DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF DOCOSAHEXAENOIC ACID-RICH OIL AS A FOOD INGREDIENT FOR INFANT FORMULA APPLICATIONS

Prepared for Linyi Youkang Biology Co., Ltd

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
Tel: 410-531-3336
Susanscho1@yahoo.com

GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF DHA-RICH OIL AS A FOOD INGREDIENT FOR INFANT FORMULA APPLICATIONS

Table of contents

Tables

Figures

PART 1. SIGNED STATEMENTS AND A CERTIFICATION

Pursuant to 21 CFR Part 170, subpart E, Linyi Youkang Biology Co., Ltd (hereinafter referred to as "Linyi Youkang Biology") submits a Generally Recognized as Safe (GRAS) notice and claims that the use of docosahexaenoic acid (DHA)-rich oil ingredients in foods, as described in Parts 2 through 7 of this GRAS notice, is not subject to premarket approval requirements of the FD&C Act based on its conclusion that the substance is GRAS under the conditions of its intended use.

1.A. Name and Address of the Notifier

Contact: Guobin Li
Company: Linyi Youkang Biology Co., Ltd
Address: Lianbang Road, Economical and Technical Development Area, Linyi City, ShangDong Province
Tel: +86-539-2650092
E-mail: liguobin.aaa@163.com

1.B. Common or Trade Name

Docosahexaenoic acid-rich oil, DHA-rich oil, docosahexaenoic acid-rich algal oil, DHA-rich algal oil, DHA algal oil, DHA-oil

1.C. Applicable Conditions of Use of the Notified Substance

1.C.1. Foods in Which the Substance is to be Used

DHA-rich oil (available in both oil and powder forms) is intended for use as a direct ingredient in exempt (pre-term) and nonexempt (term) infant formula (ages from birth to 12 months), in accordance with current good manufacturing practices (cGMP), and in combination with a source of arachidonic acid (ARA).

1.C.2. Levels of Use in Such Foods

The intended use level of DHA-rich oil is similar to all other approved uses for incorporation of DHA in infant formula (GRNs 553, and 667). Linyi Youkang Biology intends to market DHA-rich oil produced from *Schizochytrium* sp. in use as a direct ingredient in preterm and term infant formulas (ages from birth to 12 months) in combination with a safe and suitable source of ARA. DHA-rich oil may be used at a maximum use level of 1.11% of dietary fat since it has approximately 45% DHA. This level corresponds to a maximum of 0.5% of total fat as DHA. DHA-rich oil powder may be used at a maximum use level of 6.24% to provide DHA at 0.5% of total fat since powder contains 8% DHA. The ratio of DHA to ARA would range from 1:1 to 1:2. The intended use level is similar to all other approved uses for incorporation of DHA or DHA-rich oil in infant formula (GRNs 553 - stamped page 12; and 677 - page 6).

1.C.3. Purpose for Which the Substance is Used

The substance will be used as a food ingredient for infant formulas.

1.C.4. Description of the Population Expected to Consume the Substance

The population expected to consume the substance consists of pre-term and full-term infants.

1.D. Basis for the GRAS Determination:

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.E. Availability of Information

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting Susan Cho at NutraSource, Inc. at the address above. The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

1.F. Availability of FOIA Exemption

Privileged or confidential information such as trade secrets and/or commercial or financial information has been redacted from this document and the information contained in this dossier can be made publicly available if warranted. A separate dossier containing the redacted information can be made available to FDA on request if warranted. None of the data and information in Parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552.

1.G. Certification

Linyi Youkang Biology certifies that, to the best of their knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information available and obtainable by Linyi Youkang Biology, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of DHA-rich oil. Linyi Youkang Biology accepts responsibility for the GRAS determination that has been made for DHA-rich oil as described in this dossier.

1.H Name, Position/Title of Responsible Person Who Signs Dossier and Signature

(b) (6) _____ Date: September 14, 2017

Name: Guobin Li
Title: Chairman

Address correspondence to
Susan S. Cho, Ph.D., NutraSource, Inc.
Agent for Linyi Youkang Biology

1.I. FSIS/USDA Statement

Linyi Youkang Biology does not intend to add DHA-rich oil to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

PART 2. IDENTITY, MANUFACTURING, SPECIFICATIONS, AND TECHNICAL EFFECTS OF DHA

2.A.1. Identity of the Notified Substance

2.A.1.1. Common Name
Docosahexaenoic acid-rich oil, DHA-rich oil, docosahexaenoic acid-rich algal oil, DHA-rich algal oil, DHA algal oil, DHA-oil

2.A.1.2. Chemical Names
Its systematic name is *all-cis*-docosa-4,7,10,13,16,19-hexa-enoic acid, and its shorthand name is 22:6(n-3)

2.A.1.3. Chemical Abstract Service (CAS) Registry Number
6217-54-5

2.A. 1.4. Empirical Formula
Molecular formula, $C_{22}H_{32}O_2$

2.A.1.5. Molecular Weight
328.488

2.A.1.6. Structural Formula
Figure 1 shows the structure of DHA.

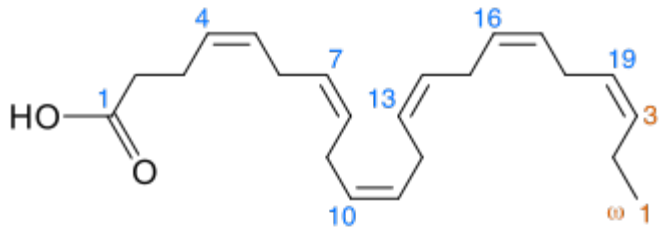

Figure 1. Structure of DHA.

2.A.1.7. Physical Properties
Density, 0.943 g/cm^3

2.A.1.8. Background
Docosahexaenoic acid is an omega-3 fatty acid (FAs) that is a primary structural component of the human brain, retina and other tissues. It can be synthesized from alpha linolenic acid or obtained directly from maternal milk, algal oil, or fish oil. Fatty acids can be desaturated endogenously up to the Δ9 position due to lack of certain enzymes in humans (Kremmyda et al., 2011). For this reason linoleic (18:2n-6) and α-linolenic (18:3n-3) acids must

be obtained from the diet and are termed essential FAs. Further elongation and desaturation of these FA to produce long-chain polyunsaturated fatty acids (PUFA) is possible, but not very efficient in humans. Examples of PUFA include ARA (20:4n-6), eicosapentaenoic (EPA; 20:5n-3), and (DHA; 22:6n-3). Thus, these FAs may be conditionally essential depending on essential FA availability.

Linyi Youkang Biology's DHA-rich oil is derived from the heterotrophic fermentation of the marine alga, *Schizochytrium* sp. DHA's structure is a carboxylic acid with a 22-carbon chain (docosa is Greek for 22 and hexa is Greek for six) *cis*-double bonds; the first double bond is located at the third carbon from the omega end (methyl terminus). Thus, it is classified as an omega-3 fatty acid. Figure 1 presents molecular structure of DHA.

2.A.2. Potential Toxicants in the Source of the Notified Substance

Potential toxicants have not been identified in DHA. High-performance liquid chromatography (HPLC) reveals that Linyi Youkang Biology's DHA-rich oil is > 45.0% pure. No pesticide residues (organochlorine and organophosphorus) and shellfish poisons have been detected in Linyi Youkang Biology's DHA-rich oil ingredients (Tables 1 to 3 and Appendix A). In addition, no significant amounts of dioxins and furans, polychlorinated biphenyls **(**PCBs), polycyclic aromatic hydrocarbons (PAHs), or solvent residues have been detected from Linyi Youkang Biology's DHA-rich oil ingredients (oil or powder form; Tables 4 to 8 and Appendix). Certificates of analysis (COA) for DHA-rich oil ingredients are presented in Appendix.

Pesticides and shellfish toxins

Three non-consecutive lots of DHA-rich oil ingredients (oil or powder forms) were screened for organochlorine pesticides, organophosphate pesticides, and shellfish toxins, domoic acid (Tables 2 to 4 and Appendix). None of these pesticides and toxins were found at levels above the detection limit in the sample analyzed (both oil and powder).

Dioxins, furans, PCBs, and PAHs

The analysis of 3 non-consecutive lots of DHA-rich oil ingredients showed that levels of dioxins, furans, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) were at levels below or close to the detection limits (Tables 3-6 for DHA-rich oil; Appendix).

