Notice to US Food and Drug Administration of the Conclusion that the Intended Use of DHA Algal Oil is Generally Recognized as Safe

Submitted by the Notifier:

Xiamen Huison Biotech Co., LDT
No 1337, Middle of Tongji Road
Tongji Industrial Area
Tong'an District, Xiamen, Peoples Republic of China

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc.
2800 E. Madison, Suite 202
Seattle WA 98112

December 20, 2018

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Susan Carlson, PhD
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Department of Health and Human Services
5001 Campus Drive
College Park, MD 20740

Dear Dr. Carlson:

In accordance with regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of Xiamen Huison Biotech Co., LTD (the notifier), the undersigned, Timothy Murbach, submits, for FDA review, the enclosed notice that DHA Algal Oil is GRAS for use in foods.

Should you have any questions or concerns regarding this notice, please contact me at 253-286-2888 or tim@aibmr.com.

Sincerely,

Timothy Murbach, ND, DABT (agent of the notifier)
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc. ("AIBMR")

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Figures and Tables

Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

Xiamen Huison Biotech Co., LTD (the notifier) is submitting a new GRAS notice in accordance with 21 CFR Part 170, Subpart E, regarding the conclusion that DHA Algal Oil is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

1.2 Name and Address of the Notifier and Agent of the Notifier

Notifier

Jane Wu
International Trading Director
Xiamen Huison Biotech Co., LTD
Number 1337, Middle of Tongji Road
Tongji Industrial Area
Tong'an District, Xiamen, Peoples Republic of China

Agent of the Notifier

Timothy Murbach, ND, DABT
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc.
2800 E. Madison
Seattle, WA 98112
Tel: (253) 286-2888
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1.3 Name of the Substance

DHA Algal Oil—a mixture of concentrated edible fatty acid triglycerides derived from *Schizochytrium* sp., strain HS01. DHA Algal Oil contains 50–60% docosahexaenoic acid (DHA; C22:6, all-*cis*- $\Delta^{4, 7, 10, 13, 16, 19}$).

1.4 Intended Conditions of Use

DHA Algal Oil is intended to be used as an ingredient in the food categories gelatin desserts or salads at a maximum concentration of 0.33% (approximately 0.2% DHA) and in vegetable oils at a maximum concentration of 3.0% (approximately 1.8% DHA) as a source of edible oils to replace other edible fats and oils normally

contained in the aforementioned categories. DHA Algal Oil will not be combined or augmented with any other oil that is rich in DHA or eicosapentaenoic acid (EPA).

DHA Algal Oil is not intended for use in foods where standards of identity would preclude such use. The ingredient is not intended for use in infant formula or any products that would require additional regulatory review by USDA.

1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of DHA Algal Oil for its intended conditions of use, stated in Part 1.4 of this report, has been made based on scientific procedures.

1.6 Not Subject to Premarket approval

Xiamen Huison has concluded that DHA Algal Oil is GRAS for its intended conditions of use, stated in Part 1.4 of this report, and, therefore, such use of DHA Algal Oil is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of Jane Wu at:

Xiamen Huison Biotech Co., LTD
Number 1337, Middle of Tongji Road
Tongji Industrial Area
Tong'an District, Xiamen, Peoples Republic of China

or will be sent to FDA upon request.

1.8 Exemption from Disclosure under the Freedom of Information Act

None of the data and information in Parts 2 through 7 of this GRAS notice are considered exempt from disclosure under the Freedom of Information Act (FOIA) as trade secret or commercial or financial information that is privileged or confidential.

1.9 Certification of Completion

We hereby certify that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of DHA Algal Oil.

December 20, 2018

Jane Wu
International Trading Director
Xiamen Huison Biotech Co., LTD

Date

Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification

DHA Algal Oil is derived from *Schizochytrium* sp., strain HS01, a proprietary unicellular walled osmotroph of the Thraustochytriaceae family that has not been genetically engineered. However, taxonomy of kingdom Chromista, as well as genera of the Labyrinthulea class, is an evolving field with multiple active hypotheses that remain as of yet, non-definitive.[1, 2] *Schizochytrium* spp. are single celled organisms with thin walls and pale yellow globulus bodies.[1] They produce large colonies by continuous binary division and form well-developed ectoplasmic nets in both seawater/pine pollen culture and enriched media. As a chemotaxonomic feature, the distinguishing polyunsaturated fatty acid profile (PUFA) is composed of about 20% arachidonic acid (AA; note; DHA contents reported by Yokoyama et al. were greater than 60%, but this was not reported as a characterizing feature), and the carotenoid content is limited strictly to beta-carotene. Finally, the 18S rRNA gene sequences are distinct. One possible taxonomic ranking of *Schizochytrium* sp., strain HS01 derived from current literature[1, 2] is shown below:

Domain, Eukaryota
Kingdom, Chromista
Subkingdom, Harosa
Infrakingdom, Halvaria
Superphylum, Heterokonta
Phylum, Bigyra
Subphylum, Sagenista
Class, Labyrinthulea
Order, Thraustochytriales
Family, Thraustochytriaceae
Genus, *Schizochytrium*
Species, *Schizochytrium* sp., strain HS01

The production strain for Xiamen Huison's DHA Algal Oil was originally harvested from mangrove leaf material at multiple locations in the mangrove forest that lies in Yunxiao town, Zhangzhou city, Fujian province, China and was obtained from the China general microbiological culture collection center (CGMCC). Xiamen Huison acclimatized *Schizochytrium* sp., strain CGMCC No. 136746 for optimal DHA yield and branded the organism as *Schizochytrium* sp., strain HS01. Identification of *Schizochytrium* sp., strain HS01 as a strain allotted to *Schizochytrium* spp. was performed by morphology and 18S rRNA sequence, and third-party verification of the species was provided by the Institute of Microbiology, Chinese Academy of Sciences.

Morphologically, *Schizochytrium* sp., strain HS01 was described as rapidly growing on sea water malt extract agar medium, forming large colonies by continuous binary

division that appeared white, turning pale orange with age. The formation of ectoplasmic nets or zoospores were not observed. The cell bodies are thin-walled, translucent, and globulus. The 18S rRNA sequence phylogenetic analysis shows an internal branch bootstrap value of 100% on a branch shared with *Schizochytrium limacinum* NIBH SR21, which is now reclassified as *Aurantiochytrium limacinum* NIBH SR21 according to Yokoyama et al. and adopted by MycoBank Database (although *Schizochytrium limacinum* is still given as the current name).[1, 3] Like *Schizochytrium*, *Aurantiochytrium* spp. are single celled organisms with thin walls, but differ in carotenoid composition and appear orange.[1] Colonies are formed by continuous binary division, but, as characterizing morphological features (which are critical differences compared to *Schizochytrium*), they tend to be small and not to develop ectoplasmic nets in both seawater/pine pollen culture and enriched media. PUFA content is comprised of <5% AA and approximately 80% DHA. Thus, it appears somewhat uncertain whether *Schizochytrium* sp., strain HS01 should be assigned to genus *Schizochytrium*, *Aurantiochytrium*, or another of the Thraustochytriaceae.

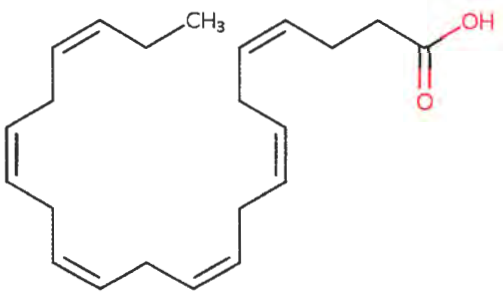

Figure 1. Chemical Structure of DHA

Schizochytrium sp., strain HS01 is high in fat content with the biomass containing >18% DHA (C22:6, all-*cis*- $\Delta^{4,7,10,13,16,19}$; IUPAC name (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid; CAS RN 6217-54-5). DHA is a long-chain omega 3 polyunsaturated fatty acid containing six double bonds and having a chemical formula of $C_{22}H_{32}O_2$ and a molar mass of 328.496 g/mol. The structural formula of DHA is shown in Figure 1.

DHA Algal Oil is concentrated to provide a minimum of 50% (and a maximum of 60%) DHA as the predominate fatty acid present in the ingredient. The only other fatty acids present at significant percentages of the final product are palmitic acid

(C16:0) and a docosapentaenoic acid isomer (C22:5, all-*cis*-$\Delta^{4, 7, 10, 13, 16}$), which are present at approximately 30 and 10%, respectively. EPA content of the oil is <1%. Other minor fatty acids present include saturated, monounsaturated, and polyunsaturated fatty acids typical of edible oils.

Fatty acids comprising DHA Algal Oil are present predominately (99.0%) as triacylglycerols (a.k.a., triglycerides) with monoglycerides, diglycerides, and free fatty acids contributing <0.5% each. The extracted and concentrated oil of *Schizochytrium* sp., strain HS01 is diluted with high oleic sunflower oil in order to adjust the DHA content to the desired specification, and antioxidants (vitamin E and citric acid) are added for stabilization. The finished Algal DHA Oil ingredient provides a typical profile of edible fatty acids as shown in Table 1. The fatty acid percentages may vary slightly; however, the DHA and EPA concentrations are controlled (see Specification Table 2).

Table 1. Typical Fatty Acid Profile of DHA Algal Oil

Fatty Acids Common Name	Lipid numbers + Δ^x	DHA Algal Oil (% w/w)
Lauric	C12:0	0.07
Myristic	C14:0	0.59
Pentadecanoic	C15:0	0.27
Palmitic	C16:0	30.50
Palmitoleic	C16:1, *cis*-Δ^9	0.23
Margaric	C17:0	0.38
Stearic	C18:0	1.19
Elaidic	C18:1, *trans*-Δ^9	0.01
Oleic	C18:1, *cis*-Δ^9	0.24
Linoleic	C18:2, all-*cis*-$\Delta^{9, 12}$	0.13
Alpha-linolenic	C18:3, all-*cis*-$\Delta^{9, 12, 15}$	0.10
Arachidic	C20:0	0.20
Eicosatrienoic	C20:3, all-*cis*-$\Delta^{8, 11, 14}$	0.16
Arachidonic	C20:4, all-*cis*-$\Delta^{5, 8, 11, 14}$	0.14
EPA	C20:5, all-*cis*-$\Delta^{5, 8, 11, 14, 17}$	0.55
Behenic	C22:0	0.15
Docosapentaenoic	C22:5, all-*cis*-$\Delta^{4, 7, 10, 13, 16}$	10.05
DHA	C22:6, all-*cis*- $\Delta^{4, 7, 10, 13, 16, 19}$	50.36
Unidentified fatty acids		4.60

2.2 Manufacturing

DHA Algal Oil is produced in a multistage heterotropic fermentation process carried out in the absence of light and under axenic conditions. Operating parameters, such as temperature, aeration, agitation, and pH, are controlled throughout the process to ensure that cell growth and oil production are reproducible to product specifications. Quality control checks are performed at every stage of production in accordance with the Hazard Analysis Critical Control Point (HACCP) system and Good Manufacturing Practice (GMP).

2.2.1 Manufacturing Overview

Strain Storage: Production begins with removal of a cryopreserved inoculum of *Schizochytrium* sp., strain HS01 from storage.

Flask Propagation: Strains are prepared and expanded via a two-stage shake flask process using a simple salt media containing glucose and yeast extract.

Seed Fermentation: Seed inoculum is transferred from the Stage 2 flask to a seed inoculum vessel and fermented through two stages over two to three days. The seed fermentation media is similar to that of the flask media with the addition of sunflower oil.

Production Fermentation: Fermentation is further upscaled to produce a production cell mass over 90 ± 4 h. The production fermentation media is similar to the seed fermentation media with the addition of monosodium glutamate. Harvested cells are observed full of oil under light microscope.

Oil Extraction: Cells are inactivated, and the walls are mechanically broken using a sand mill followed by centrifugation to extract the lipid fraction from the cell lysate.

Refining: The crude oil is heated under alkaline conditions to remove free fatty acids and impurities and then washed, vacuum dried, and crystalized and filtered to remove wax. A second crystallization and filtration step is performed to separate the winterized oil. Decoloring sand is used under normal temperature to improve the color of the oil followed by dilution with high oleic sunflower oil. Odor and oxide are removed under high temperature and pressure for 3–5 h using steam. As the final step, antioxidants (vitamin E and citric acid) are added.

Packaging: In a 100,000-class Bio-Clean area, the final product is filtered using a 1 micron filter bag and packed into aluminum drums under nitrogen. The drums are packed in corrugated cardboard boxes with top and bottom PE foam protection followed by banding.

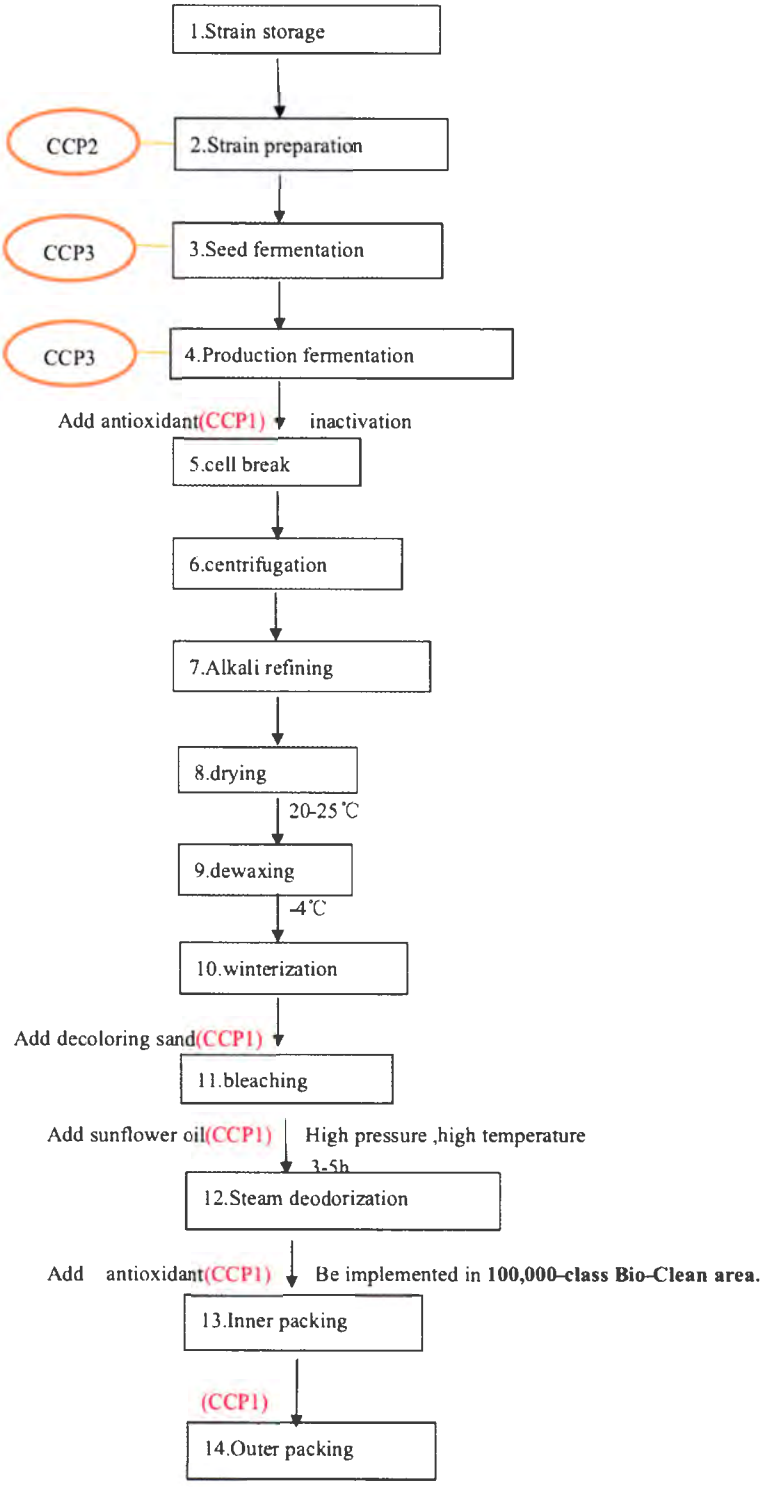

Figure 2. Manufacturing Flowchart

2.2.2 Good Manufacturing Practice

DHA Algal Oil from Xiamen Huison is produced in an FDA registered and ISO 22000 certified facility under strict adherence to a HACCP plan and compliance with current GMP standards set to comply with the U.S. Code of Federal Regulations, 21 CFR part 110. DHA Algal Oil is certified HALAL and Kosher.

