However, we note that the intended uses in Table 13 of the July 27, 2021 email update ATK Biotech provided to us includes egg, meat, poultry, and fish products.

   a. Please confirm the following statement: *The intended use of algal oil (≥35% DHA) is substitutional for other DHA-containing oils currently used in foods, excluding uses in egg, meat, poultry, and fish products; therefore, the cumulative dietary exposure to DHA is not expected to increase.*

   b. Please clarify if the excluded use in fish products refers only to Siluriformes fish, including catfish, under the purview of USDA or excludes all fish products.

   c. Please provide a clarifying statement that the uses in egg, meat, poultry, and fish products listed in Table 13 of the July 27, 2021 email update are not included among the intended uses for this GRAS notice.

2. We note that the specifications for algal oil (≥35% DHA) include a range of values for DHA (34-70%) and DPA (5-20%). It is unclear if these specifications reflect the unstandardized oil or the standardized oil or a combination of both. It is our understanding that the algal oil (≥35% DHA) added to infant formula would be minimally standardized, if at all, and would contain approximately 55% total fatty acids, whereas the algal oil (≥35% DHA) added to

conventional foods would be standardized to approximately 35% to achieve DHA levels analogous to other algal oil ingredients.

a. Please provide the fatty acid specifications for algal oil (≥35% DHA) added to infant formula based on batch analyses that were provided in the notice.

b. Please provide the fatty acid specifications for algal oil (≥35% DHA) added to conventional foods listed in 21 CFR 184.1472(a)(3) (Menhaden oil).

c. We note that in ATK Biotech's response to question 8b in the November 3, 2021 amendment, the batch analysis results for DPA in Table 2 reflect the n-3 form, while the DPA n-6 form is indicated in the FCC 12 monograph. The levels of DPA n-6 in Table 7 of the notice (12.98-14.51 mg/100 g) are much higher than those shown in Table 2 of the November 3, 2021 amendment (0.13-0.15 mg/100g). Please clarify this discrepancy.

3. ATK Biotech provides a specification for *Salmonella* serovars, listed as negative by test in 375 g. The method referenced is ISO 6579-1:2017. We note that this method is based on the analysis of a 25 g test portion. Please clarify that the analytical method used to detect *Salmonella* serovars has been validated for that purpose.

4. We note that the response to question 14 in the November 3, 2021 amendment includes the assumption that the typical caloric density of both pre-term and term infant formulas is 67 kcal/100 mL. We note that this assumption is not supported by the literature. Typical caloric density of pre-term infant formula is greater than that of term infant formula.

5. Please confirm the following statements from ATK Biotech's response to question 16c in the November 3, 2021 amendment:

    a. The referenced GRNs should be 000933 and 000934 (not 000944) in the last paragraph on p. 20.
    b. The levels of sitostanol and delta-5-avenasterol in the notice should be 12.3 mg/100 g (as stated on p. 18 of the November 3, 2021 amendment), and not 9 mg/100 g as shown in the last paragraph of the response to question 16c on p. 20.

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

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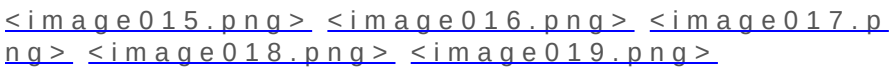

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Spherix Consulting Group

January 3, 2022

Rachel Morissette, Ph.D.
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 001008

Dear Dr. Morissette:

Below are our responses to your requests for additional information regarding GRN 001008 as stated in your email on December 15, 2021. Your requests are in italicized text and our responses are below in plain text:

1. In the Dietary Exposure section on p. 23 of the notice, ATK Biotech states:

> *"Therefore, the subject of this GRAS Notice is equivalent to the DHA-rich oils that are GRAS for use in exempt and non-exempt infant formulas for preterm and term infants, and conventional foods, and the resulting dietary exposures to this product will be the same as the dietary exposures for the subjects of GRNs 137, 553, 677, 776 and 843, which are incorporated by reference and summarized below for convenience."*