Table 1. A List of Organochlorine Pesticides Screened for DHA-Rich Oil Ingredients

Pesticide (detection limit, ppm)	Pesticide (detection limit, ppm)	Pesticide (detection limit, ppm)
Aclonifen (0.01)	Acrinathrin (0.02)	Aldrin (0.005)
Benfluralin (0.005)	Bifenox (0.02)	Binapacryl (0.02)
Bifenthrin (0.02)	Bromocyclen (0.02)	Bromoxynil-octanoate (0.01)
Butralin (0.02)	Chlordane, cis- (0.005)	Chlordane, oxy- (0.005)
Chlordane, trans- (0.005)	Chlorfenapyr (0.005)	Chlorfenprop-methyl (0.01)
Chlorfenson (0.01)	Chloroneb (0.05)	Chlorothalonil (0.01)
Chlorthal-dimethyl (0.005)	Cyfluthrin (0.02)	Cyhalothrin, lamda- (0.02)
Cypermethrin (0.02)	Cyphenothrin (0.02)	DDD, o,p- (0.005)
DDD, p,p'- (0.005)	DDE, o,p- (0.005)	DDE, p,p'- (0.005)
DDT, o,p'- (0.005)	DDT, p,p'- (0.005)	Deltamethrin (0.02)
Diallate (0.05)	Dichlobenil (0.01)	Dichlone (0.02)
Dicloran (0.005)	Dichlorobenzophenone, o,p- (0.4)	Dichlorobenzophenone, p,p- (0.04)
Dicofol, o,p- (0.04)	Dicofol, p,p- (0.04)	Dieldrin (0.005)
Dienochlor (0.02)	Dinitramine (0.01)	Dinobuton (0.02)
Endosulfan, alpha- (0.005)	Endosulfan sulphate (0.01)	Endosulfan, beta- (0.005)
Endrin (0.01)	Endrin ketone (0.01)	Esfenvalerate (0.02)
Ethalfluralin (0.01)	Etridiazole (0.01)	Fenfluthrin (0.02)
Fenpropathrin (0.02)	Fenson (0.01)	Fenvalerate (RR-/SS-Isomers)
Fenvalerate (RS-/SR-Isomers) (0.01)	Flubenzimine (0.01)	Fluchloralin (0.01)
Flucythrinate (0.02)	Flumetralin (0.01)	Fluorodifen (0.02)
Fluoroimide (0.02)	Genite (0.01)	Halfenprox (0.02)
HCH, alpha- (0.005)	HCH, beta- (0.01)	HCH, delta- (0.005)
HCH, epsilon- (0.005)	Lindane (gamma-HCH) (0.005)	Heptachlor (0.005)
Heptachlor epoxide, cis- (0.005)	Heptachlor epoxide, trans- (0.005)	Hexachlorobenzene (HCB) (0.005)
Ioxynil-octanoate (0.005)	Isobenzan (0.005)	Isodrin (0.005)
Isopropalin (0.01)	Methoxychlor (0.01)	Mirex (0.005)
Nitrapyrin (0.01)	Nitrofen (0.01)	Octachlorstyrene (0.01)
Oxyfluorfen (0.01)	Pendimethalin (0.01)	Pentachloranisole (0.01)
Pentachloroaniline (0.005)	Pentachlorothioanisolte (0.005)	Permethrin (0.02)
Plifenate (0.005)	Polychloroterpene (Camphechlor) (0.2)	Profluralin (0.005)
Propanil (0.02)	Quintozene (0.005)	S 421 (0.005)
Tau-Fluvalinate (0.02)	Tecnazene (0.005)	Tefluthrin (0.02)
Tetradifon (0.01)	Tetrasul (0.01)	Tralomethrin (0.02)
Triallate (0.02)	Trichloronat (0.01)	Trifluralin (0.005)

Table 2. A List of Organophosphorus Pesticides Screened for DHA-Rich Oil Ingredients

Pesticide (detection limit, ppm)	Pesticide (detection limit, ppm)	Pesticide (detection limit, ppm)
Acephate (0.02)	Amidithion (0.02)	Azamethiophos (0.04)
Azinphos-ethyl (0.05)	Azinphos-methyl (0.05)	Carbophenothion (0.02)
Bromfenvinphos (0.02)	Bromophos-methyl (0.02)	Bromophos-ethyl (0.02)
Butamifos (0.02)	Cadusaphos (0.02)	Carbophenothion (0.02)
Carbophenothion-methyl (0.02)	Chlorfenvinphos (0.02)	Chlormephos (0.02)
Chlorpyrifos (-ethyl) (0.02)	Chlorpyrifos-methyl (0.02)	Chlorthion (0.02)
Chlorthiophos (0.02)	Coumaphos (0.05)	Crotoxyphos (0.02)
Crufomate (0.02)	Cyanofenphos (0.05)	Cyanophos (0.02)
Demeton-S-methyl (0.05)	Demeton-S-methyl-sulfone (0.05)	Dialifos (0.05)
Diazinon (0.02)	Dicapthon (0.01)	Dichlofenthion (0.02)
Dichlorvos (0.01)	Dicrotophos (0.02)	Dimefox (0.02)
Dimethoate (0.02)	Dimethoate/Omethoate (sum) ()	Dimethylvinphos (002)
Dioxabenzofos (0.02)	Dioxathion (0.02)	Disulfoton (0.02)
Disulfoton-sulfon (0.02)	Disulfoton-sulfoxide (0.04)	Ditalimfos (0.02)
Edifenphos (0.05)	EPN (0.05)	Ethion (0.01)
Ethoprophos (0.02)	Etrimfos (0.02)	Famophos (0.05)
Fenamiphos (0.02)	Fenamiphos (sum) ()	Fenamiphos-sulfone (0.02)
Fenamiphos-sulfoxide (0.02)	Fenchlorphos (0.02)	Fenchlorphos-oxon-sulfone (0.1)
Fenitrothion (0.01)	Fensulfothion (0.02)	Fensulfothion-oxon-sulfone (0.05)
Fensulfonthion-oxon-sulfoxide (0.02)	Fensulfothion-sulfone (0.02)	Fenthion (0.01)
Fenthion-oxon (0.02)	Fenthion-oxon-sulfone (0.05)	Fenthion-oxon-sulfoxide (0.02)
Fention-sulfone (0.05)	Fenthion-sulfoxide (0.02)	Fonofos (0.02)
Formothion (0.02)	Fosthiazate (0.02)	Fosthietan (0.02)
Heptenophos (0.02)	Iodofenphos (0.02)	Iprobenfos (0.02)
Isazophos (0.02)	Isocarbofos (0.02)	Isofenphos (0.02)
Isofenphos-methyl (0.02)	Isoxathion (0.05)	Leptophos (0.05)
Malaoxon (0.02)	Malathion (0.02)	Mecarbam (0.02)
Mephosfolan (0.02)	Merphos (0.02)	Methacriphos (0.02)
Methamidophos (0.02)	Methidathion (0.02)	Mevinphos (0.02)
Monocrotophos (0.01)	Morphothion (0.05)	Naled (0.02)
N-Desethyl-pirimiphos-methyl (0.02)	Omethoate (0.02)	Oxydemeton-methyl (0.05)
Paraoxon-ethyl (0.02)	Paraoxon-methyl (0.02)	Parathion (0.02)
Parathion-methyl (0.02)	Parathion-methyl/Paraoxon-methyl (sum) ()	Phenkapton (0.02)

Phenthoate (0.02)	Phorate (0.02)	Phorate (sum) ()
Phorate-sulfone (0.02)	Phorate-sulfoxide (0.02)	Phosalone (0.04)
Phosfolan (0.02)	Phosmet (0.05)	Phosphamidon (0.02)
Piperophos (0.02)	Pirimiphos-ethyl (0.02)	Pirimiphos-methyl (0.02)
Profenofos (0.02)	Propaphos (0.02)	Propetamphos (0.02)
Prothiofos (0.02)	Prothoate (0.02)	Pyraclofos (0.05)
Pyrazophos (0.05)	Pyridaphenthion (0.02)	Pyrimitate (0.02)
Quinalphos (0.02)	Quintiofos (0.02)	Sulfotep (0.02)
Sulprofos (0.05)	Tebupirimfos (0.02)	TEPP (0.02)
Terbufos (0.02)	Terbufos (sum) ()	Terbufos-sulfone (0.01)
Tetrachlorvinphos (0.02)	Thiometon (0.02)	Thionazin (0.02)
Tolclofos-methyl (0.02)	Triamiphos (0.05)	Triazophos (0.01)
Tribufos (0.04)	Trichlorfon (0.05)	Vamidothion (0.04)

Table 3. Analytical Results for Amnesic Shellfish Poison

Amnesic Shellfish Poison, Domoic Acid, ug/g	Lot: DHY2017040401	Lot: DHY2017041201	Lot: DHY2017042001
Detection limit	< 3.0	< 3.0	< 3.0
Results	Not Detected	Not Detected	Not Detected

Table 4. List of Dioxins and Furans Tested for DHA-Rich Oil

Dioxins and Furans, pg/g	Lot: DHY2017040401	Lot: DHY2017041201	Lot: DHY2017042001
1,2,3,4,6,7,8-HeptaCDD	< 0.132	< 0.260	< 0.132
1,2,3,4,6,7,8-HeptaCDF	< 0.0927	< 0.182	< 0.0921
1,2,3,4,7,8,9-HeptaCDF	< 0.0646	< 0.127	< 0.0641
1,2,3,4,7,8-HexaCDD	< 0.0629	< 0.123	< 0.0625
1,2,3,4,7,8-HexaCDF	< 0.0977	< 0.192	< 0.0970
1,2,3,6,7,8-HexaCDD	< 0.0861	< 0.169	< 0.0855
1,2,3,6,7,8-HexaCDF	< 0.0894	< 0.175	< 0.0888
1,2,3,7,8,9-HexaCDD	< 0.0811	< 0.159	< 0.0806
1,2,3,7,8,9-HexaCDF	< 0.0662	< 0.130	< 0.0658
1,2,3,7,8-PentaCDD	< 0.0414	< 0.0812	< 0.0411
1,2,3,7,8-PentaCDF	< 0.0596	< 0.117	< 0.0592
2,3,4,6,7,8-HexaCDF	< 0.0811	< 0.159	< 0.0806
2,3,4,7,8-PentaCDF	< 0.0927	< 0.182	< 0.0921
2,3,7,8-TetraCDD	< 0.0315	< 0.0617	< 0.0313
2,3,7,8-TetraCDF	< 0.0861	< 0.169	< 0.0855
OctaCDD	< 0.960	< 1.88	< 0.954
OCtaCDF	< 0.199	< 0.390	< 0.197
WHO (2005)-PCDD/F TEQ (lower-bound)	Not Detected	Not Detected	Not Detected
WHO (2005)-PCDD/F TEQ	0.171	0.335	0.170