2.2.3 Raw Materials

Raw materials used in the production of Xiamen Huison's DHA Algal Oil are of appropriate food grade. No material of human or animal origin is used. DHA Algal Oil is non-GMO and not irradiated.

2.3 Specifications

The specifications for the food-grade product DHA Algal Oil, along with the specification methods, are listed in Table 2 below.

Table 2. DHA Algal Oil Specifications

Tested Parameters	Specification	Method
Fatty Acid Analysis		
DHA Content	50–60%	GB 26400; AOAC 996
EPA Content	< 1.0%	GB 5413.27
Free Fatty Acids	< 0.3%	ISO 660
Trans Fatty Acids	NMT 1.0%	GB 5413.36
Unsaponifiables	NMT 4%	GB/T 5535.1
Physical Characteristics		
Appearance	Clear yellow oily liquid	GB/T 5525
Moisture	NMT 0.05%	GB 5009.3
Impurities		
Insoluble Impurities	NMT 0.2%	GB/T 15688
Acid Value	NMT 1.0 mg KOH/g	GB/T 5009.37
Anisidine Value	< 10	ISO 6885
Peroxide Value	NMT 5.0 meq/kg	GB/T 5009.37
Total Oxidation Value	< 20	Calculated
Benzo(a)pyrene	NMT 10 µg/kg	GB/T 5009.27
Heavy Metals		
Arsenic	NMT 0.1 mg/kg	GB/T 5009.11
Cadmium	NMT 0.5 mg/kg	GB/T 5009.15
Lead	NMT 0.1 mg/kg	GB/T 5009.12
Mercury	NMT 0.1 mg/kg	GB/T 5009.17
Microbiological Tests		
Total Aerobic Microbial	NMT 1000 cfu/g	GB 4789.2
Total Yeast & Mold	NMT 10 cfu/g	GB 4789.15
Aflatoxin B1	Negative (µg/kg)	GB/T 18979

Abbreviations: cfu, colony forming units; GB, National Standard of People's Republic of China; GB/T, Recommendation of the National Standard of People's Republic of China; KOH, potassium hydroxide; NLT, not less than; NMT, not more than.

2.3.1 Batch Analysis

Production conformity and consistency of Xiamen Huison's DHA Algal Oil is tested in production lots. Batch analyses of five non-consecutive lots, representing 49 months of production, are shown below and are reasonably consistent and meet the product specifications for fatty acid analyses (including DHA content), physical characteristics, impurities, manufacturing impurities, heavy metals, and microbial analyses.

Table 3. DHA Algal Oil Batch Analyses

Tested Parameters	Specification	Lot No./Date of Manufacture				
		BY131203 12/12/2013	BY150106 1/19/2015	BY150713 7/17/2015	BY170603 6/22/2017	BY180114 1/18/2018
Fatty Acid Analysis						
DHA Content	50–60%	53.61%	51.41%	51.53%	53.37%	50.4%
EPA Content	< 1.0%	0.6%	0.8%	0.7%	0.7%	0.8%
Free Fatty Acids	< 0.3%	0.11%	0.10%	0.05%	0.05%	0.09%
Trans Fatty Acids	NMT 1.0%	0.16%	0.21%	0.17%	0.19%	0.171%
Unsaponifiables	NMT 4%	1.9%	2.0%	1.8%	2.1%	1.7%
Physical Characteristics						
Appearance	Clear yellow oily liquid	Conforms	Conforms	Conforms	Conforms	Conforms
Moisture	NMT 0.05%	0.020%	0.010%	0.030%	0.011%	0.010%
Impurities						
Insoluble Impurities	NMT 0.2%	0.02%	0.03%	0.02%	0.04%	< 1.0%
Acid Value	NMT 1.0 mg KOH/g	0.21 mg KOH/g	0.20 mg KOH/g	0.10 mg KOH/g	0.11 mg KOH/g	0.18 mg KOH/g
Anisidine Value	< 10	4.2	3.7	4.4	4.6	5.4
Peroxide Value	NMT 5.0 meq/kg	0.13 meq/kg	0.10 meq/kg	0.20 meq/kg	0.38 meq/kg	1.5 meq/kg
Total Oxidation Value	< 20	4.46	3.9	4.8	5.36	8.4
Benzo(a)pyrene[a]	NMT 10 µg/kg	<5 µg/kg	<5 µg/kg	<5 µg/kg	<5 µg/kg	1.3 µg/kg
Heavy Metals						
Arsenic[b]	NMT 0.1 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg
Cadmium[c]	NMT 0.5 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg
Lead[d]	NMT 0.1 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg
Mercury[b]	NMT 0.1 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg	<0.01 mg/kg
Microbiological Tests						
Total Aerobic	NMT 1000 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Total Yeast & Mold	NMT 10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Aflatoxin B1[a]	Negative (µg/kg)	<5 µg/kg	<5 µg/kg	<5 µg/kg	<5 µg/kg	<5 µg/kg

Abbreviations: cfu, colony forming units; KOH, potassium hydroxide; NLT, not less than; NMT, not more than.
[a]Limit of Detection (LoD) = 5 µg/kg (note, manufacturing update from GB/T 5009.27-2003 to GB/T 5009.27-2016 on June 27, 2017 resulted in a lower LoD for lots tested after that date); [b]LoD = 0.01 mg/kg; [c]LoD = 0.005 mg/kg; [d]LoD = 0.05 mg/kg.

2.3.2 Residual Solvent Analysis

Water is the only solvent used in the manufacture of DHA Algal Oil; hence residual solvent analysis is not necessary.

2.3.3 Microbial Analysis

During the deodorization phase of the manufacturing process, the ingredient is subjected to sterilizing temperature, pressure, and time parameters in a fully closed environment and 100,000-class Bio-clean area. Moisture content of the finished product is too low to support new microbial growth, which has been confirmed by finished product testing for total coliforms, *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Shigella* spp. to a point of statistical verification. For these reasons, specific microbial count tests are no longer included in the finished product specifications.

2.3.4 Shelf–Life Stability

A two-year shelf–life from the time of manufacture has been demonstrated as an appropriate expiration period for DHA Algal Oil based upon two four–month accelerated stability tests of DHA Algal Oil lot number BY160101 (manufactured January 20, 2016; note, lot number BY160101 is Xiamen Huison's 40% DHA Algal Oil product while the subject of this GRAS conclusion is the company's 50% DHA Algal Oil product. As the source material, raw materials, and manufacturing processes are identical and the product specifications are identical except for DHA content, there is no reason to presume the results of these stability tests should not apply to the 50% product).

The accelerated stability tests were conducted at 42 ± 2 °C, 75 ± 5% relative humidity and 62 ± 2 °C, 75 ± 5% relative humidity, respectively, under conditions of commercial packaging in an aluminum drum with nitrogen protection. At all time points, outcome measures included the product specification parameters relevant to safety and stability of the ingredient (i.e., peroxide value, anisidine value, acid value, and DHA%) analyzed using the same test methodologies used for commercial batch analysis. Microbial parameters were not considered necessary to the stability test as the high temperature and pressure employed during the deodorization phase of the manufacturing process is sufficient to sterilize the ingredient, and the low moisture content of the ingredient is sufficient to prevent subsequent microbial growth.

All parameters assessed were stable and within specification throughout the stability test at 42 °C. At 62 °C, anisidine value increased slightly after Day 58 and peroxide value decreased slightly from Day 90 forward, indicating the anisidine increase was likely due to peroxide conversion to a secondary species. Therefore, the shelf-life was based on stability up to 58 days at 62 °C.

2.4 Physical or Technical Effect

DHA Algal Oil is not intended to produce any physical or other technical effects that are relevant to the safety of the ingredient.

Part 3: Dietary Exposure

3.1 Intended Use

Xiamen Huison's DHA Algal Oil, manufactured in accordance with current Good Manufacturing Practice, is intended to be used as an ingredient in the food categories and at the addition levels shown in Table 4, as a source of edible oils to replace other edible fats and oils normally contained in the below categories. DHA Algal Oil will not be combined or augmented with any other oil that is a significant source of DHA or EPA.

DHA Algal Oil is not intended for use in foods where standards of identity would preclude such use. The ingredient is not intended for use in infant formula or any products that would require additional regulatory review by USDA.

Table 4. Intended use of DHA Algal Oil

Food Category (Typical Serving Size)	Maximum Intended Addition Level Concentration mg/g		Maximum Intended Addition Level Per Typical Serving mg/serving	
	Algal Oil	DHA	Algal Oil	DHA
Gelatin Desserts/Salads (120 g)	3.3	2.0	396	238
Vegetable Oil (14 g)	30.0	18.0	420	252

3.2 Exposure Estimates

Exposure to DHA Algal Oil from the intended use categories was estimated for the U.S. population using food consumption data from the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES). The most recent data available at the time of this writing (2013–2014) was analyzed using Creme Food Safety software 3.6 (www.cremeglobal.com). This data was obtained from 7,574 individuals that underwent two non-consecutive 24-hour dietary recall interviews (the first was collected in-person, the second by phone 3–10 days later).

WWEIA food codes that were considered most similar to the intended use categories were utilized in the assessment and were assigned the relevant intended use concentrations.

Creme software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food groups and/or individual food ingredients. Creme Food Safety performs calculations using large-scale food consumption data sets. It bases the calculated estimates on each individual's body weight from the survey, as opposed to averaged body weights. Calculations also incorporated the NHANES assigned sample weights for each individual in the survey, which measure the number of people in the population

represented by that specific subject and help to ensure that the results statistically represent the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories. The data is shown for "proposed food consumers" (which includes only data from individuals who reported consuming one or more food/beverage categories intended to contain DHA Algal Oil over the two-day survey period). Results are given as both absolute exposure (mg/day), as well as exposure relative to body weight (mg/kg bw/day).

The relative standard error (RSE; calculated by dividing the standard error of the estimate by the estimate itself and multiplying by 100) is a statistical criterion that can be used to determine the reliability of estimates as pertains to the population (the larger the RSE the less reliable the estimate).[4] RSE values greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.[4, 5] For the purpose of this GRAS conclusion, an RSE value of greater than 25% was used to indicate that the estimated value was unreliable with regard to representing the population. RSE values are shown for the 90th percentile proposed food consumers only, as these values are considered most pertinent for the exposure estimates. All of the values were considered reasonably reliable using the 25% cut-off.

Table 5. Estimated Total (Aggregate) Exposure to DHA Algal Oil (NHANES 2013–14 data; mg/day)

Population Group	Age in yrs	Proposed Food Consumers					
		N (% total)	Absolute DHA Algal Oil consumption Daily Average (mg/day)				90th% RSE Value
			Mean	Mean std err	90th%	90th% std err	
Children	2–12	68 (4.1)	194.1	17.1	388.2	61.4	15.8
Adolescents	13–18	20 (2.7)	227.5	49.3	324.5	59.6	20.2
Adults	19+	194 (4.4)	266.4	24.8	566.2	101.2	19.2
Total Population	2+	282 (4.2)	254.2	20.9	460.1	87.7	19.1

Creme run #279

Table 6. Estimated Exposure to DHA Algal Oil Relative to Body Weight (NHANES 2013–14 data; mg/kg bw/day)

Population Group	Age in yrs	Proposed Food Consumers					
		N (% total)	DHA Algal Oil consumption Daily Average (mg/kg bw/day)				90th% RSE Value
			Mean	Mean std err	90th%	90th% std err	
Children	2–12	68 (4.1)	8.82	1.03	17.67	3.46	19.6
Adolescents	13–18	20 (2.7)	3.61	0.95	5.67	0.95	20.2
Adults	19+	194 (4.4)	3.55	0.33	7.06	0.99	19.2
Total Population	2+	282 (4.2)	4.29	0.33	9.19	1.22	13.3

Creme run #279

According to the estimates for proposed food consumers above, approximately 4.2% of the U.S. total population (ages 2 years and older) were identified as potential consumers of DHA Algal Oil from the intended uses. The 90th percentile aggregate estimated exposure estimate for the total population was 460.1 mg/day (9.19 mg/kg bw/day), while the highest estimates for individual populations were 566.2 mg/day (for adults ages 19 years and older), and 17.67 mg/kg bw/day (for ages 2–12 years).

An additional Creme assessment was performed in order to compare the exposure to DHA from Xiamen Huison's DHA Algal oil to DHA from menhaden oil based on allowable menhaden oil maximum addition levels for DHA + EPA, (assuming 100% from DHA) listed in 21 CFR 184.1472 for Xiamen Huison's two intended use categories. The maximum limitations of 1% menhaden oil in gelatins and puddings (equivalent to 0.2% DHA) and 12% menhaden oil in fats and oils (equivalent to 2.4% DHA) in the regulation were established to ensure that total DHA + EPA exposure does not exceed 3.0 g/day. Results of the comparative assessment are shown in Tables 7 and 8 below.