We note that GRNs 000553, 000677, and 000776 refer to infant formula uses only. Excluding infant formula-only notices, the above statement indicates that the use of algal oil (≥35% DHA) is substitutional for algal oils in GRNs 000137 and 000843. However, we note that these notices included uses in meat and poultry, while ATK Biotech excluded uses in meat and poultry products under the purview of USDA in this notice. ATK Biotech excluded USDA-regulated foods in Form 3667 of this submission and on p. 25 of the notice stating that algal oil (≥35% DHA) will be added at the maximum use levels "with the exception of egg, meat, poultry, and fish products." However, we note that the intended uses in Table 13 of the July 27, 2021 email update ATK Biotech provided to us includes egg, meat, poultry, and fish products.

a. Please confirm the following statement: The intended use of algal oil (≥35% DHA) is substitutional for other DHA-containing oils currently used in foods, excluding uses in egg, meat, poultry, and fish products; therefore, the cumulative dietary exposure to DHA is not expected to increase.

Yes, the intended use of algal oil (≥35% DHA) manufactured by ATK Biotech is substitutional for other DHA-containing oils currently used in foods, excluding uses in egg, meat, poultry, and fish products. Therefore, the cumulative dietary exposure to DHA is not expected to increase.

b. *Please clarify if the excluded use in fish products refers only to Siluriformes fish, including catfish, under the purview of USDA or excludes all fish products.*

All fish products are excluded.

c. *Please provide a clarifying statement that the uses in egg, meat, poultry, and fish products listed in Table 13 of the July 27, 2021 email update are not included among the intended uses for this GRAS notice.*

The uses in egg, meat, poultry, and fish products were erroneously included in the Table 13 that was provided in the July 27, 2021 email update. The intended uses for the subject of this GRAS notice do not include egg, meat, poultry, and fish products. A new version of Table 13 is now provided in our response to Question 2.

2. *We note that the specifications for algal oil (≥35% DHA) include a range of values for DHA (34-70%) and DPA (5-20%). It is unclear if these specifications reflect the unstandardized oil or the standardized oil or a combination of both. It is our understanding that the algal oil (≥35% DHA) added to infant formula would be minimally standardized, if at all, and would contain approximately 55% total fatty acids, whereas the algal oil (≥35% DHA) added to conventional foods would be standardized to approximately 35% to achieve DHA levels analogous to other algal oil ingredients.*

a. *Please provide the fatty acid specifications for algal oil (≥35% DHA) added to infant formula based on batch analyses that were provided in the notice.*

b. *Please provide the fatty acid specifications for algal oil (≥35% DHA) added to conventional foods listed in 21 CFR 184.1472(a)(3) (Menhaden oil).*

The specifications for DHA (34-70%) and DPA (5-20%) were set for the unstandardized oil and on a % fatty acid basis per our response to Question 6 in FDA's request for additional information on October 8, 2021. The subject of this Notice is intended to be used in exempt and non-exempt infant formulas for term and preterm infants, and the same conventional foods as the subject of GRN 137, excluding egg, meat, poultry, and fish products. Because the use levels of the subject of GRN 137 in conventional foods are based on the DHA content of approximately 35%, the intended uses levels specified in Table 13 have now been reduced to accommodate for the amount of DHA in the subject of this Notice when unstandardized (approximately 57 % wt). The revised use levels now ensure

that the exposure to DHA from the use of the subject of this Notice in conventional foods when unstandardized will not exceed 1.5 g/day.