(upper-bound)			

Table 5. A list of PCBs Tested for DHA-Rich Oil

Polychlorinated Biphenyls	Lot: DHY2017040401	Lot: DHY2017041201	Lot: DHY2017042001
PCB 101, ng/g	< 0.166	< 0.325	< 0.164
PCB 105, pg/g	< 6.46	< 12.7	< 6.41
PCB 114, pg/g	< 0.877	< 1.72	< 0.872
PCB 118, pg/g	< 23.2	< 45.5	< 23.0
PCB 123, pg/g	< 0.662	< 1.30	< 0.658
PCB 126, pg/g	< 0.414	< 0.812	< 0.411
PCB 138, ng/g	< 0.166	< 0.325	< 0.164
PCB 153, ng/g	< 0.166	< 0.325	< 0.164
PCB 156, pg/g	< 3.64	< 7.14	< 3.62
PCB 157, pg/g	< 0.679	< 1.33	< 0.674
PCB 167, pg/g	< 1.82	< 3.57	< 1.81
PCB 169, pg/g	< 1.99	< 3.90	< 1.97
PCB 180, ng/g	< 0.166	< 0.325	< 0.164
PCB 189, pg/g	< 0.662	< 1.30	< 0.658
PCB 28, ng/g	< 0.166	< 0.325	< 0.164
PCB 52, ng/g	< 0.166	< 0.325	< 0.164
PCB 77, pg/g	< 16.6	< 32.5	< 16.4
PCB 81, pg/g	< 0.447	< 0.877	< 0.444
Total 6 ndl- PCB (lower-bound), ng/g	Not Detected	Not Detected	Not Detected
Total 6 ndl- PCB (upper-bound), ng/g	0.993	1.95	0.987
WHO (2005)-PCB TEQ (lower-bound), pg/g	Not Detected	Not Detected	Not Detected
WHO (2005)-PCB TEQ (upper-bound), pg/g	0.104	0.204	0.103

Table 6. Summary of TEQ-Totals WHO-PCDD/F and PCB

TEQ-Totals WHO-PCDD/F and PCB	Lot: DHY2017040401	Lot: DHY2017041201	Lot: DHY2017042001
WHO (2005)-PCDD/F+PCB TEQ (lower-bound), pg/g	Not Detected	Not Detected	Not Detected
WHO (2005)-PCDD/F+PCB TEQ (upper-bound), pg/g	0.275	0.539	0.273

Table 7. A list of PAHs Tested for DHA-Rich Oil

PAH, ug/kg	Lot: DHY2017 040401	Lot: DHY2017 041201	Lot: DHY2017 042001	Detection Limit
Benzo(a)anthracene	0.7	0.6	0.7	0.5
Benzo(a)pyrene	0.6	0.6	0.6	0.5
Benzo(b)fluoranthene	1.4	1.3	1.4	0.5
Chrysene	0.8	0.8	0.8	0.5
Sum PAH 4	3.5	3.3	3.5	2.0

Table 8. A List of Solvent Residues Tested for DHA-Rich Oil

Solvent Residues, mg/kg	Lot: DHY 2017040401	Lot: DHY 2017041201	Lot: DHY 2017042001	Detection Limit
1,1,1,2-Tetrachloroethane	< 0.01	< 0.01	< 0.01	0.01
1,1,1-Trichloroethane	< 0.01	< 0.01	< 0.01	0.01
1,1,2-Tricholorethane	< 0.01	< 0.01	< 0.01	0.01
1,1-Dichloroethane	< 0.05	< 0.05	< 0.05	0.05
1,2-Dichloroethane	< 0.05	< 0.05	< 0.05	0.05
2-Butanon (Methylethylketon)	< 1	< 1	< 1	1
2-Methylpentane	< 1	< 1	< 1	1
3-Methylpentane	< 1	< 1	< 1	1
Benzene	0.073	0.065	0.069	0.01
Bromodichloromethane	< 0.05	< 0.05	< 0.05	0.05
Chloroform (trichloromethane)	< 0.01	< 0.01	< 0.01	0.01
cis-Dichloroethene	< 0.05	< 0.05	< 0.05	0.05
Dibromochloromethane	< 0.05	< 0.05	< 0.05	0.05
Dichloromethane	< 0.05	< 0.05	< 0.05	0.05
Ethyl acetate	< 1	< 1	< 1	1
Ethylbenzene	< 0.01	< 0.01	< 0.01	0.01
m-/-p-Xylene	< 0.01	< 0.01	< 0.01	0.01
Methylcyclopentane	< 1	< 1	< 1	1
n-Heptane	< 1	< 1	< 1	1
n-Hexane	< 1	< 1	< 1	1
n-Pentane	< 1	< 1	< 1	1
Styrene	< 0.01	< 0.01	< 0.01	0.01
Sum 3 chlorinated solvents	Inapplicable	Inapplicable	Inapplicable	-
Technical Hexane (calculated)	Inapplicable	Inapplicable	Inapplicable	-
Tetrachloroethane	< 0.01	< 0.01	< 0.01	0.01
Tetrachloromethane	< 0.01	< 0.01	< 0.01	0.01
Toluene	< 0.01	< 0.01	< 0.01	0.01
trans-Dichloroethene	< 0.05	< 0.05	< 0.05	0.05
Tribromomethane	< 0.05	< 0.05	< 0.05	0.05
Trichloroethene	< 0.01	< 0.01	< 0.01	0.01
Xylene (ortho-)	< 0.01	< 0.01	< 0.01	0.01

2.A.3. Particle Size

DHA oil - Not Applicable

DHA-rich oil powder - NLT 90% passing a 60 mesh screen.

2.B. Method of Manufacturing

Culture

Pre-culture flasks are shaken and inoculated with one vial of *Schizochytrium* sp. Linyi Youkang Biology maintains the cultural purity, sterility, and integrity of the microalgae, *Schizochytrium* sp. Samples of the pure strain, and samples of shake flask culture, pre-culture, and main culture are checked for the detection of microbial contamination (Petri dishes with nutrient agar). The culture is transferred to the first seed fermentor (pre-cultures). This culture is subsequently transferred to additional seed fermenters until the volume is sufficient for inoculation of the main fermentor.

Fermentation

The production medium used in the manufacturing process for DHA oil is mostly based on glucose, corn steep liquor, yeast powder, potassium sulfate, and malic acid. In addition, sodium hydroxide (NaOH) and citric acid monohydrate are used as pH-adjusting agents prior to sterilization and during fermentation. All components of the culture medium meet food grade specifications or are of adequate purity for food fermentation processes.

Enzymolysis, Extraction, and Purification

After the fermentation process, sodium hydroxide is used to adjust pH to 9-10 and then alkaline protease (source: *Bacillus licheniformis*) is added for the hydrolysis of protein for 1-6 hours. Then, DHA-rich oil layer is separated from fermentation biomass by disc centrifuge, followed by a series of purification steps including water degumming, acid degumming (citric acid monohydrate), and alkali (sodium hydroxide) refining, water washing, low temperature separation for further purification of DHA-rich oil, decolorization (activated clay treatment), and deodorization (steam generator process at 0.2 MPa, 160-190 C). No organic solvents are used to extract DHA-rich oil from the fermentation biomass.

Stabilization and Packaging of DHA-Rich Oil

Natural vitamin E (0.1%) is added to purified DHA-rich oil before being packaged

Production of DHA-Rich Oil Powder

Sodium ascorbate, lecithin, ascorbyl palmitate, lactose, and modified starch (less than 4%; 21 CFR Sec. 172.892) are added to DHA-rich oil as excipients and mixed. The mixture is spray-dried to produce DHA-rich oil powder.