Table 7. Estimated Total (Aggregate) Exposure to DHA From Intended Use Categories for DHA Algal Oil and Menhaden Oil (NHANES 2013–14 data; mg/day

Population Group	Age in yrs	N (% Total)	Estimated Consumption by Consumers Daily Average (mg/day)							
			DHA from Xiamen Huison's DHA Algal Oil				DHA + EPA from Menhaden Oil (based on 21 CFR 184.1472 addition levels for Xiamen Huison's two intended use categories)			
			Mean	Mean std err	90th%	90th% std err	Mean	Mean std err	90th%	90th% std err
Children	2–12	68 (4.1)	117.4	10.4	220.7	37.4	124.1	10.3	229.4	29.4
Adolescents	13–18	20 (2.7)	137.6	29.8	196.7	35.8	146.4	30.6	197.3	48.9
Adults	19+	194 (4.4)	160.4	14.9	343.1	61.3	196.3	19.5	354.9	63.7
Total Population	2+	282 (4.2)	153.1	12.5	278.9	53.4	183.5	16.6	326.9	43.2

Creme run #278

Table 8. Estimated Exposure to DHA From Intended Use Categories for DHA Algal Oil and Menhaden Oil Relative to Body Weight (NHANES 2013–14 data; mg/kg bw/day)

Population Group	Age in yrs	N (% Total)	Estimated Consumption by Consumers (mg/kg bw/day)							
			DHA from Xiamen Huison's DHA Algal Oil				DHA + EPA from Menhaden Oil (based on 21 CFR 184.1472 addition levels for Xiamen Huison's two intended use categories)			
			Mean	Mean std err	90th%	90th% std err	Mean	Mean std err	90th%	90th% std err
Children	2–12	68 (4.1)	5.33	0.63	10.71	2.10	5.56	0.61	10.71	2.07
Adolescents	13–18	20 (2.7)	2.18	0.58	3.43	0.58	2.31	0.58	3.46	0.58
Adults	19+	194 (4.4)	2.14	0.20	4.24	0.60	2.64	0.27	5.23	0.86
Total Population	2+	282 (4.2)	2.58	0.20	5.55	0.72	3.02	0.25	6.20	1.03

Creme run #278

As expected, DHA estimated exposure levels from Xiamen Huison's DHA Algal Oil's intended uses are overall lower than maximum DHA estimated exposure levels from menhaden oil based on menhaden oil's allowable levels for the two intended use categories and using NHANES 2014 food consumption data. Exposure estimates for the total population by food consumers were 278.9 mg/day (5.55 mg/kg bw/day) and 326.9 mg/day (6.20 mg/kg bw/day) for Xiamen Huison's DHA Algal Oil and menhaden oil, respectively.

Lastly, DHA overall dietary consumption by the U.S. population was derived using Creme software. DHA concentrations were assigned to all relevant 2013–2014 NHANES food codes using composition data from the United States Department of Agriculture (USDA)'s Food and Nutrient Database for Dietary Studies (FNDDS).

The FNDDS database provides information on the amount of approximately 60 food constituents (including DHA) per 100 g of each NHANES food code. The DHA exposure data was then derived using analysis by Creme software. The Daily Average dietary DHA exposure results for the total population (ages 2+) at the mean were 58 mg/day and 0.83 mg/kg bw/day, and at the 90th percentile were 138 mg/day and 1.98 mg/kg bw/day.

Part 4: Self-limiting Levels of Use

There are no known inherent self-limiting levels of use.

Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for DHA Algal Oil is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered pivotal information. To the best of our knowledge, DHA Algal Oil was not commonly used in foods prior to 1958.

Part 6: Narrative

6.1 Absorption, distribution, metabolism, and excretion (ADME)

6.1.1 General Fatty Acid Biochemistry

The normal body weight of mammals (including humans) is comprised of 5–25% lipids, of which up to 90% are present in triacylglycerols (TAG).[6]TAGs are comprised of three fatty acids (carboxylic acids with a hydrocarbon chain/tail) esterified to a glycerol backbone, and the majority of TAGs are present as stored energy reserves (approximately 400,000 kJ in a typical 70 kg human) in adipose tissue while smaller amounts act as shock absorbers around organs or circulate in the blood and lymph tissues to transport fatty acids and other lipids to and from tissues; additionally fat acts as an efficient thermal insulator. The primary sources of utilizable TAGs are dietary intake, de novo synthesis (primarily in the liver), and the adipose tissue storage depot.

When ingested, due to their insolubility in aqueous solutions, TAGs are emulsified by bile salts in the small intestine, forming micelles, which are amphipathic and facilitate hydrolysis (digestion) by water-soluble enzymes (primarily pancreatic lipase) and absorption. Digestion yields a mixture of free fatty acids (FFA), mono- and diacylglycerols (MAG and DAG, respectively), and glycerol, with less than 10% of ingested TAGs remaining unhydrolyzed in the intestines. Following absorption at the intestinal mucosa epithelium, the hydrolysis products are resynthesized as TAGs within the enterocyte and secreted into the lymph system complexed to proteins as lipoprotein structures known as chylomicrons, which are comprised of approximately 86% TAG, for transport to peripheral tissues (e.g., heart, muscle, and adipose).

TAGs synthesized in the liver by the de novo pathway are packaged in very low-density lipoproteins (VLDL), which contain about 51% TAG (and a different composition of apoproteins compared to chylomicrons), that are secreted into the blood stream (also for delivery to peripheral tissues), and to a much lesser extent (about 6%) low-density lipoproteins. TAGs in both chylomicrons and VLDL are hydrolyzed by the lipoprotein lipase enzyme at capillary surfaces within peripheral tissues for delivery of free fatty acids to tissue cells where, depending on tissue type, they are either resynthesized into TAGs for storage or oxidized for energy (glycerol is returned to the liver for recycling or catabolism).

Use of TAG stored in adipose tissue is under hormonal regulation. Hormones initiate a phosphorylation signaling cascade resulting in activation of hormone-sensitive lipase (a.k.a., TAG lipase) and subsequent hydrolysis of one of the outer fatty acids from the glycerol backbone. This allows DAG lipase followed by MAG lipase to act sequentially resulting in the complete hydrolysis of the TAG molecule and liberation of three FFAs and one glycerol per TAG. FFAs move from the adipocyte to the blood stream by passive diffusion where they complex with

albumin and are circulated to distant tissues for cellular uptake (also primarily by passive diffusion; released glycerol molecules are primarily taken up and catabolized in the liver).

Regardless of the proximate origin (e.g., ingestion, de novo synthesis, or liberation from storage) of FFAs, in addition to their utilization as an energy substrate or resynthesis into TAGs, some contribute to the synthesis of phospholipids within local cell membranes where they serve structural, regulatory, or transport (e.g., lipoprotein components) roles.[6, 7]

Oxidation of FFAs for energy takes place within the mitochondria via the β-oxidation pathway.[6] Metabolic activation of FFAs for β-oxidation requires the addition of coenzyme A (CoA) to the carboxylic acid head of the FFA, which is catalyzed by a fatty acyl-CoA ligase, resulting in the formation of an acyl-thioester CoA conjugate (or acyl-CoA). Short- and medium-chain fatty acids move freely through the mitochondrial membrane and interact with their specific ligases within the mitochondrial matrix. However, FFAs with 13 or more carbons in their hydrocarbon tail require a specific transport system due to the impermeability of the inner mitochondrial membrane to long-chain fatty acids (LCFA) and acyl-CoAs. LCFA specific ligases are present in the outer mitochondrial membrane, where an acyl-CoA is formed and then enters the carnitine shuttle system in which carnitine acyltransferase I and carnitine acyltransferase II enzymes, respectively, replace CoA with carnitine on the outer membrane (forming an intermediate fatty acyl-carnitine) and carnitine with CoA on the inner membrane releasing the acyl-CoA within the matrix. Carnitine acyltransferase I also plays a role in regulation of fatty acid metabolism as it is strongly inhibited by the first intermediate product in de novo fatty acid synthesis; thus, shutting down β-oxidation of LCFA under conditions that favor synthesis.

β-Oxidation is a four-reaction cycle, as described below (see Figure 3) with each cycle shortening the acyl chain by two carbons and in so doing, releasing a molecule of acetyl-CoA (which then enters the citric acid cycle and is fully oxidized to carbon dioxide (CO_2)) and generating of one molecule each of reduced flavin adenine dinucleotide ($FADH_2$), and reduced nicotinamide adenine dinucleotide (NADH). The pathway name, β-oxidation, is derived from the fact that each step begins with the oxidation of the β-carbon. As an energy source, fatty acids yield 37 kJ/g (or approximately 8.2 adenosine triphosphates (ATP) for every full oxidation to CO_2), more than double that yielded from carbohydrate or protein, and are the major energy source for most cells (brain cells are a notable exception under typical American dietary conditions).

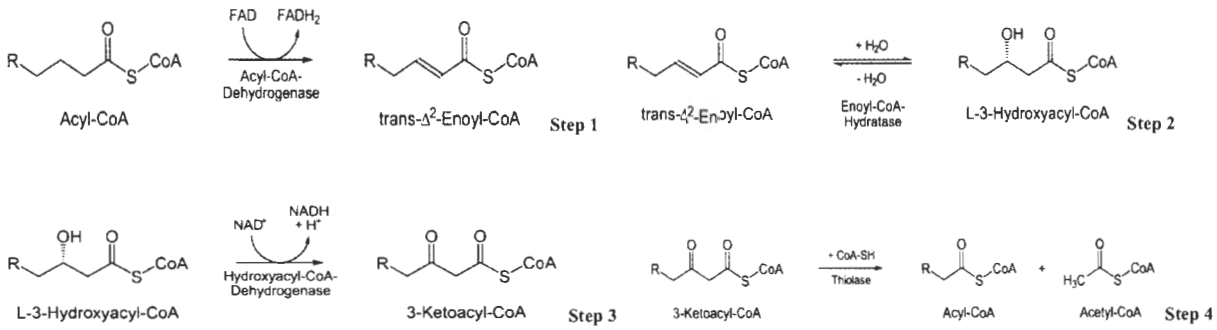

Figure 3. β-Oxidation Cycle

Step 1: Dehydrogenation. A fatty acyl-CoA is dehydrogenated between the α- and β-carbons by fatty acyl-CoA dehydrogenase, resulting in the formation of a *trans*-Δ2-enoyl-CoA and reduction of FAD to give $FADH_2$.

Step 2: Hydration. An enoyl-CoA hydratase catalyzed hydration of the *trans*-Δ2-enoyl-CoA β-carbon produces the Step 2 intermediate product, L-3-hydroxyacyl-CoA.

Step 3: Dehydrogenation. Next, 3-hydroxyacyl-CoA dehydrogenase catalyzes an NAD^+-dependent dehydrogenation of the newly formed 3-hydroxy group to give a 3-ketoacyl-CoA and NADH.

Step 4: Thiolytic cleavage. In the final reaction of the cycle, cleavage of the α—β bond by the thiol group of a second CoA molecule (CoA-SH) is catalyzed by β-ketothiolase, releasing acetyl-CoA.

The above cycle continues with the newly formed acyl-CoA, now two carbons shorter than its starting length, until the acyl-CoA is fully oxidized, assuming the starting FFA was fully saturated or contained only *trans* double bonds. In the case of *cis*-unsaturated fatty acids, such as DHA, β-oxidation proceeds until a *cis* double bond is reached at the Δ3 or Δ4 carbon of the newly formed acyl-CoA product, at which point additional reactions are required as the enoyl-CoA hydratase enzyme can only act on *trans* double bonds. These reactions are catalyzed by enoyl-CoA isomerase and 2,4-dienoyl-CoA reductase.

When the previous β-oxidation cycle results in the formation of a *cis*-Δ3-enoyl-CoA, enoyl-CoA isomerase converts it to a *trans*-Δ2-enoyl-CoA and β-oxidation then proceeds from Step 2. When the previous β-oxidation cycle results in the formation of a *cis*-Δ4-enoyl-CoA, Step 1 of the next β-oxidation cycle results in a *trans*-Δ2-*cis*-Δ4-dienoyl-CoA that is acted on by 2,4-dienoyl-CoA reductase to produce *cis*-Δ3-enoyl-CoA, which then undergoes the enoyl-CoA isomerase reaction described above.

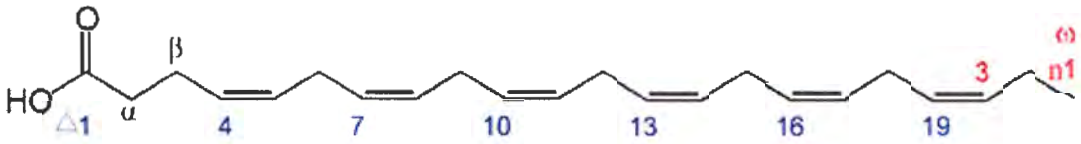

Figure 4. DHA Numbered Structure (linear depiction)

Thus, in the case of DHA (C22:6, all-*cis*- $\Delta^{4,7,10,13,16,19}$), with its first double bond at the Δ4 position (see Figure 4), β-oxidation begins with Step 1 followed by the sequential 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase catalyzed reactions and then Steps 2, 3, and 4 to complete the first cycle, resulting in a 20 carbon enoyl-CoA with its first double bond at the Δ5 position (i.e., C20, all-*cis*-$^{\Delta 5,811,14,17}$-pentaenoyl-CoA). A second cycle of regular β-oxidation results in C18, all-*cis*-$^{\Delta 3,6,9,12,15}$-pentaenoyl-CoA. Cycle three requires the insertion of the enoyl-CoA isomerase reaction resulting in C16, all-*cis*-$\Delta^{4,7,10,13}$-tetraenoyl-CoA. The next six cycles proceed as two rounds of three cycles exactly as the first three, leaving as the 9th cycle product, a four-carbon acyl-CoA that is then oxidized in one final round of regular β-oxidation. Thus, the complete β-oxidation of DHA requires 10 cycles (four regular, three with the addition of both the 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase reactions, and three with the addition of the enoyl-CoA isomerase reaction only) and results in the generation of 11 acetyl-CoA to enter the citric acid cycle.

In the case of FFAs with odd-numbered carbon chains, the substrate of the final β-oxidation cycle is a five-carbon acyl-CoA, resulting in the release of one acetyl-CoA and one propionyl-CoA (3 carbons). Propionyl-CoA is carboxylated by propionyl-CoA carboxylase to form D-methylmalonyl-CoA that is converted by methylmalonyl-CoA epimerase to its stereoisomer L-methylmalonyl-CoA that is then converted to the citric acid cycle substrate, succinyl-CoA, catalyzed by L-methylmalonyl-CoA mutase.