Table 13. Maximum Intended Use Levels of DHA-rich oil from *Schizochytrium limacinum*

Category of Food	Maximum Intended Use Level (%)[1]
Cereals	0.71
Baked goods and baking mixes	0.88
Fats and oils (not including infant formula)	2.12
Milk products	0.88
Cheese products	0.88
Frozen dairy products	0.88
Condiments	0.88
Soup mixes	0.53
Snack foods	0.88
Nut Products	0.88
Gravies and sauces	0.88
Plant protein products	0.88
Processed vegetable juices	0.18
Hard candy	1.77
Soft candy	0.71
Jams and jellies	1.24
Dairy product analogs	0.88
Nonalcoholic beverages	0.09
Pastas	0.35
Processed fruit juices	0.18
White granulated sugar	0.71
Sugar substitutes	1.77
Chewing gum	0.53
Gelatins and puddings	0.18
Confections and frostings	0.88
Sweet sauces, toppings, and syrups	0.88

[1]Determined by dividing the DHA content of the subject of GRN 137 (35%) by the average DHA content of the subject of this Notice (57%) and then multiplying the quotient by the intended use levels specified in GRN 137.

c. *We note that in ATK Biotech's response to question 8b in the November 3, 2021 amendment, the batch analysis results for DPA in Table 2 reflect the n-3 form, while the DPA n-6 form is indicated in the FCC 12 monograph. The levels of DPA n-6 in Table 7 of the notice (12.98-14.51 mg/100 g) are much higher than those shown in Table 2 of the November 3, 2021 amendment (0.13-0.15 mg/100g). Please clarify this discrepancy.*

Yes, the levels of DPA n-3 on an area % basis were mistakenly provided in the November 3, 2021 amendment. The level of DPA n-6 for the same five batches on an area % basis is approximately 14.5 +/- 0.65 (Table 1).

Table 1. Comparison of Long Chain Polyunsaturated Fatty Acid Composition in the *Schizochytrium limacinum* (TKD-1)-Derived Oil with the Long Chain Polyunsaturated Fatty Acid Ranges Specified in the FCC Monograph for Algal (*Schizochytrium* sp.) Oil

Fatty Acid	FCC Specification	Lot Number					Average ± Standard Deviation
		DD20191206	DD20191213	DD20191220	DD20200101	DD20200110	
Docosapentaenoic acid n-6 (DPA n-6; area %)	10.5 – 16.5	15.3	13.5	14.6	14.6	14.6	14.5 ± 0.65

3. *ATK Biotech provides a specification for Salmonella serovars, listed as negative by test in 375 g. The method referenced is ISO 6579-1:2017. We note that this method is based on the analysis of a 25 g test portion. Please clarify that the analytical method used to detect Salmonella serovars has been validated for that purpose.*

   This is a typographical error. The specification should be "Negative/25 g".

4. *We note that the response to question 14 in the November 3, 2021 amendment includes the assumption that the typical caloric density of both pre-term and term infant formulas is 67 kcal/100 mL. We note that this assumption is not supported by the literature. Typical caloric density of pre-term infant formula is greater than that of term infant formula.*

   Yes, the typical caloric content of preterm infant formula is 80 kcal/100 ml (Martinez and Ballew, 2011).

5. *Please confirm the following statements from ATK Biotech's response to question 16c in the November 3, 2021 amendment:*

   a. *The referenced GRNs should be 000933 and 000934 (not 000944) in the last paragraph on p. 20.*

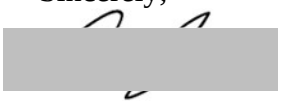
      Yes, the referenced GRNs should be 000933 and 000934.

   b. *The levels of sitostanol and delta-5-avenasterol in the notice should be 12.3 mg/100 g (as stated on p. 18 of the November 3, 2021 amendment), and not 9 mg/100 g as shown in the last paragraph of the response to question 16c on p. 20.*

      Yes, the levels of sitostanol and delta-5-avenasterol in the last paragraph of the response to question 16c on p. 20 should be 12.3 mg/100 g.

Should you need any additional information, please feel free to contact me at 240-367-6089 or dconze@spherixgroup.com.

Sincerely,



Dietrich B. Conze, Ph.D.
Managing Partner

Reference

Martinez, J.A., and Ballew, M.P. (2011). Infant formulas. Pediatr Rev *32*, 179–189.