Linyi Youkang Biology uses a Hazard Analysis and Critical Control Points (HACCP)-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

Figure 2. Manufacturing Flow Diagram of DHA-rich Oil Ingredients

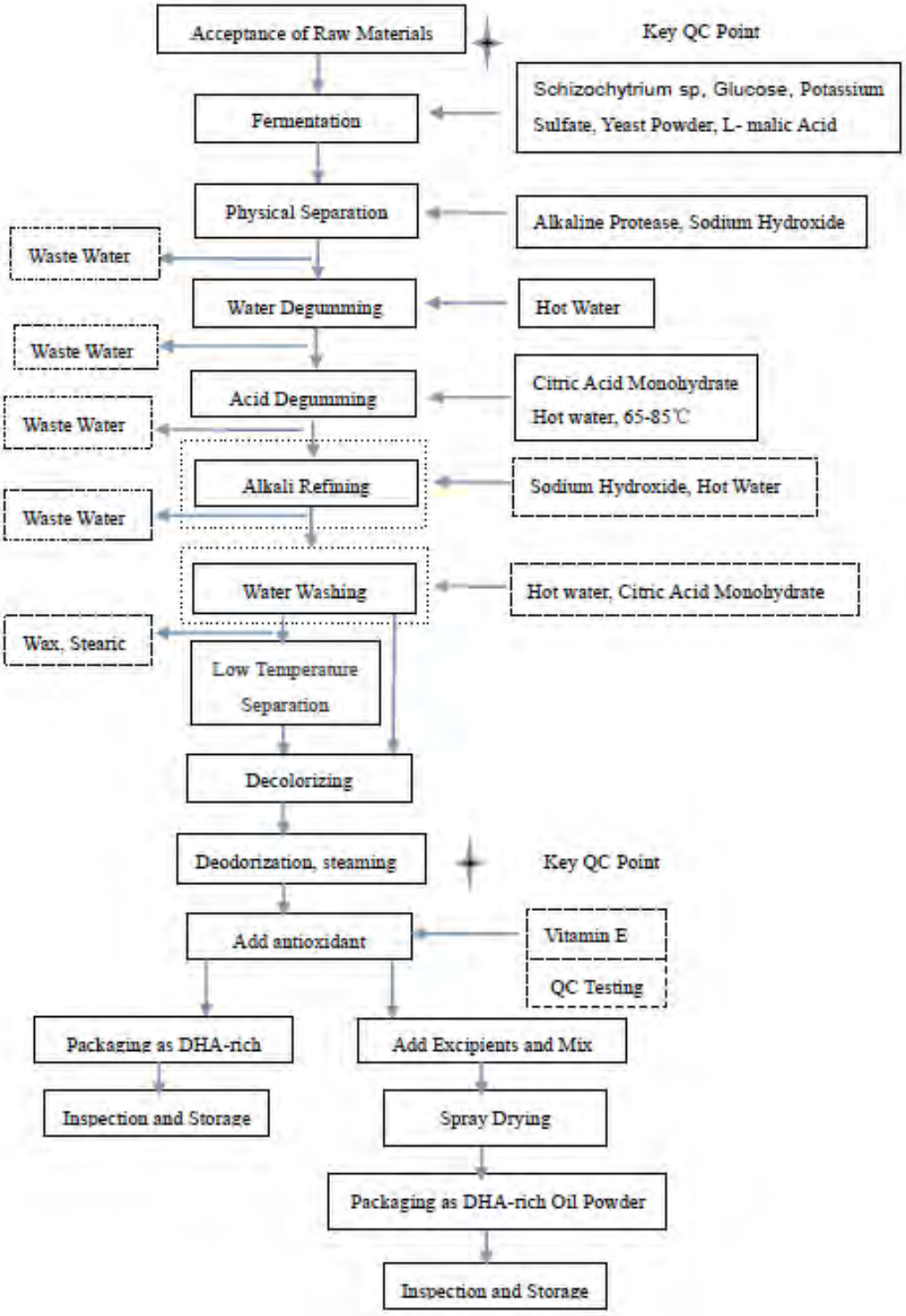

Table 9. Raw Materials Used in Fermentation

Ingredient	CAS number	Regulatory status
Yeast powder	8013-01-2	21CFR 172.896
Corn syrup powder (corn steep liquor)	66071-94-1	21CFR 184.1033
Glucose	50-99-7	21 CFR 168.121
Potassium sulfate	7778-80-5	21CFR 184.1643
Malic acid	97-67-6	21CFR 184.1069
Citric acid monohydrate	5959-29-1	21CFR 184.1033
Magnesium sulfate heptahydrate	10034-99-8	21CFR 184.1443
Ammonium sulfate	7783-20-2	21CFR 194.1143
Dipotassium hydrogen phosphate	7758-11-4	21CFR 182.6245
Calcium chloride	10043-52-4	21CFR 184.1193
Copper sulfate	7758-98-7	21CFR 184.1261
Zinc sulfate	7733-02-0	21CFR 182.8997
Cobalt chloride	7646-79-9	21CFR582.80
Manganese chloride	7773-01-5	21CFR 184.1446
Nickel sulfate	10101-97-0	Nickel-184.1537
Vitamin B12	68-19-9	21CFR 184.1945
Biotin	58-85-5	21CFR 182.8159
Thiamine hydrochloride	67-03-8	21CFR 184.1875
Monosodium glutamate	32221-81-1	21CFR 182.1500

Table 10. Processing Aids

Processing aids	CAS number	Regulatory status
Tocopherols	1406-66-2	21CFR 184.1890
Activated clay (Bentonite)	1302-78-9	21CFR 184.1155
Silicon dioxide	14808-60-7	21CFR 172.480
Excipients for Powder Form		
Sodium ascorbate	134-03-2	21CFR 182.3731
Lecithin	8002-43-5	21CFR 184.1400
Ascorbyl palmitate	137-66-6	21CFR 182.3149
Lactose	9004-34-6	21CFR 168.122
Starch sodium octenyl succinate	66829-29-6	21 CFR 172.892

Characterization of the Source Organism

The principle of production method (via algal production) is similar to that described by other companies, whose production method for DHA-rich oil via *Schizochytrium* sp. received a no objections letter from the FDA (GRN 553 and 677).

DHA-rich oil is derived from the heterotrophic fermentation of the marine alga, *Schizochytrium* sp. *Schizochytrium* sp. is a thraustochytrid and a member of the Chromista kingdom (Stramenopilia). There are no reports of this organism producing toxic chemicals or being pathogenic. 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. *Schizochytrium* sp. microorganisms are widespread and are commonly found in marine environments throughout the world. There have never been any reports of toxic compounds produced by these microorganisms.

Table 11. Taxonomic Classification of *Schizochytrium* sp.

Class	Scientific Classification
Kingdom	Stramenoplia
Division	Eucaryotes
Subdivision	Chromista
Class	Labyrinthista
Order	Labyrinthulea
Family	Thraustochytriaceae
Genus	*Schizochytrium*

Alkaline protease used in the processing of DHA-rich oil was isolated from a non-pathogenic bacterium, *Bacillus licheniformis*. It has an activity unit of 200,000 IU/g and a lead content of less than 0.03 ppm.

2.C. Specifications and Composition

Tables 12-1 and 12-2 show specifications and COAs for DHA-rich oil. Tables 13-1 and 13-2 present specifications and COAs for DHA-rich oil powder. Three non-consecutive lots of DHA-rich oil and powder samples were analyzed for DHA, acid value, peroxide value, free fatty acids, trans fatty acids, heavy metals, and microbiology to ensure that Linyi Youkang Biology's DHA-rich oil ingredients meet the specifications and are free from contaminations.

Tables 14 and 15 show fatty acid profiles of Linyi Youkang Biology's DHA-rich oil in comparison with those described in GRN 553 and 677. All three DHA-rich oils are derived from *Schizochytrium* sp. The mean DHA content was higher in the current notice than GRN 677 or GRN 553 (current notice vs. GRN 553 vs. GRN 667=50.7 vs. 43.3 vs. 40%). Other fatty acid profiles are similar to each other. DHA-rich oil is a free flowing, yellow oil, predominantly triglycerides (>95.4%; data not shown) with some diglycerides (3.9%), monoglycerides (1.1%) and glycerol (<1.0%) as is typical for food-grade vegetable oils. Table 16 shows fatty acid profiles of Linyi Youkang Biology's DHA-rich oil powder. Powder form is less concentrated in DHA content since it is diluted with excipients.

Table 17 presents other nutrients present in DHA-rich oil. There are negligible amounts of other nutrients such as carbohydrates and protein.