Because of the current popularity of various low carbohydrate diets, a discussion of metabolism of fat for use as fuel would not be complete without a brief mention of the ketogenic pathway. This pathway is also prominent during starvation or intentional prolonged fasting states. When acetyl-CoA enters the citric acid cycle, it combines with oxaloacetate to form citrate and drive the cycle. When dietary carbohydrates are limited or unavailable the supply of citric acid cycle intermediates becomes limited resulting in reduced flux through the cycle and accumulation of acetyl-CoA derived from β-oxidation. When levels of acetyl-CoA rise, the β-ketothiolase reaction (Step 4 of β-oxidation) reverses resulting in generation of acetoacetyl-CoA from two acetyl-CoAs. The β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase enzyme catalyzes the formation of HMG-CoA by the addition of a third acetyl-CoA to acetoacetyl-CoA, which, within the mitochondria, is split by HMG-CoA lyase to form acetoacetate and acetyl-CoA. Acetoacetate may

be reduced to form D-β-hydroxybutyrate in an NADH-dependent reaction catalyzed by β-hydroxybutyrate dehydrogenase or in small amounts, may spontaneously degrade to yield acetone. Collectively, acetoacetate, β-hydroxybutyrate, and acetone are referred to as ketone bodies. Ketogenesis occurs primarily in the liver and the ketone bodies are then transported to peripheral tissues where they can be utilized for energy production.

6.1.2 DHA Pharmacokinetics

In male and female rats supplemented with a DHA algal oil (40–50% DHA) derived from *Crypthecodinium cohnii* at 0.5 and 1.25 g/kg bw/day by gavage for 91–93 days, DHA and EPA levels were statistically significantly and dose dependently increased while AA levels were statistically significantly and dose dependently decreased in all body tissues.[8] The observed changes were most pronounced in the liver followed by the heart and brain. The increases in EPA were hypothesized as due to retro-conversion of DHA (as EPA was not present in the test item or vehicle control oil) while the decreases in AA were thought due to decreased biosynthesis from its essential fatty acid precursor, linoleic acid, secondary to DHA mediated downregulation of delta-6 desaturase.

Toxicokinetic (TK) parameters were evaluated during a 90-day oral toxicity study in a subset of three rats/sex/group administered a highly purified (90%) DHA ethyl ester (EE) by gavage at doses of 1.3, 2.5, or 5.0 g/kg bw/day (providing 1.2, 2.25, and 4.5 g/kg bw/day DHA).[9] An additional group of three rats/sex administered a DHA (40–50%) TAG oil (same test item evaluated by Arterburn et al. discussed above) at 5.0 g/kg bw/day (equivalent to 2.0 g/kg bw/day DHA) was also evaluated. Note, while the text reported three rats/sex/group, the data table reported six. Both test items were derived from the microalgae *C. cohnii*. Blood was collected 1, 2, 4, 6, 8, 12, and 24 h after dosing on study Days 1, 28, and 91. As TK profiles were similar on Days 28 and 91, the authors reported Day 91 data only. There was a dose-related increase in mean C_{max} and $AUC_{(0-24)}$ in male animals receiving DHA EE while in females, the increases were only dose-related for $AUC_{(0-24)}$. C_{max} and $AUC_{(0-24)}$ in the DHA TAG oil group males were similar to those of the mid-dose DHA EE males while for the DHA TAG oil females, C_{max} was more closely aligned with the results of the low-dose DHA EE females, and $AUC_{(0-24)}$ was more closely aligned with the results of the DHA EE high-dose females. The authors concluded that DHA EE and DHA TAG oil were well absorbed and bioavailable; however, the relationship of bioavailability to DHA EE dose varied between the sexes.

Tissue distribution of DHA ingested in TAG, FFA, EE, and phospholipid (PL) forms was investigated in male Balb/c mice fed low- (LF) or high-fat (HF) diets containing DHA and EPA in a 2.6:1 ratio at a total of 0.7% of the diets for one week.[10] The TAG supplement was derived by combining a fish oil and a DHA algal oil provided by Xiamen Huison. With the exception of the effect of LF-TAG and LF-FFA diets on brain DHA concentration, all forms tended to increase absolute

DHA concentration in the liver, adipose tissue, skeletal muscle, and brain. Statistically significant increases in DHA concentrations were observed as follows: liver, LF-PL and HF-TAG and -PL; adipose; not reported; skeletal muscle, all forms in the LF-diet and HF-EE and -PL; brain, HF-FFA, -EE, and -PL. Similar effects were observed on fatty acid composition of tissues as a % of total fatty acids with statistically significant increases observed as follows: liver, LF-TAG, -FFA, and -PL and HF-FFA, -EE, and -PL; adipose, all forms in both LF- and HF-diets; skeletal muscle, all forms in the LF-diet only; brain, HF-FFA, -EE, and -PL. Consistent with other studies, EPA also tended to increase while AA tended to decrease slightly. The authors concluded the TAG and PL forms were the most efficient at increasing DHA tissue content.

The bioavailability of DHA in TAG form from a DHA-rich algal oil was investigated in a randomized double-blinded placebo-controlled trial in 20 subjects with cystic fibrosis (a population in which imbalances of omega-3 and -6 fatty acids have been reported).[11] Subjects were 8–20 years old and received either 50 mg/kg bw/day DHA or a corn/soy oil placebo for six months. DHA levels were assessed in plasma phospholipids. A 5-fold statistically significant increase was observed over the treatment period in a time-dependent, saturable manner in the treatment group while remaining stable at baseline levels in the placebo group. Erythrocyte and rectal tissue DHA levels were also statistically significantly increased (4- and 5-fold, respectively) in the treatment group during the study indicating tissue accretion. Consistent with the preclinical literature, concomitant decreases in AA levels and AA/DHA ratio were also observed as were increases in EPA (the test item did not contain EPA) and total omega-3 fatty acid levels.

In another human study in healthy subjects, DHA plasma concentrations were increased 150–160% in 4 healthy males following four weeks of supplementation with an EE fish oil providing 750 mg DHA and 930 mg EPA daily.[12] EPA levels were also increased while AA levels were decreased by 3–15%, consistent with the results observed in rats. At the end of the four-week washout period, all levels had returned to baseline.

In a single-blinded placebo-controlled trial involving 18 healthy male Japanese subjects, following single doses of 2 or 4 g of an oil containing DHA and EPA in FFA form, C_{max} for DHA was 37.2 and 91 µg/mL, respectively, at t_{max} 5.0 hours for both doses, and AUC_{0-t} (where t is the time of last measurable concentration over 48 hours) was 0.167 and 0.459 mg•h/mL, respectively.[13] Results were similar in an open-label arm of the trial in six healthy male Caucasian subjects given a single 4 g dose with C_{max}, t_{max}, and AUC_{0-t} of 68.6 µg/mL, 5.0 hours, and 0.936 mg•h/mL, respectively. In a repeated-dose phase of the same study using the same groups and doses administered daily for 14 consecutive days, steady state concentrations of DHA were achieved by Day 17, and $C_{ss,max}$ (at steady state) and dosing interval at steady state $AUC_{tau,ss}$ were 64.2 and 106 µg/mL and 0.631 and 1.02 mg•h/mL, respectively, for the respective 2 and 4 g doses in Japanese subjects and 106 µg/mL

and 1.14 mg•h/mL, respectively, for the 4 g dose in Caucasian subjects. The accumulation ratio (R_{ac}) for C_{max} and AUC_{0-24} tended to be larger with the 2 g dose compared to the 4 g dose, and the R_{ac} was similar between Japanese and Caucasian subjects consuming the 4 g dose. Thus, DHA was dose-dependently bioavailable and generally comparable among Japanese and Caucasian subjects.

DHA is also bioavailable when consumed with or in various foods. In a randomized single-blinded crossover (21-day washout) design in 11 healthy adult male and female subjects, approximately 1.3 g DHA (provided as *Schizochytrium* sp. derived 35% DHA TAG oil) in yogurt was bioavailable with an AUC_{0-48} of 7.67 mg•h/mL (derived from supplemental material with units assumed as they were not provided by the authors).[14] C_{max} and t_{max} were represented graphically in supplemental material and appeared to correspond to an approximately 0.1% increase in whole blood DHA levels and 6 hours, respectively. DHA EEs were also bioavailable when consumed in sausage,[15] and bioavailability of DHA FFAs was increased when consumed with a meal as compared to fasting consumption.[16]

The bioequivalence of DHA oil derived from *C. cohnii* and *Schizochytrium* sp. in a blend of AA was investigated in five groups of six piglets/sex/group provided test diets containing 0, 0.32 or 0.96% DHA from one or the other source for 21 days. Blood was collected for fatty acid analysis just prior to sacrifice and heart, liver and brain tissues were homogenized.[17] Dose-dependent increases were observed in group plasma, erythrocyte, heart, liver, and brain DHA levels from both DHA sources compared to controls; however, there were no differences in levels between the sources. Thus, it was concluded that DHA derived from *C. cohnii* and *Schizochytrium* sp. are bioequivalent.

The bioequivalences of two different sources of DHA were compared in capsules as well as in food matrix in the form of DHA fortified snack bars in a randomized double-blinded placebo-controlled trial.[18] DHA TAG oil derived from either *C. cohnii* or *Schizochytrium* sp. was administered to eight groups of 12 healthy adult male and female subjects randomly assigned to receive capsules containing 200, 600, or 1000 mg of DHA from one or the other source, snack bars containing 465 mg DHA from *Schizochytrium* sp., or a corn/soy oil placebo for 28 days. Plasma phospholipid and erythrocyte DHA levels were compared at baseline and following two and four weeks of supplementation. While blinding was maintained for all DHA containing and placebo capsules, the snack bar treatment could not be blinded. Treatment with DHA capsules from both sources resulted in linear dose-dependent statistically significant increases in plasma phospholipid and erythrocyte DHA levels compared to placebo. No statistically significant differences within dose levels were observed between the two sources of DHA at any timepoint or dose level, and DHA levels in the snack bar group were similar to those observed in both 600 mg DHA capsule groups. Most of the increase in plasma phospholipid DHA content occurred within the first two weeks while the increase in erythrocyte DHA content was slower and steady over the 4-week treatment period. There were no

changes from baseline in DHA levels in the placebo group over the course of the study. Based on the bioequivalence assessment, *C. cohnii* and *Schizochytrium* sp. derived DHA oil capsules are bioequivalent sources of DHA at all dose levels tested per FDA standards. Formal bioequivalence assessment could not be performed with respect to the snack bar because the dose was not within 5% of a capsule dose; however, increases compared to the 600 mg doses appear similar when viewed graphically, and, thus, were considered grossly equivalent on a per DHA consumed basis. Similar to other studies, a statistically significant dose-dependent decrease was observed for AA. This relationship was linear for the *C. cohnii* and quadratic for the *Schizochytrium* sp. oil, resulting in maintenance of AA levels at the high dose. Modest dose-related increases in EPA were not statistically significant compared to placebo.

The bioequivalence of DHA from *C. cohnii* and cooked salmon was investigated in a randomized open-label parallel group clinical trial in which 32 healthy adult subjects were randomized to consume 600 mg DHA daily from one or the other source for 14 days.[19] Baseline DHA levels were comparable among all treatment groups. Both the algal TAG oil and the salmon statistically significantly increased plasma phospholipid DHA levels compared to baseline while no statistically significant differences were observed between the treatments. In erythrocytes, the algal TAG oil statistically significantly increased DHA levels over baseline while in the salmon consuming group, the increase in DHA levels did not reach statistical significance; however, the between group difference was not statistically significant. As with other studies, increases and decreases were observed in EPA and AA levels, respectively. The post hoc bioequivalence assessment according to FDA standard demonstrated that the algal TAG oil and cooked salmon are bioequivalent sources of DHA.

6.2 Toxicology Studies

A large number of toxicological studies of DHA oil have been published and are summarized in text and/or tabular format below. Of the located studies, those produced using *Schizochytrium* spp. were considered most relevant to this safety assessment. Nonetheless, toxicological studies of DHA oil from other sources were also considered relevant the basis of safety of DHA Algal Oil. Unpublished toxicological studies on the article of commerce that is the subject of this GRAS Notice are also summarized below, and while relevant, are viewed as corroborative evidence as they are not generally available to qualified experts throughout the scientific community.

6.2.1 *Schizochytrium* spp. Derived DHA Oil Toxicological Studies

Toxicological evaluations of five different test items derived from *Schizochytrium* spp. were located and are summarized below:

- The biomass of a DHA-rich *Schizochytrium* sp. (DRM; reported comprised of approximately 41% oil of which 22–29% is DHA) was evaluated in an OECD 471 bacterial reverse mutation test, an OECD 476 CHO AS52/XPRT gene mutation assay, an OECD 473 in vitro mammalian chromosomal aberration assay, an OECD 474 in vivo mammalian micronucleus test,[20] a 13-week oral toxicity study in rats,[21] an escalating dose study in castrated male pigs,[22] developmental toxicity studies in rats and rabbits,[23] and a one-generation reproductive and developmental toxicity study in rats.[24] These studies were described in GRN No. 137 on pages 10–13 and 15–16, which are incorporated herein by reference.
- An Algal Oil (AO; 37% DHA and 16% EPA with the addition of antioxidants ascorbyl palmitate and tocopherol) from a *Schizochytrium* sp. was evaluated in an OECD 471 bacterial reverse mutation test, an OECD 473 in vitro mammalian chromosomal aberration test, an OECD 474 in vivo mammalian micronucleus test, an OECD 408 90-day oral toxicity study in rats.[25] These studies were described in GRN No. 553 on pages 44–49[a], which are incorporated herein by reference.
- A DHA-rich Algal Oil (DRAO; 40–45% DHA and up to 10% EPA) from a *Schizochytrium* sp. was evaluated in an OECD 471 bacterial reverse mutation test, an OECD 473 in vitro mammalian chromosomal aberration test, an OECD 474 in vivo mammalian micronucleus test, an OECD 408 90-day oral toxicity study in rats combined with an OECD 422 reproductive and developmental toxicity study,[26] and a 21-day bioequivalence study in pigs.[17] These studies were described in GRN No. 553 on pages 34–44[a], which are incorporated herein by reference.
- DHA-rich Algal Oil (OCN-T18-AO; 39–42% DHA and 41–44% total omega 3) from *Schizochytrium* sp., strain OCN-T18 was evaluated in an OECD 471 bacterial reverse mutation test, an OECD 473 in vitro mammalian chromosomal aberration test, an OECD 474 in vivo mammalian micronucleus test, an OECD 425 acute oral toxicity study in rats, an OECD

[a] It is unclear which sets of studies described in GRN No. 553 apply to which ingredient. The reporting and citations in GRN No. 553 do not match exactly with the cited publications. It appears the reporting of these studies may not be correct or may be reversed in whole or in part in GRN No. 553. In this GRAS Notice, the studies are accurately described in accordance with the citations to the published literature; the incorporation by reference of the GRN No. 553 page numbers may be in part or in whole reversed and/or some studies included in the incorporation by reference may be studies other than those described in the cited literature in this GRAS Notice.