From: Dietrich Conze
To: West-Barnette, Shayla
Cc: Kathy Brailer; Morissette, Rachel; Claire Kruger
Subject: [EXTERNAL] Re: clarification requested for GRN 1008
Date: Thursday, February 3, 2022 5:20:25 PM
Attachments: ATK Response to FDA on GRN1008 2-3-22.pdf

Hi Shayla,

Attached are our responses to FDA's recent requests for clarification. If you have any questions, please let me know.

Regards.
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

> On Jan 31, 2022, at 3:16 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Dear Dietz,
>
> Our chemist had a few clarifying questions for GRN 001008. Please see below. I will be out of the office until Feb. 7, so feel free to send the responses to Shayla West-Barnette (shayla.westbarnette@fda.hhs.gov) if you have them ready before I return. She will send them to the review team.
>
> In the questions we sent to you on Oct. 8, 2021, we asked for clarification of the fatty acid specifications for the ingredient that is the subject of GRN 001008. In the Jan. 3, 2022 amendment you noted that the specifications for DHA (34-70%) and DPA n-6 (5-20%) were set for the unstandardized algal oil from *Aurantiochytrium limacinum* TKD-1. Our understanding based on the batch analyses is that the mean concentrations of DHA and DPA in the unstandardized algal oil are 57% and 14% of the total fatty acids,

respectively. Based on the reported mean DHA concentration of 57% and the assumption of a DHA level of 57% in the dietary exposure estimates, we would refer to this oil as "algal oil (57% DHA)". We also understand that the oil may be standardized with sunflower oil to achieve levels as low as 35% DHA in the algal oil, but that the unstandardized oil, as produced by fermentation, contains at least 50% DHA. We note that an oil containing 34% DHA contains other fatty acids in higher amounts than DHA —this type of oil has not been described in the notice. It is our understanding, based on the batch analyses and the assumptions used in the dietary exposure estimates, that the DHA level in the unstandardized oil ranges from approximately 53-61% of total fatty acids, while the mean level of DHA from the batch analyses is 57%. We request the following information:

A. Please provide specifications for DHA and DPA n-6 that represent the range of these fatty acids (reported to be 53-61% and 13-14.5%, respectively, in Table 7 of GRN 001008) in the unstandardized oil.

B. Please provide statements confirming or clarifying the following:
   1. If the concentration of DHA in unstandardized algal oil exceeds 57%, the intended use levels in the food categories listed in 21 CFR 184.1472(a)(3) (Menhaden oil) will be reduced proportionately to ensure that the estimated dietary exposure to DHA from the use of the algal oil will not exceed 1.5 g/p/d DHA.
   2. The oil may be standardized to 35% DHA with a food-grade oil. If standardized to 35% DHA, the intended uses in the food categories listed in 21 CFR 184.1472(a)(3) (Menhaden oil) will be no more than 29% of the levels specified in that regulation.
   3. The intended use of algal oil from *A. limacinum* TKD-1 is substitutional for other DHA-containing oils on the market and estimated dietary exposure is not expected to change based on infant formula or conventional food uses.
   4. You previously noted in the Nov. 3, 2022 amendment that if algal oil from *A. limacinum* TKD-1 is used with a source of eicosapentaenoic acid (EPA) in conventional foods, the total dietary exposure to DHA will be no more than 1.5 g/p/d and the total dietary exposure to DHA and EPA combined will be no more than 3.0 g/p/d. Please confirm that statement is still accurate.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

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

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Spherix Consulting Group

February 3, 2022

Shayla West-Barnett, Ph.D.
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 001008

Dear Dr. West-Barnett:

Below are our responses to your requests for additional information regarding GRN 001008 as stated in your email on January 31, 2022. Your requests are in italicized text and our responses are below in plain text.