Table 12-1. Specifications of DHA-Rich Oils*

Parameter	Specifications			Method Analysis for the current notice
	Current notice	GRN 667	GRN 553	
DHA, %	>45	>35	>35	AOAC 996.06
Acid value, mg KOH/g	< 0.5	<0.5	<0.5	DCF C-V 2
Free fatty acid, calc as % oleic acid	< 0.1	NA	<0.4 (total FFA)	
Trans fatty acids, %	<1.0	NA	<3.5	AOCS2a-94
Unsaponifiable matter, %	<1.0	<3.5	<3.5	ISO 18609
Peroxide value, meq/kg	<5.0	<5.0	<5.0	AOCS Cd 8b-90:2003
EPA, %	NA	NA	<10.0	AOAC 996.06
Copper (Cu), mg/kg	<0.5	<0.1	<0.1	BS EN ISO 17294-2 2004 mod.
Iron (Fe), mg/kg	<0.2	<0.2	<0.2	
Lead (Pb), mg/kg	< 0.1	< 0.1	< 0.1	
Arsenic (As), mg/kg	< 0.1	< 0.1	< 0.1	
Cadmium (Cd), mg/kg	< 0.1	NA	< 0.1	
Mercury (Hg), mg/kg	< 0.01	< 0.1	< 0.04	BS EN 13806:2002
Moisture (direct drying method), g/100 g	<0.1	< 0.05	< 0.02	ISO 662:1998
Ash, g/100 g	< 0.1	NA	< 0.1	AOAC 941.12
Coliforms, cfu/ml	< 1	NA	< 1	ISO 4832:2006
Molds, cfu/ml	< 1	NA	< 1	ISO 21527:2008
Yeast, cfu/ml	< 1	NA	< 1	
Salmonella, /25 g	Not Detected	NA	Not Detected	ISO 6579:2002

*DHA-Rich Oils listed in this table are derived from *Schizochytrium* sp. AOAC = Association of Official Analytical Chemists; AOCS = American Oil Chemist's Society; cfu = Colony Forming Units

Table 12-2. Summary of Analytical Values for DHA-Rich Oil

Parameter	Analytical values supporting specifications		
	DHY2017 040401	DHY2017 041201	DHY2017 042001
DHA, %	50.664	50.427	50.433
Acid value, mg KOH/g	<0.2	< 0.2	< 0.2
Free fatty acid, calc as % oleic acid	< 0.1	< 0.1	< 0.1
Trans fatty acids, %	0.25	0.08	0.09
Unsaponifiable matter, %	1.0	1.0	1.0
Peroxide value, meq/kg	0.85 (retest value)	0.57 (retest value)	0.51 (retest value)
Moisture, g/100 g	<0.1	<0.1	<0.1
Copper (Cu), mg/kg	0.19	<0.1	<0.1

Iron (Fe), mg/kg	<0.1	<0.1	<0.1
Lead (Pd), mg/kg	< 0.05	< 0.05	< 0.05
Arsenic (As), mg/kg	< 0.1	< 0.1	< 0.1
Cadmium (Cd), mg/kg	< 0.01	< 0.01	< 0.01
Mercury (Hg), mg/kg	< 0.005	< 0.005	< 0.005
Ash, g/100 g	< 0.1	< 0.1	< 0.1
Coliforms, cfu/ml	< 1	< 1	< 1
Moulds, cfu/ml	< 1	< 1	< 1
Yeast, cfu/ml	< 1	< 1	< 1
Salmonella, /25 g	Not Detected	Not Detected	Not Detected

Table 13-1. Specifications of DHA-Rich Oil Powder

Parameter	Specifications	Method Analysis
DHA, %	>8.0	AOAC 996.06
Acid value, mg KOH/g	< 0.5	DCF C-V 2
Free fatty acids, as % oleic acid	<0.1	DCF C-V 2
Peroxide value, meq/kg	<5.0	AOCS Cd 8b-90:2003
Mercury (Hg), mg/kg	< 0.01	BS EN 13806:2002
Lead (Pd), mg/kg	< 0.05	BS EN ISO 17294-2 2004 mod.
Arsenic (As), mg/kg	< 0.1	BS EN ISO 17294-2 2004 mod.
Cadmium (Cd), mg/kg	< 0.01	BS EN ISO 17294-2 2004 mod.
Moisture and volatile matter content, g/100 g	<5.0	ISO 662:1998
Ash, g/100 g	<2.0	AOAC 941.12
Coliforms, cfu/g	< 10	ISO 4832:2006
Molds, cfu/g	< 10	ISO 21527:2008
Yeast, cfu/g	< 10	ISO 21527:2008
Salmonella, /25 g	Not Detected	ISO 6579:2002
Aerobic plate count, cfu/g	< 10	ISO 4833-1:2013

Table 13-2. Analytical Values of DHA-Rich Oil Powder*

	Analytical values		
Parameter	2017020901	2017021501	2017022401
DHA, %	8.98	8.91	8.80
Acid value, mg KOH/g	< 0.2	<0.2	<0.2
Free fatty acids, as % oleic acid	<0.1	<0.1	<0.1
Peroxide Value, meq/kg	1.78	< 0.05	0.66
Mercury (Hg), mg/kg	< 0.005	< 0.005	< 0.005
Lead (Pd), mg/kg	< 0.05	< 0.05	< 0.05
Arsenic (As), mg/kg	< 0.1	< 0.1	< 0.1
Cadmium (Cd), mg/kg	< 0.01	< 0.01	< 0.01
Mercury (Hg), mg/kg	< 0.005	< 0.005	< 0.005
Moisture and volatile matter	2.38	2.44	2.42

content, g/100 g			
Ash, g/100 g	1.58	1.60	1.55
Coliforms, cfu/g	< 10	< 10	< 10
Molds, cfu/g	< 10	< 10	< 10
Yeast, cfu/g	< 10	< 10	< 10
Salmonella, /25 g	Not Detected	Not Detected	Not Detected
Aerobic plate count, cfu/g	< 10	< 10	< 10

*Samples were taken from 3 non-consecutive batches.

Table 14. Fatty Acid Profile of DHA-Rich Oil

Fatty Acid Profile, g/100 g	Lot: DHY 2017040401	Lot: DHY 2017041201	Lot: DHY 2017042001	Mean
C 6:0 (Caproic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 8:0 (Caprylic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 10:0 (Capric acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 12:0 (Lauric acid)	0.10	0.10	0.10	0.10
C 14:0 (Myristic acid)	0.82	0.82	0.82	0.82
C 14:1 (Myristoleic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 15:0 (Pentadecanoic acid)	0.06	0.06	0.06	0.06
C 15:1 (Pentadecenoic acid)	0.07	0.07	0.07	0.07
C 16:0 (Palmitic acid)	20.86	21.05	20.97	20.96
C 16:1 (Palmitoleic acid)	0.51	0.50	0.50	0.51
C 17:0 (Margaric acid)	0.08	0.08	0.08	0.08
C 17:1 (Heptadecenoic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 18:0 (Stearic acid)	1.29	1.31	1.30	1.30
C 18:1 (Oleic acid)	0.28	0.27	0.27	0.27
C 18:1n7 (Vaccenic acid)	0.51	0.51	0.51	0.51
C 18:2n6 (Linoleic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 18:3n3 (alpha-Linolenic acid)	0.14	0.14	0.14	0.14
C 18:3n6 (gamma-Linolenic acid)	0.09	0.09	0.09	0.09
C 20:0 (Arachidic acid)	0.29	0.29	0.29	0.29
C 20:1 (Eicosenoic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 20:2n6 (Eicosodienoic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 20:3n3 (Eicosatrienoic acid)	1.35	1.34	1.34	1.34
C 20:3n6 (homo-gamma-Linolenic acid)	0.21	0.21	0.21	0.21
C 20:4n6 (Arachidonic acid)	0.15	0.13	0.14	0.15
C 20:5n3 (Eicosapentaenoic acid)	0.70	0.70	0.69	0.70
C 21:0 (Heneicosanoic acid)	0.031	0.029	0.059	0.04
C 22:0 (Behenic acid)	0.15	0.15	0.15	0.15
C 22:1n9 (Erucic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 22:2n6 (Docosadienoic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 22:6n3 (Docosahexaenoic acid)	50.66	50.43	50.43	50.66
C 22-5n3 (Docosapentaenoic acid)	0.10	0.11	0.11	0.11

C 22-5n6 (Docosapentaenoic acid)	10.37	10.30	10.33	10.33
C 23:0 (Tricosanoic acid)	< 0.02	< 0.02	< 0.02	< 0.02
C 24:0 (Lignoceric acid)	0.14	0.16	0.15	0.15
C 24:1 (Nervonic acid)	0.40	0.42	0.40	0.41
Monounsaturated fat	1.76	1.78	1.76	1.76
Omega-3 fatty acids	54.02	53.79	53.80	53.87
Omega-6 fatty acids	10.88	10.80	10.84	10.84
Polyunsaturated fat	64.90	64.60	64.65	64.73
Saturated fat	23.84	24.06	23.99	23.96
Total fat	90.52	90.43	90.40	90.45

Based on AOAQC 996.06; Detection limit=0.02% for all FAs.