407 14-day oral toxicity study in rats, an OECD 408 90-day oral toxicity study in rats,[27] an OECD 414 prenatal developmental toxicity study in rats, and an OECD 415 one-generation reproduction toxicity study in combination with a second 90-day oral toxicity study in rats.[28] These studies were described in GRN No. 677 on pages 33–35, which are incorporated herein by reference.

- A DHA-rich oil (DRO; >40% DHA) derived from a *Schizochytrium* sp. was evaluated in an OECD 471 bacterial reverse mutation test, an FDA Redbook IV.C.1.b in vitro mammalian chromosomal aberration test, an FDA Redbook IV.C.1.d in vivo mammalian micronucleus test, an FDA Redbook IV.C.2 acute oral toxicity study in rats, an FDA Redbook IV.C.3.a 28-day repeated-dose oral toxicity study in rats, an FDA Redbook IV.C.4.a 90-day repeated-dose oral toxicity study in rats,[29] an FDA Redbook IV.C.9.a reproductive toxicity study in rats, and an FDA Redbook IV.C.9.b developmental toxicity study in rats.[30] These studies were described in GRN No. 731 on pages 28, 29, 31, and 32 and in GRN No. 732 on pages 31–35, which are incorporated herein by reference (note, GRN Nos. 731 and 732 are currently under evolution and pending response by FDA). The reproductive and developmental studies published by Falk et al., are also summarized in GRN No. 776 on page 24 and Appendix 2 page 5 and GRN No. 777 on page 22 and Appendix 2 page 5, which are incorporated herein by reference.

Table 9 below provides a comparison of the composition of Xiamen Huison's DHA Algal Oil to the test items described above while Table 10 provides a summary of the genetic toxicity studies, and Table 11 provides a summary of the general and reproductive and developmental oral toxicity studies.

Table 9. Typical Fatty Acid Profiles of *Schizochytrium* spp. DHA Oils

Common Name	Fatty Acids Lipid numbers + Δ^X or *n*	DHA Algal Oil* (wt%)	DRM (wt%**)	AO (wt%)	DRAO (wt%)	OCN-T18-AO (wt%)	DRO (wt%)
Lauric	C12:0	0.07	0.43	NR	NR	1.10	0.05
Myristic	C14:0	0.59	12.57	NR	1.6	13.77	0.38
Pentadecanoic	C15:0	0.27	ND	NR	NR	NR	NR
Palmitic	C16:0	30.50	30.06	NR	19.5	26.57	17.78
Palmitoleic	C16:1, *cis*-Δ^9	0.23	6.86	NR	NR	2.47	0.15
Margaric	C17:0	0.38	NR	NR	NR	NR	NR
Stearic	C18:0	1.19	0.89	NR	1.5	0.80	1.20
Elaidic	C18:1, *trans*-Δ^9	0.01	NR	NR	NR	NR	NR
Oleic	C18:1, *cis*-Δ^9	0.24	ND	NR	18.3	0.43	2.68
Vaccenic	C18:1, *trans*-Δ^{11}	NR	3.81	NR	NR	2.10	0.16
Linoleic	C18:2, all-*cis*-$\Delta^{9, 12}$	0.13	NR	NR	1.4	0.07	6.29
Alpha-linolenic	C18:3, all-*cis*-$\Delta^{9, 12, 15}$	0.10	NR	NR	NR	0.20†	NR
Stearidonic	C18:4, all-*cis*-$\Delta^{6, 9, 12, 15}$	NR	ND	NR	NR		0.18
Arachidic	C20:0	0.20	NR	NR	NR	NR	NR
Gondoic	C20:1, *cis*-Δ^{11}	ND	NR	NR	<1	NR	NR
Eicosatrienoic	C20:3, all-*cis*-$\Delta^{8, 11, 14}$	0.16	0.49	NR	NR	0.10	0.25
Arachidonic	C20:4, all-*cis*-$\Delta^{5, 8, 11, 14}$	0.14	ND	NR	<1	0.23	0.39
Eicosatetraenoic	C20:4, all-*cis*-$\Delta^{8, 11, 14, 17}$	NR	ND	NR	NR	0.47‡	0.44
Eicosatetraenoic	C20:4, *n*-7	NR	0.59	NR	NR		NR
EPA	C20:5, all-*cis*-$\Delta^{5, 8, 11, 14, 17}$	0.55	0.53	16	8.0	0.87	0.39
Behenic	C22:0	0.15	NR	NR	NR	NR	NR
Docosapentaenoic	C22:5, all-*cis*-$\Delta^{4, 7, 10, 13, 16}$	10.05	9.31	NR	2.4	7.90ⁱ	NR
Docosapentaenoic	C22:5, all-*cis*-$\Delta^{7, 10, 13, 16, 19}$	ND	NR	NR	<1		10.71
DHA	C22:6, all-*cis*- $\Delta^{4, 7, 10, 13, 16, 19}$	50.36	27.17	37	42.6	40.23	41.37
Unidentified fatty acids		4.60	NR	NR	NR	NR	17.58

Abbreviations: AO, Algal Oil from a *Schizochytrium* sp.; DRAO, DHA-rich Algal Oil from a *Schizochytrium* sp.; DRM, DHA-rich *Schizochytrium* sp. biomass; DRO, DHA-rich oil from a *Schizochytrium* sp.; ND, not detected; NR, not reported; OCT-T18, DHA-rich Algal Oil from *Schizochytrium* sp., strain OCN-T18.

*The subject of this GRAS notice; **Oil fraction only; †Specific octadecatetraenoate isomer not reported; ‡Specific eicosatetraenoate isomer not reported; ⁱSpecific docosapentaenoate isomer not reported.

Table 10. Summary of *Schizochytrium* spp. derived DHA Genetic Toxicity Studies

Author, Date	Test Item	Test System	Concentration / Dose	Outcome
Bacterial Reverse Mutation Tests				
Hammond et al., 2001[20]	DRM	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA102	0, 5, 15, 50, 150, & 500 µg/plate ± S9 (PIM & PM)	Not mutagenic
Fedorova-Dahms, et al., 2011[25]	AO	*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvrA*	Several up to 5000 µg/plate ± S9 (PIM only)	Not mutagenic
Fedorova-Dahms et al., 2001[26]	DRAO	*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvrA*	Several up to 5000 µg/plate ± S9 (PIM only)	Not mutagenic
Schmitt et al., 2012[27]	OCN-T18-AO	*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvrA*	0, 313, 625, 1250, 2500, & 5000 µg/plate ± S9 (PIM; initial and confirmatory tests)	Not mutagenic
Lewis, et al., 2017[29]	DRO	*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 *uvrA*	0.062, 0.185, 0.556, 1.667, 2.5, 3.75, & 5 mg/plate ± S9 (PIM & PM)	Not mutagenic
In vitro Mammalian Cell Gene Mutation Test Using the *xprt* Gene				
Hammond et al., 2001[20]	DRM	CHO AS52 cells	0, 200, 500, 1000, 2000, & 5000 µg/mL + S9 0, 200, 700, 850, 900, & 1000 µg/mL – S9	Not mutagenic
In vitro Mammalian Chromosomal Aberration Test				
Hammond et al., 2001[20]	DRM	HPBL	125, 250, 500 & 750 µg/mL 4, 20, & 44h treatment – S9 & 4h treatment + S9	Not clastogenic
Fedorova-Dahms, et al., 2011[25]	AO	HPBL	Several up to 5 µL/mL Experiment I: 4 h treatment ± S9 Experiment II: 4h treatment + S9 & 24h treatment – S9	Not clastogenic
Fedorova-Dahms et al., 2001[26]	DRAO	HPBL	Several up to 5 µL/mL Experiment I: 4 h treatment ± S9 Experiment II: 4h treatment + S9 & 24h treatment – S9	Not clastogenic

Author, Date	Test Item	Test System	Concentration / Dose	Outcome
Schmitt et al., 2012[27]	OCN-T18-AO	HPBL	Initial test: 0, 235, 336, 480 µg/mL (3h treatment – S9) & 0, 480, 686, 980 µg/mL (3h treatment + S9) Confirmatory test: 0, 500, 750, 1000 µg/mL (22h treatment – S9) & 0, 750, 1000, 1500 µg/mL (3h treatment + S9)	Not clastogenic
Lewis, et al., 2017[29]	DRO	HPBL	Phase I: 0, 1.25, 2.5, & 5.0 mg/mL (4h treatment ± S9) Phase II: 1.25, 2.5, & 5.0 mg/mL (24h treatment – S9; 4h treatment + S9)	Not clastogenic
In vivo Mammalian Micronucleus Test				
Hammond et al., 2001[20]	DRM	CD-1 mice (male) (bone marrow)	0, 500, 1000, and 2000 mg/kg bw	Not genotoxic
Fedorova-Dahms, et al., 2011[25]	AO	Mice (peripheral blood)	0 and 2000 mg/kg bw	Not genotoxic
Fedorova-Dahms et al., 2001[26]	DRAO	Mice (peripheral blood)	0 and 2000 mg/kg bw	Not genotoxic
Schmitt et al., 2012[27]	OCN-T18-AO	Sprague-Dawley (Hsd:SD, Harlan) rats (male) (bone marrow)	0, 500, 1000, 1500, or 2000 mg/kg bw	Not genotoxic
Lewis, et al., 2017[29]	DRO	Male and female wistar rats (bone marrow)	0, 1000, 2000, & 5000 mg/kg bw (two treatments)	Not genotoxic

Abbreviations: CHO, Chinese hamster ovary; HPBL, human peripheral blood lymphocytes; PIM, plate incorporation method; PM, pre-incubation method.

As summarized in Table 10 above, the biomass of a DHA-rich *Schizochytrium* sp. as well as the oils derived from four different *Schizochytrium* spp. have been evaluated for in vitro and in vivo genetic toxicity in accordance with internationally harmonized recommendations for standard genotoxicity test batteries. Evaluations conducted for each substance were a bacterial reverse mutation test, an in vitro mammalian chromosomal aberration test, and an in vivo mammalian micronucleus test (the biomass only was additionally evaluated in an in vitro mammalian cell gene mutation test using the *xprt* gene). In all the performed tests, results were negative for genotoxic potential of the test items under the applied conditions. While all studies performed were based on OECD and/or US FDA Redbook test guidelines, there were some deviations. Nonetheless, when considered in totality, the results are consistent across tests, and we conclude that *Schizochytrium* spp. lack concern for genotoxic potential.

Table 11. Summary of *Schizochytrium* spp. derived DHA Oral Toxicity Studies

Author, Date	Test Item	Study Duration	Dose Groups	Groups	Outcomes/NOAEL
Acute Oral Toxicity Studies					
Schmitt et al., 2012[27]	OCN-T18-AO	Single dose/ 14-day observation (OECD 425)	5000 mg/kg bw x 2	1 & 2 female Sprague-Dawley albino rats	LD_{50} >5,000 mg/kg bw
Lewis, et al., 2016[29]	DRO	Single dose/ 14-day observation (RB IV.C.2 fixed dose)	5000 mg/kg bw x 2	5 female Wistar rats/group	LD_{50} >5,000 mg/kg bw
Short-Term Oral Toxicity Studies					
Schmitt et al., 2012[27]	OCN-T18-AO	14 days	0, 10000, 25000, & 50000 ppm in diet	5 Hsd:SD Sprague-Dawley rats/sex/group	Well tolerated up to 50000 ppm
Fedorova-Dahms et al., 2014[17]	DRAO	21 days	0, 0.32 & 0.96% DHA in diet	6 Domestic Yorkshire Crossbred piglets/sex/group	Well tolerated up to 0.96% (~ 117.9 mg/kg bw/day)
Lewis, et al., 2016[29]	DRO	28 days	0, 1000, 2500, & 5000 mg/kg bw/day by gavage	10 Wistar rats/sex/group + corn oil positive control	NOAEL 5000 mg/kg bw/day DRO (~ 2069 mg/kg bw/day DHA)
Subchronic Oral Toxicity Studies					
Hammond et al., 2001[21]	DRM	13 weeks	0, 400, 1500, &4000 mg/kg bw/day in diet	20 Crl:CD(SD)BR rats/sex/group + FO positive control group	NOAEL 4000 mg/kg bw/day DRM (~ 413 mg/kg bw/day DHA)
Abril et al., 2003[22]	DRM	42 days (120 days low-dose only)	See footnote[b]	29 Landrace and Large White castrated male growing pigs	No adverse effects up to 5.745 kg DRM/pig (~ 1281 g DHA/pig)

[b] Three control diets were administered over the course of the study as commonly done in commercial swine operations. A starter diet from Days 0 to 37, a grower diet from Days 37 to 79, and a finisher diet from days 79 to 120. For treatment group one (TG1), DRM was added to all three control diets at 1.10%. For treatment groups two (TG2), three (TG3), and four (TG4), DRM was added only to the finisher diets at concentrations of 1.10, 3.30, and 5.51%, respectively, from Days 79 to 106 and then at decreased concentrations of 0.39, 1.17, and 1.94% from Days 107 to 120. The dietary interventions were targeted to provide overall exposures of 785 g DHA at a constant dose in TG1 and 250, 750, and 1250 g DHA over the last 42 days in TG2, TG3, and TG4, respectively. The latter three diets were designed to reflect commercial feeding regimes at 1-, 3-, and 5-fold, respectively, the anticipated commercial doses for swine operations.