In the questions we sent to you on Oct. 8, 2021, we asked for clarification of the fatty acid specifications for the ingredient that is the subject of GRN 001008. In the Jan. 3, 2022 amendment you noted that the specifications for DHA (34-70%) and DPA n-6 (5-20%) were set for the unstandardized algal oil from Aurantiochytrium limacinum TKD-1. Our understanding based on the batch analyses is that the mean concentrations of DHA and DPA in the unstandardized algal oil are 57% and 14% of the total fatty acids, respectively. Based on the reported mean DHA concentration of 57% and the assumption of a DHA level of 57% in the dietary exposure estimates, we would refer to this oil as "algal oil (57% DHA)". We also understand that the oil may be standardized with sunflower oil to achieve levels as low as 35% DHA in the algal oil, but that the unstandardized oil, as produced by fermentation, contains at least 50% DHA. We note that an oil containing 34% DHA contains other fatty acids in higher amounts than DHA—this type of oil has not been described in the notice. It is our understanding, based on the batch analyses and the assumptions used in the dietary exposure estimates, that the DHA level in the unstandardized oil ranges from approximately 53-61% of total fatty acids, while the mean level of DHA from the batch analyses is 57%. We request the following information:

A. *Please provide specifications for DHA and DPA n-6 that represent the range of these fatty acids (reported to be 53-61% and 13-14.5%, respectively, in Table 7 of GRN 001008) in the unstandardized oil.*

ATK Biotech has put in place DHA and DPA content specifications for the unstandardized oil of 45 to 70 % (g/100 g) and 10 to 20 % (g/100 g), respectively.

751 Rockville Pike, Unit 30-B
Rockville, MD 20852
301-557-0375; info@spherixgroup.com
http://spherixgroup.html

B. Please provide statements confirming or clarifying the following:

1. *If the concentration of DHA in unstandardized algal oil exceeds 57%, the intended use levels in the food categories listed in 21 CFR 184.1472(a)(3) (Menhaden oil) will be reduced proportionately to ensure that the estimated dietary exposure to DHA from the use of the algal oil will not exceed 1.5 g/p/d DHA.*

   ATK Biotech confirms that if the concentration of DHA in the unstandardized algal oil exceeds 57%, the intended use levels in the food categories listed in 21 CFR 184.1472(a)(3) (Menhaden oil) will be reduced proportionately to ensure that the estimated dietary exposure to DHA from the use of the algal oil will not exceed 1.5 g/p/d DHA.

2. *The oil may be standardized to 35% DHA with a food-grade oil. If standardized to 35% DHA, the intended uses in the food categories listed in 21 CFR 184.1472(a)(3) (Menhaden oil) will be no more than 29% of the levels specified in that regulation.*

   ATK Biotech confirms that if the oil is standardized to 35% with a food-grade oil, then the intended uses in the food categories listed in 21 CFR 184.1472(a)(3) (Menhaden oil) will be not more than 29% of the levels specified in that regulation.

3. *The intended use of algal oil from A. limacinum TKD-1 is substitutional for other DHA-containing oils on the market and estimated dietary exposure is not expected to change based on infant formula or conventional food uses.*

   ATK Biotech confirms the intended use of algal oil from *A. limacinum* TKD-1 is substitutional for other DHA-containing oils on the market and that the estimated dietary exposure is not expected to change based on infant formula or conventional food uses.

4. *You previously noted in the Nov. 3, 2022 amendment that if algal oil from A. limacinum TKD-1 is used with a source of eicosapentaenoic acid (EPA) in conventional foods, the total dietary exposure to DHA will be no more than 1.5 g/p/d and the total dietary exposure to DHA and EPA combined will be no more than 3.0 g/p/d. Please confirm that statement is still accurate.*

   Yes, the statement is still accurate.

Should you need any additional information, please feel free to contact me at 240-367-6089 or dconze@spherixgroup.com.

Sincerely,

Dietrich B. Conze, Ph.D.
Managing Partner

Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30B, Rockville, MD 20852
301-557-0375; info@spherixgroup.com