Table 15. Comparison of Fatty Acid Profiles of DHA-rich Oils

	Current notice	GRN 553	GRN 677
DHA specifications, %	>45	>35	>35
Actual content, %	50.66	43.34	
Fatty Acid Profile, g/100 g			
C 6:0 (Caproic acid)	< 0.02	NA	NA
C 8:0 (Caprylic acid)	< 0.02	NA	NA
C 10:0 (Capric acid)	< 0.02	NA	NA
C 12:0 (Lauric acid)	0.10	<0.10	0.91
C 14:0 (Myristic acid)	0.82	1.18	11.87
C 14:1 (Myristoleic acid)	< 0.02	<0.10	<0.10
C 15:0 (Pentadecanoic acid)	0.06	0.24	0.52
C 15:1 (Pentadecenoic acid)	0.07	NA	NA
C 16:0 (Palmitic acid)	20.96	13.78	25.43
C 16:1 (Palmitoleic acid)	0.51	<0.10	3.42
C 17:0 (Margaric acid or Heptadecanoic acid)	0.08	<0.10	<0.10-0.15
C 18:0 (Stearic acid)	1.30	1.65	0.82
C 18:1 (Oleic acid)	0.27	25.00	4.77
C 18:1n7 (Vaccenic acid)	0.51	0.26	NA
C 18:2n6 (Linoleic acid)	< 0.02	2.01	0.33
C 18:3n3 (alpha-Linolenic acid)	0.14	<0.10	NA
C 18:3n6 (gamma-Linolenic acid)	0.09	NA	0.23
C 20:0 (Arachidic acid)	0.29	0.32	<0.10
C 20:1 (Eicosenoic acid)	< 0.02	<0.1	<0.01-<0.10
C 20:2n6 (Eicosodienoic acid)	< 0.02	0.13	NA
C 20:3n3 (Eicosatrienoic acid)	1.34	NA	NA
C 20:3n6 (homo-gamma-Linolenic acid)	0.21	<0.1	1.18
C 20:4n6 (Arachidonic acid)	0.15	0.69	NA
C 20:5n3 (Eicosapentaenoic acid; EPA)	0.70	6.22	NA
C 21:0 (Heneicosanoic acid)	0.04	NA	NA
C 22:0 (Behenic acid)	0.15	0.35	<0.10

C 22:1n9 (Erucic acid)	< 0.02	NA	NA
C 22:2n6 (Docosadienoic acid)	< 0.02	0.53	NA
C 22:6n3 (Docosahexaenoic acid)	50.66	43.34	NA
C 22-5n3 (Docosapentaenoic acid)	0.11	0.76	NA
C 22-5n6 (Docosapentaenoic acid)	10.33	2.53	7.81
C 23:0 (Tricosanoic acid)	< 0.02	NA	NA
C 24:0 (Lignoceric acid)	0.15	0.14	<0.10
C 24:1 (Nervonic acid)	0.41	<0.10	NA
Monounsaturated fat	1.76		
Omega-3 fatty acids	53.87		
Omega-6 fatty acids	10.84		
Polyunsaturated fat	64.73		
Saturated fat	23.96		
Total fat	90.45		

Table 16. Fatty Acid Profile of DHA-Rich Oil Powder

Fatty Acid Profile, g/100g	Lot: 2017020901	Lot: 2017021501	Lot: 2017022401	Mean
C 6:0 (Caproic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 8:0 (Caprylic acid)	0.047	0.044	0.040	0.02
C 10:0 (Capric acid)	0.039	0.034	0.038	0.02
C 12:0 (Lauric acid)	< 0.020	< 0.020	< 0.020	0.02
C 14:0 (Myristic acid)	0.134	0.133	0.131	0.133
C 14:1 (Myristoleic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 15:0 (Pentadecanoic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 15:1 (Pentadecenoic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 16:0 (Palmitic acid)	8.407	8.407	8.243	8.352
C 16:1 (Palmitoleic acid)	0.077	0.075	0.074	0.075
C 17:0 (Margaric acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 17:1 (Heptadecenoic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 18:0 (Stearic acid)	0.273	0.272	0.266	0.270
C 18:1 (Oleic acid)	0.041	0.040	0.040	0.040
C 18:1n7 (Vaccenic acid)	0.067	0.066	0.065	0.066
C 18:2n6 (Linoleic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 18:3n3 (alpha-Linolenic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 18:3n6 (gamma-Linolenic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 20:0 (Arachidic acid)	0.053	0.054	0.052	0.053
C 20:1 (Eicosenoic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 20:2n6 (Eicosodienoic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 20:3n3 (Eicosatrienoic acid)	0.204	0.200	0.198	0.200
C 20:3n6 (homo-gamma-Linolenic acid)	0.037	0.036	0.036	0.030
C 20:4n6 (Arachidonic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 20:5n3 (Eicosapentaenoic acid)	0.096	0.095	0.096	0.096

C 21:0 (Heneicosanoic acid)	0.027	< 0.020	< 0.020	< 0.020
C 22:0 (Behenic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 22:1n9 (Erucic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 22:2n6 (Docosadienoic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 22:6n3 (Docosahexaenoic acid)	8.983	8.912	8.805	8.900
C 22-5n3 (Docosapentaenoic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 22-5n6 (Docosapentaenoic acid)	1.972	1.959	1.936	1.956
C 23:0 (Tricosanoic acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 24:0 (Lignoceric acid)	< 0.020	< 0.020	< 0.020	< 0.020
C 24:1 (Nervonic acid)	0.055	0.062	0.059	0.058
Total fat	20.73	20.61	20.28	20.54

Based on AOAC 996.06.

Table 17. Other Nutrients in DHA-Rich Oil

	Lot: DHY2017 040401	Lot: DHY2017 041201	Lot: DHY2017 042001	Mean
Protein, g/100 g	< 0.1 (k=6.25)	< 0.1 (k=6.25)	< 0.1 (k=6.25)	<0.1
Dietary fiber, g/100 g	< 0.5	< 0.5	< 0.5	<0.5
Total fat, g/100 g	99.1	99.6	99.9	99.5
Energy				
Energy kcal (calculated), kcal/100g	896	898	900	898
Energy kJ (calculated), kJ/100g	3682	3692	3698	3691
Carbohydrates, g/100 g				
Carbohydrates (available)	0.90	0.40	0.10	0.46
Total carbohydrates	0.90	0.40	0.10	0.46
Sugar Profile, g/100 g				
Fructose	< 0.1	< 0.1	< 0.1	< 0.1
Galactose	< 0.1	< 0.1	< 0.1	< 0.1
Glucose	< 0.1	< 0.1	< 0.1	< 0.1
Lactose	< 0.1	< 0.1	< 0.1	< 0.1
Maltose	< 0.1	< 0.1	< 0.1	< 0.1
Monosaccharides and disaccharides	< 0.1	< 0.1	< 0.1	< 0.1
Sucrose	< 0.1	< 0.1	< 0.1	< 0.1
Sterols, mg/100 g				596

2.D. Intended technical effects

DHA-rich oil ingredients will be used as nutritional ingredients in infant formulas.

PART 3. EXPOSURE ESTIMATES

3.A. Exposure Estimates

Linyi Youkang Biology's DHA-rich oil may be used at a maximum use level of 1.11% of dietary fat since it has at least 45% DHA. This level corresponds to a maximum of 0.5% of total fat as DHA. DHA-rich oil powder may be used at a maximum use level of 6.24% to provide DHA at 0.5% of total fat since powder contains 8% DHA. The intended use level is similar to all other approved uses for incorporation of DHA in infant formula (GRNs 553, and 667). Consistent to other GRAS notices, the ratios of ARA:DHA is expected to be in the range of 2:1 to 1:1 (GRN 41 – FDA, 2001a; GRN 94 – FDA, 2001b; GRN 379, FDA, 2011; GRN 553 – FDA, 2015; and GRN 677 – FDA, 2017).

Assuming human infants consume about 100 to 120 kcal/kg bw/day, of which fat comprises about 50%, an infant will consume about 50-60 kcal/kg bw/day of fat, or about 5.5-6.7 g of fat/kg bw/day (1 g fat=9 kcal). The DHA-rich oil maximum intake of 1.11% of daily fat (or oil powder maximum intake of 6.24% of daily fat) for an infant would correspond to 69-83 mg DHA-rich oil/kg bw/day providing 27-33 mg/kg bw/day of DHA which is consistent with current DHA recommendations for term and preterm infants of 10-60 mg DHA/kg bw depending on gestational age (Koletzko et al., 2014).

3.B. Food Sources of DHA

Human milk provides small quantities of DHA and ARA, usually less than 1% of total fatty acids (Brenna et al., 2007). Fish oil and egg yolks also are known to be excellent sources of naturally occurring DHA.

Summary of consumption data

The intended use will result in 69-83 mg DHA-rich oil/kg bw/day providing 27-33 mg/kg bw/day of DHA which is consistent with current DHA recommendations for term and preterm infants of 10-60 mg DHA/kg bw depending on gestational age. These levels far below the safe intake levels determined from animal toxicity studies (details are found in Section 6.B.3). The No-Observed Adverse Effect-Level (NOAEL) was determined to be 5,000 mg/kg bw/day in a subchronic toxicity study in rats. After applying a safety margin of 100, it can be concluded that doses of up to 50 mg/kg bw/day are safe. Overall, intended use will result in EDIs at levels below those associated with any potential side effects.

PART 4. SELF LIMITING USE LEVELS

No known self-limiting levels of use are associated with the DHA-rich oil ingredients. However, the ratio of ARA:DHA is expected to be in the range of 2:1 to 1:1.

PART 5. HISTORY OF CONSUMPTION

EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

The statutory basis for the conclusion of GRAS status of DHA-rich oil derived from *Schizochytrium* sp. in this document is not based on common use in food before 1958. The GRAS determination is based on scientific procedures. As described in Part 3 of this document, DHA is a naturally occurring food component. It is reasonable to conclude that it was present in food prior to 1958.

PART 6. BASIS FOR GRAS DETERMINATION

6.A. Current Regulatory Status

As shown in Table 18, algal DHA-rich oil derived from *Schizochytrium* sp. (GRNs 553 and 677) received a GRAS notice status with U.S. FDA for infant formula applications. Other sources of the DHA-rich oils include *Crypthecodinium cohnii* (GRN 41), and tuna oils (GRN 94 and 379).