Author, Date	Test Item	Study Duration	Dose Groups	Groups	Outcomes/NOAEL
Fedorova-Dahms et al., 2011[25]	AO	90 days	0, 0.5, 1.5, & 5% in diet	10 Sprague-Dawley rats/sex/group + menhaden oil positive control	NOAEL 5% AO (~ 1165 (M) or 1669 (F) mg/kg bw/day DHA)
Fedorova-Dahms et al., 2011[26]	DRAO	In utero + 90 days post weaning† + 30-day recovery	0, 0.5, 1.5, & 5% in diet	10 Sprague-Dawley rats/sex/group + FO positive control	NOAEL 5% DRAO (~ 1756 mg/kg bw/day DHA; 2086 mg/kg bw/day DHA+EPA for positive control)
Schmitt et al., 2012[27]	OCN-T18-AO	13 weeks + 4-week recovery	0, 10000, 25000, & 50000 ppm in diet	10 Hsd:SD Sprague-Dawley rats/sex/group + tuna oil positive control	NOAEL 50000 ppm OCN-T18-AO (~ 1330 (M) or 1480 (F) mg/kg bw/day DHA)
Schmitt et al., 2012[28]	OCN-T18-AO	In utero + 90 days post weaning‡	0, 10000, 25000, & 50000 ppm in diet	20 Crl:CD(SD) Sprague-Dawley rats/sex/group + tuna oil positive control	NOAEL (F) 25000 ppm OCN-T18-AO due to bw effects NOAEL (F) 50000 ppm OCN-T18-AO (~ 1419 mg/kg bw/day DHA)
Lewis, et al., 2016[29]	DRO	90 days + 14-day recovery	0, 1000, 2500, & 5000 mg/kg bw/day by gavage	20 Wistar rats/sex/group + corn oil positive control	NOAEL 5000 mg/kg bw/day DRO (~ 2069 mg/kg bw/day DHA)
Reproductive and Developmental Oral Toxicity Studies					
Hammond et al., 2001[23]	DRM	GD 6–15 dosing /observed through GD 20	0, 0.6, 6, & 30% in diet	25 female Crl:CD(SD)BR Sprague-Dawley rats/group	NOEL for maternal & developmental toxicity 30% DRM (~ 5.28 g/kg bw/day DHA)
Hammond et al., 2001[23]	DRM	GD 6–19 dosing /observed through GD 29	0, 180, 600, & 1800 mg/kg bw/day by gavage	22 female SPF New Zealand White rabbits/group + FO positive control	NOEL for developmental toxicity 1800 mg/kg bw/day DRM (~ 432 g/kg bw/day DHA) NOEL for maternal toxicity 600 mg/kg bw/day DRM due to bw effects and slight increase in SA.
Hammond et al., 2001[24]	DRM	F_0 males: 10 weeks prior to mating through 3 weeks post-mating F_0 females: 2 weeks prior to mating through lactation day 21	0, 0.6, 6, & 30% in diet	30 Crl:CD(SD)BR Sprague-Dawley rats/sex/group	NOAEL for reproductive performance & pup development 30% DRM (~ 1500 (M) or 1800 (F) mg/kg bw/day DHA)

Author, Date	Test Item	Study Duration	Dose Groups	Groups	Outcomes/NOAEL
Fedorova-Dahms et al., 2011[26]	DRAO	28 days prior to mating through gestation (F_0 males) or PND 22 (F_0 females)†	0, 0.5, 1.5, & 5%	13 male and 26 female Sprague-Dawley rats/sex/group + FO positive control	NOAEL for reproductive & developmental toxicity 5% DRAO
Schmitt et al., 2012[28]	OCN-T18-AO	GD 6–19	0, 400, 1000, & 2000 mg/kg bw/day by gavage	25 female Crl:CD(SD) Sprague-Dawley rats/group	NOAEL for maternal toxicity & embryo/fetal development 2000 mg/kg bw/day ONC-T18-AO (~ 805 mg/kg bw/day DHA)
Schmitt et al., 2012[28]	OCN-T18-AO	F_0 males: 70 days prior to mating through mating F_0 females: 14 days prior to mating through weaning‡	0, 10000, 25000, & 50000 ppm in diet	30 Crl:CD(SD) Sprague-Dawley rats/sex/group	NOAEL for reproductive & developmental toxicity 50000 ppm OCN-T18-AO (at least ~ 941 mg/kg bw/day DHA)
Falk et al., 2017[30]	DRO	GD 6–20	0, 1000, 2500, & 5000 mg/kg bw/day by gavage	24 female Wistar rats/ group + corn oil positive control	NOAEL for maternal toxicity & embryo/fetal development 5000 mg/kg bw/day DRO (~ 2069 mg/kg bw/day DHA)
Falk et al., 2017[30]	DRO	F_0 generation dosing commenced 84 (M) & 14 (F) days prior to mating* & continued until weaning in both sexes	1000, 2500, & 5000 mg/kg bw/day by gavage	20 male & 24 female Wistar rats/ group + corn oil positive control	NOAEL NOAEL for reproductive & developmental toxicity 5000 mg/kg bw/day DRO (~ 2069 mg/kg bw/day DHA)

Abbreviations: ~, approximately; F, female; FO, fish oil; GD, Gestation Day; M, male; PND, Post-natal Day; RB, US FDA Redbook.
†OECD 408 90-day oral toxicity study conducted in combination with an OECD 422 reproduction and development toxicity study; ‡OECD 408 90-day oral toxicity study conducted in combination with an OECD 415 one-generation reproduction toxicity study; *Males were dosed for at least one complete spermatogenic cycle (84 days) prior to mating, Females were dosed for two complete estrous cycles (14 days) prior to mating.

Table 11 above summarizes the published acute, short-term, subchronic, and reproduction and developmental toxicity studies that have been conducted on the biomass of a DHA-rich *Schizochytrium* sp. as well as the oils derived from four different *Schizochytrium* spp.. While the fatty acid fractions of each of the above test items differ somewhat from one to another and to that of Xiamen Huison's DHA Algal Oil (the article of commerce that is the subject of this GRAS notice), DHA is the predominant fatty acid in each, most other fatty acids are present in small

amounts, and those present in amounts more than 10% of the oil fraction composition are generally similar among each of the test items (see Table 9). Moreover, for the most part, the body acts on all fatty acids in essentially the same way (see Subpart 6.1), and therefore, these differences are not expected to present differential toxicological concerns when used in foods in a substitutional manner based on total DHA and EPA combined (in order to ensure exposure to DHA and EPA is maintained within the levels determined by FDA, with respect to menhaden oil, to be protective of possible adverse effects). While all studies performed were based on OECD and/or US FDA Redbook test guidelines, there were some deviations in some of the studies. Nonetheless, when considered in totality, the results and conclusions are consistent across studies, as well as with the broader literature on DHA and other polyunsaturated fatty acids (PUFA), and we are in general agreement with the conclusions drawn.

With respect to effects observed in some of the studies summarized in Table 11 that were considered attributable to the test item but non-adverse, these, for the most part, were considered species specific effects relevant only in rats. For example, test item-related histopathological lesions observed by Hammond et al.[21] in the hearts of study animals were characterized as cardiomyopathy (small foci mononuclear inflammatory cells and degeneration of cardiac myofibers) of minimal severity and were observed at a statistically significantly increased frequency in high-dose DRM males compared to untreated and FO controls (and also observed in all other groups (including controls) at lower incidence). This was considered to be a species-specific effect due to the high polyunsaturated fat (PUFA) content of the test diets. The observed lesions were identical to lesions observed in early stages of spontaneous cardiomyopathy associated with aging in many strains of laboratory rats, and the development of cardiomyopathy in rats is also known to be increased by diets containing high levels of PUFA[31, 32] as well as by diets inadequate in various nutrients (e.g., vitamin E).[33, 34] However, similar lesions have not been observed in other species in response to a diet containing similar amounts of oils high in PUFAs,[35-37] and among rats, SD strain males are particularly susceptible.[38-40] It is well-established that tissue phospholipid composition is a reflection of dietary fatty acid intake[31]; therefore, the extensive accumulation of omega 3 fatty acids in cardiac phospholipids is an expected observation in the rat and was not considered adverse. In addition, also in contrast to metabolism in other animals, long-chain fatty acids are known to be resistant to β-oxidation in rats and to inhibit the citric acid cycle. Furthermore, *Schizochytrium* spp. are not known to produce any known cardiotoxins instead demonstrating only the presence of substances that are normal components of the human diet (see Subpart 6.5). Increases in alkaline phosphatase, liver weights, and/or histological observations of lipid containing, non-degenerative, non-inflammatory vacuoles in livers (and sometimes adrenal glands and kidneys) of treated (test item and positive controls) rats were also observed in some studies and were attributed to species specific physiological adaptive responses to a high-PUFA diet due to their occurrence in studies of other PUFA-

containing test items and in the positive control groups and their general reversibility during recovery periods.

With respect to observed effects that were considered attributable to the test item and adverse, in the developmental toxicity study in rabbits by Hammond et al.,[23] the mid-dose was considered to be the NOEL for maternal toxicity due to observations in the high-dose group of statistically significantly decreased mean body weight gain and food consumption from GDs 12–19, and also when considered for the treatment period as a whole (GD 6–19), together with a slight, non-significant increase in spontaneous abortion. However, because body weight gain and food consumption were affected similarly in both the high-dose DRM and FO positive control groups, with the effects more pronounced in the FO group, and spontaneous abortion frequency was also similar (and occurs in rabbits more frequently than in other laboratory species), we conclude that the observed maternal toxicity may be considered reflective of a general effect of high-fat consumption rather than a specific effect of DRM and the high-dose group may be appropriately considered as a NOAEL.

6.2.2 Non-*Schizochytrium* spp. derived DHA Studies

A number of additional toxicological studies have been performed on DHA containing oils derived from sources other than *Schizochytrium* spp. Summaries of these published studies are provided in Tables 12 & 13 below.

Table 12. Summary of Non-*Schizochytrium* spp. derived DHA Genetic Toxicity Studies

Author, Date	Test Item	Source	Study Type	Concentration / Dose	Test System	Outcome
Blum et al., 2007a[41]	DHA (45%) algal oil	*Ulkenia* sp. SAM2179	BMRT	0.5, 1.25, 2.5, 3.75, & 5 mg/plate ± S9 (PM) 0.062, 0.185, 0.556, 1.667, & 5 mg/plate ± S9 (PIM)	*S. typhimurium* TA97, TA98, TA100, and TA102 (PM) *S. typhimurium* TA1535, TA1537, TA98, and TA100, and *E. coli* WP2 *uvrA* (PIM)	Not mutagenic
			CAT	1.25, 2.5, & 5 mg/mL ± S9	Chinese hamster fibroblast cells.	Not clastogenic

Abbreviations: BMRT, bacterial reverse mutation test; CAT, in vitro mammalian chromosomal aberration test; Conc, concentration; PIM, plate incorporation method; PM, pre-incubation method.

Table 13. Summary of Non-*Schizochytrium* spp. derived DHA Oral Toxicity Studies

Author, Date	Test Item	Source	Study Duration	Dose Groups	Groups	Outcomes/NOAEL
Wibert et al., 1997[42]	DHA & AA oil blend to approximate human breast milk ratio	*C. cohnii* & *Mortierella alpine*, respectively	4 weeks	Low -(5%) & high-fat (13.1%) control diets. Test item added to high-fat diet at expense of canola oil at 1.8, 6.0, & 12.0%	10 rats/sex/group	NOAEL: 12.0% of diet
Burns et al., 1999[43]	DHA & AA oil blend to approximate human breast milk ratio	*C. cohnii* & *M. alpine*, respectively	Premating–weaning of F_0 dams; 90-days post weaning of F_1 pups	Low -(5%) & high-fat (13.1%) control diets. Test item added to high-fat diet at expense of canola oil at 1.8, 6.0, & 12.0%	F_0: 28F & 14M rats/group; F_1 pups: 20 rats/sex/group	Maternal weight gain (wk 3 of gestation) and litter size lower in the high-fat controls compared to low-fat controls; treated groups were comparable to high-fat controls. All other parameters comparable among groups in both studies. DHA/AA NOAEL considered 120 g/kg diet. a
Arterburn et al., 2000[8]	DHA (40–50%) oil	*C. cohnii*	91–93 days	0, 0.5, & 1.25 g/kg bw/day (gavage) + untreated control	20 rats/sex/group	NOAEL for general & neurotoxicity: 1.25 g/kg bw/day
Blum et al., 2007a[41]	DHA (45%) algal oil / DHA (26.7%) fish oil combined	*Ulkenia* sp. SAM2179 / tuna	90 days + 28-day recovery	0, 0/2000, 500/1500, 1000/1000, & 2000/0 mg/kg bw/day (gavage) resulting in DHA exposure of 0, 540, 630, 720, & 900 mg/kg bw/day	15 rats/sex/group + five rats/sex in recovery groups (control and high-doses only)	NOAELs: 2000 mg/kg bw/day (equivalent to 900 & 540 mg/kg bw/day DHA, respectively, from the algal and tuna oil test items).

Author, Date	Test Item	Source	Study Duration	Dose Groups	Groups	Outcomes/NOAEL
Blum et al., 2007b[44]	DHA (45%) algal oil	*Ulkenia* sp. SAM2179	1-gen repro tox: 10 wk prior to mating through mating (M) or lactation (F)	0, 1.5, 3.0, & 7.5% of diet; (7.5% corn oil added to commercial diet for control diet); control and test diets contained 11.6, 5.6, 7.1, & 11.6% total fat, respectively.	28 rats/sex/group	NOAEL F_0 toxicity: 3.0% due to yellow fat disease/steatitis (species specific disorder not reported in humans); NOAEL F_0 reproductive performance & F_1 pup survival and development: 7.5% (equivalent to 3.4 (M-premating), 4.0 (F-premating), 7.9 (F-gestation), & 7.8 (F-lactation) g/kg bw/day.
Hadley et al., 2010[9]	DHA EE (90%) & DHA (40–50%) oil	*C. cohnii*	90 days + 1-month recovery	0 (corn oil vehicle/control) DHA EE: 1.3, 2.5, 5.0 g/kg bw/day (1.2, 2.25 & 4.5 g/kg bw/day DHA) DHA oil: 5.0 g/kg bw/day (2.0 g/kg bw/day DHA) (gavage)	10 rats/sex/group + five rats/sex in recovery groups (control and high-dose only)	NOAEL DHA EE: 2.5 mg/kg bw/day (due to marked infiltration of the mesenteric lymph node) NOAEL DHA oil: 5.0 mg/kg bw/day.
Dahms et al., 2016[45]	DHA EE (90%)	*C. cohnii*	9 months + 2-month recovery	0, 150, 1000, & 2000 mg/kg bw/day (gavage)	Three Beagles/sex/group (main phase) + five Beagles/sex/group (recovery phase)	NOAEL 2000 mg/kg bw/day

Abbreviations: 1-gen repro tox, one-generation reproductive toxicity study; AA, arachidonic acid; EE, ethyl ester; F, female; M, male

6.2.3 Unpublished Studies on the Article of Commerce

As part of the requirements for a National Health Care Product License ("blue hat") in China, Xiamen Huison has conducted a battery of toxicological studies on their DHA Algal Oil ingredient derived from *Schizochytrium* sp., strain HS01 ingredient (i.e., the article of commerce that is the subject of this safety assessment):

- DHA Algal Oil was not mutagenic in a bacterial reverse mutations test in *S. typhimurium* tester strains TA97a, TA98, TR100, and TA102 at concentrations up to 5000 µg/plate with and without metabolic activation,
- DHA Algal Oil was not genotoxic in an in vivo mammalian (Kunming mice) bone marrow micronucleus test at doses up to 10.00 g/kg bw,
- DHA Algal Oil was not mutagenic an in vivo mammalian (Kunming mice) sperm deformity test at doses up to 10.00 g/kg bw,
- A maximum tolerated dose of >18.74 g/kg bw DHA Algal Oil was established in an acute oral toxicity test in male and female ICR mice,
- In a 30-day repeated-dose oral toxicity test in Sprague-Dawley rats, the NOAEL was concluded to be 1.67 g/kg bw/day, the highest dose tested.

The above results corroborate the results of toxicological studies conducted on other DHA oil containing ingredients derived from other *Schizochytrium* spp. and other sources.