Table 18. Regulatory Approvals for Use of DHA in Infant Formula

Year Approved	Country	Submission
2001	USA	GRN 41; DHASCO (DHA-rich single-cell oil from *Crypthecodinium cohnii* for use in infant formula)
2006	USA	GRN 94; Docosahexaenoic acid-rich oil from tuna (DHA-rich tuna oil)
2011	USA	GRN 379; DHA from tuna oil
2015	USA	GRN 553; Algal oil (40% DHA) derived from *Schizochytrium* sp.
2017	USA	GRN 677; Algal oil (>35% DHA) derived from *Schizochytrium* sp.

6.B. Review of Safety Data

As the DHA-rich oil in this GRAS notice has similar specifications and composition compared to the DHA-rich oils derived from *Schizochytrium* sp. in the previous FDA GRAS notices (GRN 553 -stamped pages 40-58; GRN 677 - pages 28-41; Table 12-1 of the current notice), it is recognized that the information and data in GRN notices are pertinent to the safety of the DHA-rich oil in this GRAS notice. Therefore, this notice incorporates, by reference, the safety and metabolism studies discussed in the previous GRAS notices, and will not discuss previously reviewed references in detail. Additionally, this notice discusses additional animal studies that have been published since the FDA's last review in 2017 (GRN 677). The subject of the present GRAS notice is DHA-rich oil derived from *Schizochytrium* sp. (both oil and powder forms).

Our literature search has focused on the papers published between January 2011 and July 2017.

6.B.1. Metabolic Fate of DHA (mostly adopted from Kremmyda et al., 2011; Kroes et al., 2003; Martin et al., 1993)

DHA is mainly found in the form of triglycerides (TG), although they also occur in phospholipids in breast milk (Martin et al., 1993). In general, dietary TG undergo enzymatic hydrolysis in the upper intestine to free FAs and 2-monoglycerides. These products are then integrated into bile acid micelles for diffusion into the interior of the intestinal epithelial cells for subsequent incorporation into new or reconstituted TGs (Kroes et al., 2003). These reconstructed TGs enter the lymph in the form of chylomicrons for transport to the blood, which allows distribution and incorporation into plasma lipids, erythrocyte membranes, platelets, and adipose

tissue. The chylomicron-contained TGs are hydrolyzed by lipoprotein lipase during passage through the capillaries of adipose tissue and the liver to release free FAs to the tissues for metabolism or for cellular uptake, with subsequent re-esterification into TGs and phospholipids for storage as energy or as structural components of cell membranes. The metabolism of FAs occurs in the mitochondria following their transport across the mitochondrial membrane in the form of acylcarnitine. Fatty acids are metabolized predominantly via beta-oxidation, a process that involves a shortening of the FA carbon chain and the production of acetic acid and acetyl CoA, which combines with oxaloacetic acid and enters the citric acid cycle for energy production. The degree of transport of FAs across the mitochondrial membrane is contingent upon the length of the carbon chain; FAs of 20 carbons or more are transported into the mitochondria to a lesser degree than shorter chain FAs. Therefore, long chain FAs, such as DHA, may not undergo mitochondrial beta-oxidation to the same extent (Kroes et al., 2003). Instead they are preferentially channeled into the phospholipid pool where they are rapidly incorporated into the cell membranes of the developing brain and retina.

Fatty acids can be desaturated endogenously up to the Δ9 position due to lack of certain enzymes in humans (Kremmyda et al., 2011). For this reason, linoleic acid (18:2n-6) and linolenic acid (18:3n-3) acids must be obtained from the diet and are termed essential FAs. Further elongation and desaturation of these FA to produce long chain PUFA is possible, but not very efficient in humans. Examples of PUFA include ARA (20:4n-6), eicosapentaenoic (EPA; 20:5n-3), and DHA (22:6n-3). Thus, these FAs may be conditionally essential depending on essential FA availability. Genetic variation in human desaturase genes affects FA metabolism, plasma lipid profiles, and risk of disease development.

6.B.2. Studies on Mutagenicity and Genotoxicity of DHA Derived from *Schizochytrium sp*.

Table 19 summarizes the results of mutagenicity and genotoxicity studies of DHA derived from *Schizochytrium* sp. Due to the abundance of papers, this mutagenicity and genotoxicity review limits the studies on the DHA-rich oils derived from *Schizochytrium* sp. only, instead of covering DHA from various sources. In a recent study by Lewis et al. (2016), the safety of DHA-rich oil from *Schizochytrium* sp. was confirmed by testing for gene mutations, clastogenicity, and aneugenicity. The results of all mutagenicity and genotoxicity tests were negative.

Recent Studies

Bacterial reverse mutation assays for DHA-rich oil (Lewis et al., 2016)

None of the revertant colonies exceeded three times the mean of the solvent control in the presence or absence of metabolic activation when treated with DHA-rich oil. There was no dose-related increase over the range tested for any of the five tester strains used. Based on these results, DHA-rich oil is not mutagenic.

In vitro chromosomal aberration tests using human blood peripheral lymphocyte with DHA-rich oil (Lewis et al., 2016)

In Phase I, the cultures were treated for 4 h with DHA- rich oil and the mean number of percent aberrant cells was determined in the presence of metabolic activation and in the absence of metabolic activation for concentrations of 0.00 (water control), 0.00 (vehicle control), 1.25,

2.5, and 5.0 mg DHA-rich oil/mL and positive controls, respectively. For Phase II, test item treatment concentrations were 1.25, 2.5, and 5.0 mg DHA-rich oil/mL culture in the presence and in the absence of metabolic activation (2%). The duration of exposure was 24 h. The mean percentage of aberrant cells was determined in the absence and presence of metabolic activation. Treatment with 600 mg/mL ethyl methane sulfonate in the absence of metabolic activation, and 30 mg/mL cyclophosphamide in the presence of metabolic activation (2%) resulted in a significant increase in percent aberrant cells. The analysis did not reveal any statistically significant results for DHA-rich oil. Under these experimental conditions, DHA-rich oil did not induce chromosomal aberration and was not genotoxic in the presence (1 and 2%) and in the absence of metabolic activation.

Mammalian erythrocyte micronucleus tests for DHA-rich oil (Lewis et al., 2016)

Wistar rats treated with DHA-rich oil at all doses exhibited mean % polychromatic erythrocytes (PCE) to normochromatic erythrocytes and individual frequencies of micronucleated PCE that were similar to the values for the vehicle control group. The data indicated no evidence of test article related to bone marrow toxicity.

Studies Reviewed in Previous GRAS Notices

GRN 553 and 677 reported that bacterial reverse mutation assays (Hammond et al., 2002; Fedorova et al., 2011a; 2011b; Schmitt et al., 2012a), chromosome aberration assays (Hammond et al., 2002; Fedorova et al., 2011a; 2011b; Schmitt et al, 2012a), *in vivo* micronucleus tests in mice and rats (Hammond et al., 2002; Fedorova et al., 2011a; 2011b; Schmitt et al, 2012a), and *in vitro* CHO AS52/XPRT gene mutation assay (Hammond et al., 2002) did not show any mutagenicity or genotoxicity of DHA-rich algal oil and DHA-rich microalgae (DRM). Individual studies are summarized in Table 18.

Table 19. Genotoxicity Studies Showing No Mutagenicity or Genotoxicity of DHA-Rich Oils

Test concentrations	Test system	Substance	Reference
Recently published studies			
62, 185, 556, 1,667, 2,500, 3,750, and 5,000 ug/plate, plate incorporation and preincubation ± S9	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. coli* WP2 uvrA	DHA-rich oil	Lewis et al., 2016
Phase I: Concentration of 0.0, 0.0625, 0.125, and 0.25 mg/mL; Phase II: 0, 1.25, 2.5, and 5,0 mg/mL culture in presence and absence of metabolic activation (2%)	Human blood peripheral lymphocytes	DHA-rich oil	Lewis et al., 2016
1000, 2500, and 5000 mg/kg bw/day	Polychromatic erythrocytes in bone marrow of treated rats	DHA-rich oil	Lewis et al., 2016
Studies reviewed in GRN 677 and 553			
2000-5000 ug/plate	*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537, and *E. coli* WP2 *uvrA;* w/ and w/o S9 activation	DHA-rich oil	Fedorova-Dahms et al., 2011a; 2011b

5-500 ug/plate	*S. typhimurium* TA 98, TA 100, TA 102, TA 1535, TA 1537 w/ and w/o S9 activation	DHA-rich microalgae	Hammond et al., 2002
500-5,000 ug/plate	*S. typhimurium* TA 100 and *E. coli* WP2 w/ and w/o S9 activation	DHA-rich oil	Schmitt et al., 2012a
NA	Human lymphocytes, 4 or 24 h, w/ and w/o metabolic activation	DHA-rich oil	Fedorova-Dahms et al., 2011a; 2011b
0.13 and 0.25 ug/ml	Human peripheral blood lymphocytes	DHA-rich microalgae	Hammond et al., 2002
13.6, 19.4, 27.7. 39.5, 56.5, 80.7, 115, 165, 235, 336, 480, 686, 980, 1,400, and 2,000 ug/ml	Human peripheral blood lymphocytes	DHA-rich oil	Schmitt et al., 2012a
2,000 mg/kg	Murine PCE in peripheral blood	DHA-rich oil	Fedorova-Dahms et al., 2011a; 2011b
500, 1,000, and 2,000 mg/kg	Murine PCE in bone marrow	DHA-rich microalgae	Hammond et al., 2002
500, 1,000, 1,500, and 2,000 mg/kg	Rat PCE in bone marrow	DHA-rich oil	Schmitt et al., 2012a
10-5000 ug/ml, and different S9 concentrations (0, 1, 5, and 10%)	CHO AS52/XPRT gene mutation assay	DHA-rich microalgae	Hammond et al., 2002

PCE=polychromatic erythrocytes

Overall, studies consistently showed that all preparations of DHA-rich oils were not mutagenic or genotoxic.