6.3 Additional Scientific Studies

6.3.1 Human Studies

A number of human studies investigating effects of DHA from a variety of sources including *Schizochytrium* spp. have been conducted and published and are summarized in Table 14 below. Adverse events reported in these studies were limited to mild effects (e.g., headache, GI symptoms) and, generally, did not differ in incidence to those reported in control groups or were determined unlikely to be related to the administered test item(s).

Table 14. Summary of Clinical Trials

Author, Date	Dose & Description	Dosing Duration	Subjects	Adverse Effects
Maki et al., 2005[46]	1.52 g DHA (*Schizochytrium sp.*) daily (olive oil control)	6 weeks	57 healthy adults	There was no significant difference between groups for treatment-emergent adverse events.
Jensen et al., 2005[47] & Jensen et al., 2010[48]	Algal TAG 41.7% DHA by weight, approximately 200 mg DHA/day (soy/corn oil control)	4 months	227 breastfeeding women	No safety outcomes included; no AEs reported
Clandinin et al., 2005[49]	Algal-DHA (17 mg DHA/100 kcal from algal oil; Tuna-DHA (17 mg DHA/100 kcal); (control formula without DHA)	Sole nutrition up to 57 wks PMA, primary nutrition PMA 57–92 wks	361 pre-term infants	No increase in morbidity or AEs. Three deaths in the tuna group during initial hospitalization were determined unrelated to study formula.
Lloyd-Still et al., 2006[11]	Algal oil 50 mg DHA per kg per day (1 to 4.2 g DHA) (corn/soy oil control)	6 months	20 subjects (8 to 20 y) with cystic fibrosis	AEs did not differ from placebo. Well tolerated.
Sanders et al., 2006[50]	4 g DHA from *Schizochytrium* sp. (olive oil control)	4 weeks	79 adults	No AEs or pathological changes in hematology or other biochemical indices.
Theobald et al., 2007[51]	0.7 g DHA/day from *C. chonii* (olive oil control)	2 phases of 3 months duration	38 healthy adults	Well tolerated, blood counts and liver tests unaffected in both groups.
Arterburn et al., 2007[18]	200, 600, 465, or 1000 mg DHA daily from *C. chonii* or *Schizochytrium* sp. (corn/soy control)	28 days	96 healthy adults	No clinically significant AEs reported; eructation was significantly associated with supplementation but did not cause discontinuation.
Arterburn et al., 2008[19]	205 mg DHA/day from *C. chonii* (cooked salmon control)	2 weeks	32 healthy adults	Reported AEs in both groups were minor and transient (headache, eructations, mild-to-moderate gastrointestinal disturbances).

Author, Date	Dose & Description	Dosing Duration	Subjects	Adverse Effects
Van Biervliet et al, 2008[52]	500 mg algal TAG 40% DHA (sunflower seed oil control)	1 year	17 cystic fibrosis patients 6 y and older	Two patients reported eructations with a fishy taste. One dropped out unrelated to treatment.
Hoffman et al., 2008[53]	Soy formula + 17 mg DHA/100 kcal from algal oil and 34 mg AA/100 kcal from fungal oil (soy formula control)	14 to 120 days	244 healthy term infants	Discontinuation rates due to AEs did not differ from controls. AEs reported in 6 treatment subjects determined unrelated to test item.
Wien et al., 2010[54]	10:1 or 2:1 micro algae oil, 0.20/0.72 g EPA/DHA per 2400 kcal/d (no control)	3 8-week study periods (24 treatment sequences)	24 healthy adults	No safety outcomes included; no AEs reported.
Vanlint & Ried, 2012[55]	400 mg algal DHA daily (corn oil control)	12 months	37 osteopenic adults	No SAEs reported; minor AEs did not differ among groups.
Richardson et al., 2012[56]	600 mg algal DHA/day (corn/soybean oil control)	16 weeks	362 healthy children $\leq 33^{rd}$ percentile in reading	No SAEs reported. Two minor AE dropouts in active group. Six weeks after completion, 1 child reported hair loss. No side effects as assessed by Barkley scale.
Alicandro et al., 2013[57]	100 mg/kg/day or 1 g/day algal derived TAG (germ oil control)	12 months total (1 month/100 mg, 11 months/1 g)	41 children with cystic fibrosis	Well tolerated and without relevant GI symptoms as compared to control. One subject had mildly increased liver enzymes, concurrent with itraconazole/claritromycine therapy.
Kagan et al., 2013[58]	Algal oil (*Nannochloropsis oculata*) ~1.5 g EPA only; krill oil 1.02 g EPA/0.54 g DHA	Single dose of each product	10 healthy males	No indication of intolerance with either product.
Uhl et al., 2013[59]	510 mg microalgal DHA/day	29 days	13 healthy adults	No AEs occurred

Author, Date	Dose & Description	Dosing Duration	Subjects	Adverse Effects
Purcell et al., 2014[60]	5 g DHA from *C. chonii* (AO) or FO; (high-oleic acid sunflower oil (HOSO) control); 4 test meals containing AO or FO alone or in combination with HO.	Single dose of each test meal in a crossover design	16 healthy men	No AEs reported.
Hughbanks-Wheaton et al., 2014[61]	30 mg/kg/d DHA, total dose range 600–3600 mg (corn/soy oil control)	4 years	52 males with X-linked retinitis pigmentosa	Treatment emergent AEs were minor and sporadic in occurrence and duration with exception of one case of recurrent GI symptoms who discontinued participation in year 2.
Lane et al., 2014[14]	Approximate dose of 1200 mg DHA nanoemulsion from *schizochytrium* sp. (non-microemulsion DHA control)	Single dose, crossover design	11 healthy adults	AE reporting not included in paper.
Scholtz et al., 2015[62]	600 mg algal DHA (corn/soybean oil control)	Last 2 trimesters of pregnancy	205 pregnant women with FADS genotype	AEs not included in outcomes.
Yurko-Mauro et al., 2015[63]	Krill oil, fish oil/ethyl ester, or fish oil/TAG; Total dose EPA + DHA 1.3 g/day	28 days	66 healthy adults	No adverse effects on safety evaluation parameters (e.g., vitals, physical exam). Eleven AEs reported by 9 subjects, none of which were serious (e.g., headache, GI symptoms).

Author, Date	Dose & Description	Dosing Duration	Subjects	Adverse Effects
Kohler et al., 2017[15]	80 g/day sausage supplemented with approximately 250 mg EPA + DHA from anchovy oil (non-supplemented sausage control)	8 weeks	44 healthy adults	All documented AEs were mild and regarded as not related to the intervention product (influenza, mild URI).
Shimada et al., 2017[16]	FFA capsule providing approximately 2200 mg EPA & 800 mg DHA	3 treatment periods of 1 day	42 healthy Japanese males	Diarrhea reported by 11 subjects, 1 episode of pre-syncope. No AEs were serious. No clinically remarkable findings (lab tests, vital signs, EEG, or physical findings).
Noda, et al., 2018[13]	FFA 2 g or 4 g EPA + DHA per day	14 days	18 healthy Japanese males, 6 healthy Caucasian males	No SAE, discontinuation due to AE, or changes in clinical exams. Elevated ALT in 1–2 persons in all groups.

Abbreviations: TAG, triacylglycerol; AE, adverse event; PMA, post-menstrual age; AA, arachidonic acid; SAE, serious adverse event; GI, gastrointestinal; FO, fish oil; FADS, fatty acid desaturase; URI, upper respiratory infection; FFA, free fatty acid; ALT, alanine aminotransferase.

Additionally, no adverse reactions or effects on blood and urine parameters were observed in an unpublished clinical trial in which 10–15-year-old children were administered 500 mg daily of Xiamen Huison's DHA Algal Oil ingredient for 30 consecutive days.

6.4 Authoritative Safety Opinions

6.4.1 FDA

Menhaden oil has been affirmed GRAS for its uses as listed in regulation at 21 CFR §184.1472. The specific limitations on use of menhaden oil were established to ensure that total DHA + EPA exposure does not exceed 3.0 g/person/day; the level of exposure determined by FDA to be protective of possible adverse effects.[64]

6.4.2 European Food Safety Authority

The European Food Safety Authority (EFSA) set an Adequate Intake (AI) of 250 mg per day combined EPA and DHA for adults and children 2 to 18 years of age with an additional 100–200 mg per day DHA for pregnant and lactating women. AI of DHA for infants >6 months of age and children <24 months of age was set as 100 mg per day.[65]

EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA) concluded that data was insufficient to set Tolerable Upper Intake Levels for EPA, DHA, or *n*-3 docosapentaenoic acid (DPA) alone or in combination.[66] The Panel opined that long-term intake up to 5 g/day of EPA and DHA combined does not present a health concern (as long as oxidative stability is preserved) and that supplemental intake up to 1 g/day of DHA alone also does not present a health concern in the general population. The use of a DHA and EPA rich algal oil from *Schizochytrium* sp. as a novel food ingredient (NFI) was authorized in a range of foodstuffs at levels ranging from 80 to 600 mg/g (or absolute levels in food supplements and meal replacements) by the United Kingdom in accordance with Article 4(2) of Regulation (EC) No 258/97.[67] NDA was asked to opine on a request to extend the use of this oil in food supplements intended for the normal adult population, excluding pregnant and lactating women, at levels up to 3 g/day (the current approved level was 0.25 g/day in the general population and 0.45 g/day in pregnant and lactating women). The Panel concluded the proposed use extension of the NFI would not result in the level of 5 g/day of EPA and DHA combined, previously concluded by the Panel as safe, to be exceeded.

A DHA-rich oil from the *Schizochytrium* sp. specified to contain >32% DHA has also been approved by the European Commission (EC) as an NFI in various food stuffs at levels ranging from 200 to 600 mg/g (or absolute levels in food supplements and meal replacements,[68] with uses extended in 2009,[69] and Xiamen Huison's 35% DHA Algal Oil from *Schizochytrium* sp., strain WZU477 (note, this organism has since been replaced by Xiamen Huison with the current organism *Schizochytrium* sp., strain HS01 that is the source of the 50% DHA oil that is the subject of this GRAS Notice) was authorized as an NFI based on an argument of substantial equivalency to this ingredient.[70, 71]

6.5 Non-pathogenicity and Non-toxicogenicity

Pang et al. reported that several mangrove dwelling members within the class Labyrinthulomycetes (referred to as Labyrinthulea in Subpart 2.1 of this report) are "parasitic or pathogenic, causing serious diseases such as eelgrass wasting disease and the hard clam disease Quahog Parasite Unknown (QPX) in North America and Europe."[72] In addition to these reports, Bower reported that *Labyrinthuloides haliotidis* is pathogenic to juvenile abalone.[73] Due to changes and continuing debate regarding taxonomic classification within kingdom Chromista and class Labyrinthulea, it is not entirely clear where *Labyrinthuloides haliotidis* is currently assigned within the class.

The pathogen responsible for eelgrass (an underwater flowering plant) wasting disease was identified as a single *Labyrinthula* sp. using techniques of cell culture and disease inoculation following Koch's postulates; a total of 145 disease tests were performed.[74] A specific epithet was not assigned due to uncertainties in assignment based on morphological characteristics within the genus. *Labyrinthula*

sp. are members of the order Labyrinthulales and, thus, related to *Schizochytrium* at the class level.[1]

Using small-subunit (ssu) rDNA analysis and BLAST (basic local alignment search tool), Ragan et al. were able to positively assign QPX to phylum Labyrinthulomycota (possibly synonymous with Bigyra as used in Subpart 2.1 of this report) and determined that its closet relative is the Thraustochytriales member *Thraustochytrium pachydermum*.[75] Similarly, Yokoyama and Honda confirmed this relationship based on 18S rRNA analysis.[1] In their phylogenetic analysis based on molecular genetics (ssu rDNA), Stokes et al. were also able to classify QPX as belonging to phylum Labyrinthulomycota but could not confirm the specific species or whether all QPX organisms belong to a single species.[76] They did however, confirm that QPX is not Thraustochytriales species *Schizochytrium aggregatum*, *Thraustochytrium aureum,* or *T. striatum*. It is also noteworthy that infection with QPX is only known to occur in waters of eastern North America and, while lethal to clams, we found no reports of QPX affecting humans, and according to The Rhode Island Marine and Estuarine Invasive Species Site, "QPX has no effects on humans."[77]

With respect to organisms with pathogenic or toxicogenic potential towards humans, our searches of the scientific literature failed to locate any known reports or concerns related to *Schizochytrium* spp. or the order Thraustochytriales or phylum Bigyra, in general. Ryan et al., in their book chapter on *Safety Evaluation of Single Cell Oils and the Regulatory Requirements for Use as Food Ingredients*, also reported that they were unable to locate any reports of pathogenicity of, or toxin production by, *Schizochytrium* spp.[78]

Members of kingdom Chromista known to produce toxins diverge from *Schizochytrium* at the level of phylum or higher. Reports are associated with superclass Dinoflagellata (within superphylum Alveolata), which share commonality with *Schizochytrium* at the level of infrakingdom; species of the Prymnesiophyceae, which includes the genus, *Prymnesium*, in phylum Haptista, and, therefore, related to *Schizochytrium* at the level of subkingdom; species of the class Bacillariophyceae (diatoms), in phylum Gyrista and, therefore, related to *Schizochytrium* at the level of superphylum; and species of the genus *Ochromonas*, also in phylum Gyrista.[2, 79-81]

Nonetheless, all of the toxicogenic organisms discussed above are only distantly taxonomically related to species of the order Thraustochytriales to which *Schizochytrium* sp., strain HS01 belongs, and therefore, there is no scientific justification that any of the toxins produced by these organisms are a cause for concern with respect to *Schizochytrium* spp. The lack of any reports of pathogenicity of, or toxin production by, *Schizochytrium* spp. indicate that *Schizochytrium* spp., including *Schizochytrium* sp., strain HS01, may be concluded as non-pathogenic and non-toxicogenic without the need of any specific toxin testing (i.e., there are no known toxins for which to test. Additionally, in the toxicological studies on a

Schizochytrium sp. biomass discussed in Subpart 6.2 above, if this closely related species possessed pathogenic or toxicogenic potential, some indication of this would have been expected. Thus, based on our searches of the public domain and the absence of toxic effects in a battery of formal toxicological investigations on a related organism there is no reason to suspect a pathogenic or toxicogenic potential of *Schizochytrium* sp., strain HS01.

6.6 Allergenicity

DHA Algal Oil is not genetically engineered and does not contain or have added, and is manufactured in a facility free of, all eight major allergens (milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) identified, and required to be disclosed in labeling, in the Food Allergen Labeling and Consumer Protection Act (FALCPA). Additionally, DHA Algal Oil does not contain gluten, celery, mustard, or sesame seeds or any derivatives or products of the aforementioned. DHA Algal Oil does not contain sulfur dioxide and sulfites at concentrations of 10 mg/kg or 10 mg/L expressed as sulfur dioxide.

No reports of allergic reactions to DHA, DHA algal oils derived from *Schizochytrium* spp., or to *Schizochytrium* spp. or Thraustochytrids in general were found in our investigations. A single case report was located of an allergic reaction associated with ingestion of 4 g daily (2 capsules twice daily) for four days of a prescription fish oil, Lovaza®, containing a mixture of predominately EPA and DHA fatty acid ethyl esters derived from several fish sources occurring in an individual with a documented seafood allergy.[82] The labeling of Lovaza® contains the following warning/precaution: "**Fish Allergy** LOVAZA contains ethyl esters of omega-3 fatty acids (EPA and DHA) obtained from the oil of several fish sources. It is not known whether patients with allergies to fish and/or shellfish, are at increased risk of an allergic reaction to LOVAZA. LOVAZA should be used with caution in patients with known hypersensitivity to fish and/or shellfish." Thus, it is likely that this reaction, if due to Lovaza®, was related to residual fish proteins, which are not contained in DHA Algal Oil. Overall, the potential for allergic reactions to Xiamen Huison's DHA Algal oils to occur is considered very low.

6.7 History of Consumption

In their articles describing toxicity studies conducted on DHA-rich microalgae from *Schizochytrium* sp., Hammond et al. stated, "Direct consumption by man of thraustochytrids, especially those of the genus *Schizochytrium*, is primarily through consumption of mussels and clams. Indirect consumption, through the marine food chain (fish and shellfish), is more widespread"[20, 21, 23, 24] although they provided no citations. Our literature searches did not discover any quantitative or qualitative data regarding consumption of *Schizochytrium* sp. by humans.

As described in Part 3, with respect to DHA, based on food consumption data from the WWEIA dietary component of NHANES, following assignment of DHA concentrations to all relevant NHANES (2013–2014) food codes using composition data from the USDA FNDDS, current per capita consumption of DHA in the proportion of the US population that reported consuming DHA containing foods is approximately 58 mg/day at the mean and 138 mg/day at the 90th percentile. In some subpopulations, much higher DHA consumption has been reported. For example, mean DHA intake in Yup'ik Eskimos, who consume a traditional diet high in marine foods, has been reported to be 3.7 and 2.4 g/day in men and women, respectively.[83]

6.8 Past Sales and Reported Adverse Events

According to Xiamen Huison, approximately 154,500 kg of the company's DHA Algal Oil have been sold over the lifetime of the product. The majority of these sales, 153,000 kg, have occurred between January 1, 2013 and the date of this report. Xiamen Huison declares that no serious adverse event reports associated with the consumption of this ingredient to date have been received by the company.

No FDA letters regarding concern for safety to companies that market products containing DHA or *Schizochytrium* spp. or Thraustochytrids in general were located. A search of MedWatch, FDA's adverse event reporting program and FDA's Recalls, Market Withdrawals, & Safety Alerts search engine did not uncover any mention of products containing DHA or *Schizochytrium* spp. or Thraustochytrids in general products. A search of FDA's Center for Food Safety and Applied Nutrition (CFSAN) Adverse Event Reporting System (CAERS) found 141 of 92,232 (0.15%) adverse event reports (AER) listed in the system for the period of time spanning January 2004 through September 2017 that were associated with products containing DHA. Of these 141 AERs, 98 were categorized (as specified by the reporter) as serious adverse events (SAE). Three non-serious AERs were associated with topical use of cosmetic products. Of the remaining 138 AERs, the listed product, as categorized by the reporter, was reported as "suspect" in 126 adverse events (AE), of which 88 were categorized as SAE, and the remaining 12, of which 10 were categorized as SAE, were reported as concomitant (AERs may specify consumption of multiple products). The major food categories involved were dietary supplements (71 AERs) followed by baby food (61 AERs). Of the remaining six, five were associated with milk products and one with a soy milk product. The majority reported various gastrointestinal symptoms while only seven were reported as hypersensitivity reactions. Twenty-two involved hospitalization (15 dietary supplements and 7 baby foods) and two deaths (both associated with infant formulas and reported as suspect) were reported. The vast majority involved products containing multiple other ingredients in addition to DHA, and based on product descriptions, only nine are suspected of being DHA oil only products with two specifically identified as algal DHA products (one categorized by the reporter as suspect, one as concomitant, and both as non-serious). We note that reported AEs

associated with products that contain DHA but for which DHA was not listed brands and/or product names reported would not have been located in our searches. Most importantly, AERs are only associations, and reported products may not be causally related to the AE. CFSAN notes the following:

> "*The adverse event reports about a product and the total number of adverse event reports for that product in CAERS only reflect information AS REPORTED and do not represent any conclusion by FDA about whether the product actually caused the adverse events. For any given report, there is no certainty that a suspected product caused a reaction. Healthcare practitioners, firms, agencies, consumers, and others are encouraged to report suspected reactions; however, the event may have been related to a concurrent underlying condition or activity or to co-consumption of another product, or it may have simply occurred by chance at that time.*"

Additionally, it is noted that AERs vary in quality and reliability and CAERS may contain duplicate reports. All of the above databases were accessed on April 17, 2018.

6.9 Current Regulatory Status

A thorough search for the current regulatory status of algal oil and/or DHA, relevant to their use in food in the United States, was conducted. A summary of the pertinent search results is shown below:

- Pursuant to 21 CFR §184.1472 menhaden oil is GRAS for human consumption with specific limitations in a variety of food categories in order to ensure that intake of EPA or DHA does not exceed a combined daily intake of 3.0 grams/person/day. Included among the intended use food categories are Fats and oils, including margarine, dressings for salads, butter, salad oils, shortenings and cooking oils, excluding use in infant formula, (21 CFR §170.3(n)(12)) at a maximum use level of 12.0% (approximately 2.4% EPA+DHA) and Gelatins, puddings, and fillings, including flavored gelatin desserts, puddings, custards, parfaits, pie fillings, and gelatin base salads (21 CFR §170.3(n)(22)) at a maximum use level of 1.0% (approximately 0.2% EPA+DHA) [Note, menhaden oil contains approximately 8% DHA and 12% EPA].
- An FDA GRAS notice (GRN No. 137) for algal oil derived from *Schizochytrium* sp. received FDA's no objections letter indicating no current challenge to the safety of the ingredient for its intended use as the sole source of DHA in the same food categories listed in 21 CFR §184.1472(a)(3) at levels not to exceed 29% of those in any of the listed food categories. Additionally, the algal oil would not be combined or augmented with any other oil that is rich in DHA or EPA. The algal oil contains approximately 35 percent (by weight) DHA, 3 percent EPA, 24% palmitic acid, 13.5%

docosapentaenoic acid, and 10% myristic acid and is manufactured to food grade specifications using pure culture grown in a heterotrophic fed-batch fermentation process followed by concentration, drying, hexane extraction, centrifugation and/or filtration, and winterization. The winterized oil is heated and treated with acid followed by treatment with caustic, centrifugation, bleaching, and deodorizing. Finally, antioxidants are added and the oil is packaged and each lot is subjected to batch analysis.

- Four FDA GRAS notices (GRN Nos. 553, 677, 776 & 777) for algal oil derived from *Schizochytrium* sp. received FDA's no objections letter for their intended use in various infant formulas.
- An FDA GRAS notices (GRN No. 731) for algal oil derived from *Schizochytrium* sp. has been submitted and is currently under FDA review and pending a response for its intended use in various infant formulas.
- An FDA GRAS notices (GRN No. 732) for algal oil derived from *Schizochytrium* sp. has been submitted and is currently under FDA review and pending a response for its intended use as the sole source of DHA in the same food categories listed in 21 CFR §184.1472(a)(3) at levels not to exceed 22.22% of those in any of the listed food categories. Additionally, the algal oil would not be combined or augmented with any other source of DHA.
- Several other FDA GRAS notices for oils derived from other species of algae have also received no objection letters for various intended uses (oils with high DHA or EPA concentrations for use in infant formula or in the food categories specified in 21 CFR §184.1472(a)(3)); oils with low levels or no DHA and EPA for various other uses).
- Several FDA GRAS notices for fish oils or fungal oils have received no objections letters for use in food categories specified in 21 CFR §184.1472(a)(3) or infant formula.

6.10 Basis for the GRAS Conclusion

DHA Algal Oil has been the subject of a thorough safety assessment as described above. The totality of evidence supporting the safety of DHA Algal Oil is comprised of data and information that establish the safety of DHA Algal Oil under the conditions of its intended use and data and information that is corroborative of safety. 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 Xiamen Huison's conclusion of GRAS status of DHA Algal Oil for its intended use.

6.10.1 Data and Information that Establish Safety

The scientific data, information, and methods establishing the safety of the intended use of Xiamen Huison's DHA Algal Oil are:

- The establishment of identity and non-pathogenicity and non-toxicogenicity of the *Schizochytrium* sp., strain HS01 as well as the characterization and fatty acid profile of the derived DHA Algal Oil demonstrating a composition of commonly consumed edible fatty acids;
- The method of manufacture and specifications, demonstrating the safe production and the quality control standards of DHA Algal Oil;
- The exposure analysis demonstrating the intended use of Xiamen Huison's DHA Algal Oil will not result in increased exposure to DHA or exposure to DHA and EPA combined above the limit established by US FDA;
- Well established ADME data demonstrating the well-known and accepted ways in which the body acts on edible fatty acids in general as well as ADME specific to DHA, including DHA derived from *Schizochytrium* spp.;
- Genetic and oral toxicity (including reproduction and developmental toxicity) studies demonstrating the safety of the biomass of a *Schizochytrium* sp. high in DHA content;
- Genetic and oral toxicity (including reproduction and developmental toxicity) studies demonstrating the safety of the high-DHA content oils extracted from a variety of sources including *Schizochytrium* spp.
- Clinical trials on DHA from various sources (including *Schizochytrium* spp.) at doses higher than the estimated exposure for DHA Algal Oil without SAEs and with minor AEs not attributable to the test items and/or, generally, with similar incidence in control groups.

Because ingredients that are comprised of edible fatty acids provide macronutritive content to the diet, their use in foods will necessarily be at relatively high levels. It is not feasible to test such uses in laboratory animals at doses many-fold greater than the level of exposure in humans. This is especially the case with respect to the rat, which does not tolerate high levels of dietary fat as well as humans and some other species. Nonetheless, many preclinical toxicological studies have been conducted on DHA-rich edible fatty acid ingredients and have not raised toxicological concerns. Additionally, many human studies on DHA-rich edible fatty acid ingredients have been conducted and have not resulted in cause for concern at provided dose levels higher than the estimated exposure for DHA Algal Oil.

The well-known physiological processes by which the human body acts on edible oils are described in Subpart 6.1 and, additionally, the specific pharmacokinetics of DHA-rich oils, including ingredients derived from *Schizochytrium* spp., were described and are reasonably consistent among humans and laboratory animals.

Most importantly, as part of its affirmation of GRAS status of the intended uses of menhaden oil, codified at 21 CFR §184.1472, FDA established that a limit on exposure to combined EPA + DHA mean levels of 3 g/person/day (approximately equivalent to 43 mg/kg bw/day in a 70 kg human) is protective of possible adverse effects. DHA Algal Oil is comprised of 50–60% DHA and is not a significant source of EPA; therefore, the exposure analysis in Subpart 3.2 compared exposure to DHA from DHA Algal Oil to exposure to EPA + DHA from menhaden oil for Xiamen Huison's intended use of DHA Algal Oil, which is a small subset of the intended use of menhaden oil as provided by 21 CFR §184.1472. For the comparison, Xiamen Huison's intended use was considered entirely substitutive to the use of menhaden oil in the selected categories as addition levels are equivalent to or lower than menhaden oil in terms of EPA + DHA content, and DHA Algal Oil is not intended for use in combination with, or augmented by, any other oil that is a significant source of DHA or EPA.

Estimates of total aggregate exposure to DHA + EPA for the total population by food consumers at the 90th percentile were 278.9 mg/day (5.55 mg/kg bw/day) and 326.9 mg/day (6.20 mg/kg bw/day) for DHA Algal Oil and menhaden oil, respectively. Thus, the substitutive use of DHA Algal Oil in the food categories of its intended use is not expected to result in a material increase in the combined exposure to DHA + EPA from those food categories, either individually or in aggregate. Furthermore, because the intended use food categories of DHA Algal Oil comprise only a small subset of the intended use food categories of menhaden oil, the intended substitutive use of DHA Algal Oil in these limited categories, at levels consistent with menhaden oil equivalents, is not expected to result in any increase in the total aggregate exposure to DHA + EPA combined, which is expected to remain within the limits of exposure (3 g/person/day) established by FDA in 21 CFR §184.1472. As such, the totality of evidence supporting the safety of the ingredient as described in this subpart supports a conclusion that the intended use of Xiamen Huison's DHA Algal Oil is reasonably certain to be safe.

6.10.2 Data and Information that is Corroborative of Safety

The safety of Xiamen Huison's DHA Algal Oil is corroborated by a battery of unpublished toxicological studies and a clinical trial on the article of commerce and the history of human consumption of approximately 154,500 kg of Xiamen Huison's DHA Algal Oil with no serious adverse events reported.

6.10.3 General Recognition

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 7 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 DHA 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.

6.11 Data and Information that are Inconsistent with the GRAS Conclusion

We have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

6.12 Information that is Exempt from Disclosure under FOIA

There are no data or information in this GRAS notice that are considered exempt from disclosure under FOIA as trade secret or commercial or financial information that is privileged or confidential.

Part 7: Supporting Data and Information

Initial literature searches for the safety assessment described in Part 6 of this GRAS notice were conducted during September 2017. Additional literature searches were conducted during the course of time spanning January 2018 through July 2018 and again during the course of time spanning September 2018 through December 6, 2018.

7.1 Data and Information that are *not* Generally Available

The following data and information, relevant to the safety of the intended uses of DHA Algal Oil and discussed in Part 6 of this report, are not generally available:

- The battery of unpublished toxicological studies conducted on DHA Algal Oil that are briefly summarized in Subpart 6.2.10 of this report. Nonetheless, the studies were completely negative with regard to genetic and general oral toxicological effects and, therefore, are corroborative to the safety conclusion.
- The unpublished clinical trial on DHA Algal Oil that is briefly summarized in Subpart 6.3.
- The data and information, in part or in whole, upon which the opinions of EFSA and regulatory actions by the EC summarized in Subpart 6.4.2 are based.
- The statement of Xiamen Huison regarding sales data and the absence of any serious adverse event reports received by the company. These do not contribute to forming part of the basis for the safety conclusion as they provide no information as to the specific population(s) that consumed the ingredients or the amounts or durations of consumption on a per capita basis.

The above-identified information that is not generally available is corroborative information that is not absolutely necessary to establish the safety of DHA Algal Oil for its intended use. We believe that qualified experts throughout the scientific community would be able to conclude that DHA Algal Oil is not harmful under the conditions of its intended use without access to this corroborative information.

7.2 References that *are* Generally Available

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