6.B.3. Animal Toxicity Studies of DHA-Rich Oils derived from *Schizochytrium* sp.

The results of various animal toxicity studies are summarized in Table 20. Due to the abundance of papers reporting no adverse effects of DHA in animals, this animal toxicity review includes the studies on DHA-rich oils derived from *Schizochytrium* sp. only, instead of DHA from various sources.

Acute Toxicity Study on Linyi Youkang Biology's ARA-Rich Oil

Gao (2017) evaluated acute toxicity of DHA-rich oil in rats. DHA-rich oil was administered to 10 young rats (5 males and 5 females) by oral gavage at the dosage of 15.2 g/kg body weight (bw). Animals were observed for 14 days to monitor changes in body weight, clinical signs, as well as food consumption. At the end of the study, all surviving animals were sacrificed and major organs were examined. No animal died during the 14-day observation period and no clinical signs of abnormality were observed at the maximum dose of 15.2 g/kg bw. Furthermore, no significant differences in mean body weight, food consumption, or organ

weights were found among the test group and control groups (water control and sunflower oil vehicle control). No treatment-related abnormalities were observed in macroscopic examinations of organs. In summary, this study found that the mean lethal dose (LD_{50}) of DHA-rich oil was far above 15.2 g/kg bw.

Other DHA-Rich Oil Derived from *Schizochytrium sp.*

In a study by Lewis et al. (2016), the safety of DHA-rich oil from *Schizochytrium sp.* was evaluated by conducting 28-day and 90-day dietary studies in Wistar rats. The 28-day and 90-day studies involved dietary exposure to 1,000, 2,500, and 5,000 mg/kg bw/day of the DHA-rich oil and two control diets: water and corn oil (vehicle control). There were no treatment related effects of DHA-rich oils on clinical observations, body weight, food consumption, behavior, hematology, clinical chemistry, coagulation, urinalysis parameters, or necropsy findings. Increases in cholesterol and TG levels were considered related to a high oil diet and non-adverse. In a series of toxicity studies (28-day subacute toxicity and 90-day subchronic toxicity), the NOAEL for the DHA-rich oil from *Schizochytrium* sp. was determined to be 5,000 mg/kg bw/day, the highest dose tested. The DHA oil contained 41.4% DHA.

A recent study by Falk et al. (2017) investigated the reproductive and developmental toxicity of dietary exposure to DHA-rich oil (41.4% DHA) from *Schizochytrium* sp. In a developmental toxicity study, pregnant Wistar rats were untreated (control) or administered corn oil (vehicle control), 1,000, 2,500, or 5,000 mg/kg bw/day of DHA-rich oil via gavage from gestation days 6 through 20. In the reproductive toxicity study, male and female Wistar rats were administered vehicle control (corn oil), or 1,000, 2,500, or 5,000 mg/kg bw/day of DHA-rich oil via gavage throughout the mating period, pregnancy, and the nursing and lactation period. Differences in the number of fetuses, fetal skeletal malformations, and external and visceral anomalies in the developmental study and mortality, clinical signs, fertility indices, physical observations, gross necropsy findings, and gestation period length in the reproductive toxicity study were not dose-related or significantly different from control groups, and were not considered to be treatment related. The NOAEL for maternal toxicity and embryo/fetal development and for paternal or maternal treatment-related reproductive toxicity for the DHA-rich oil administered by gavage was 5,000 mg/kg bw/day.

In addition, GRN 553 and 677 reported that the NOAELs for DHA-rich algal oil were found to be 3,250 -7,464 mg/kg bw/day (or 1,300- 2,985 mg DHA/kg bw/day) in rats from subchronic toxicity or developmental toxicity studies (Fedorova-Dahms et al., 2011a; 2011b; Hammond et al., 2001a; Schmitt et al., 2012a; 2012b). For DHA-rich microalgae (DRM), the NOAELs were estimated to be 3,162-4,000 mg/kg bw/day from asubchronic toxicity study in pigs (Abril et al., 2003; Hammond et al., 2001a), 20,000 -22,000 mg/kg bw/day (or 1,800 mg DHA/kg bw/day) from a single generation reproduction or developmental toxicity studies in rats (Hammond et al., 2001b, 2001c), and 1,800 mg/kg bw/day from a developmental toxicity in rabbits (Hammond et al., 2001b). Individual studies are summarized in Table 21.

Conclusion:

Based on the studies summarized above, for purposes of safety evaluation, a NOAEL of 5,000 mg/kg bw/day was chosen for DHA-rich oils (or 2,000 mg/kg bw/day for DHA) in rats.

Table 19. Animal Toxicity Studies of DHA-rich Algal Oil or DHA-rich Microalgae from *Schizochytrium* sp.

Dose	Study Design (Route of administration)	Duration	Species	Material Studied	Primary Observations	NOAEL (mg/kg bw/d)	Reference
Linyi Youkang Biology's DHA-rich oil							
15.2 g/kg bw/d	Oral gavage	Single dose	Rat	DHA-rich oil	Clinical signs of abnormality	LD50>>>15,200	Gao et al., 2017
Studied published since FDA's review in 2017							
1,000, 2,500, or 5,000 mg/kg bw/d	Subchronic toxicity (oral gavage)	90 d	Rat	DHA-rich oil	No treatment-related adverse effects	5,000	Lewis et al., 2016
	Developmental toxicity (oral gavage)	Gestat-ion days 6 to 20	Rat	DHA-rich oil	No treatment-related adverse effects	5,000	Falk et al., 2017
Studies reviewed in previous GRAS notices							
5000 mg/kg/d	Acute oral toxicity (gavage)	14 d	Rat	DHA-rich oil	No treatment-related adverse effects	LD_{50}>5,000	Schmitt et al., 2012a
1, 2.5, or 5% in diet	Acute oral toxicity (diet)	14 d	Rat	DHA-rich oil	No treatment-related adverse effects	M, 3,258; F, 3,542	Schmitt et al., 2012b
0.5, 1.5, or 5% in diet	Subchronic toxicity (diet)	90 d	Rat	DHA-rich oil[1]	Reduced food consumption in all treatment and fish oil control groups; attributed to high fat content rather than treatment.	3,246	Fedorova-Dahms et al., 2011a
1, 2.5, or 5% in diet	Subchronic toxicity (diet)	90 d	Rat	DHA-rich oil[1]	No treatment-related adverse effects	M, 3,305; F, 3,679	Schmitt et al., 2012a
400, 1,500, or 4,000	Subchronic toxicity (diet)	13 wk	Rat	DHA-rich microalgae	No treatment-related adverse effects	4,000	Hammond et al., 2001a
1.17, 3.39, or 5.75 kg DRM per pig over 42	Subchronic toxicity (diet)	42-120 d	Pig	DHA-rich microalgae	No treatment-related adverse effects (598, 261, 756, and 1,281 g DHA per pig during expt. period)	DHA-rich microalgae, 1,368; DHA, ~305	Abril et al., 2003

d; 2.68 kg DRM over 120 d							
0.6, 6.0, or 30% DRM in diet	Developmental safety (diet)	15 d	Rat	DHA-rich microalgae	No treatment-related adverse effects	22,000	Hammond et al., 2001b
180, 600, or 1,800 mg/kg/d	Developmental toxicity (gavage)	30 d	Rabbit	DHA-rich microalgae	High-dose (1,800) DHA oil and fish oil groups: F0-reduced food consumption and body weight	Maternal, 600; Develop: 1800	Hammond et al., 2001b
0.5, 1.5, or 5% in diet	Developmental toxicity of mothers (diet)	15 d	Rat	DHA-rich oil	No treatment-related adverse effects	4260	Fedorova-Dahms et al., 2011b
0.5, 1.5, or 5% in diet	Subchronic toxicity of F1 (diet)	90 d	Rat	DHA-rich oil[1]	No treatment-related adverse effects	4260	Fedorova-Dahms et al., 2011b
400-2,000	Developmental toxicity (gavage)	20 d	Rat	DHA-rich oil[3]	No treatment-related adverse effects	2000	Schmitt et al., 2012b
1, 2.5, or 5% in diet	Subchronic and reproductive toxicity of first generation (diet)	75-90 d	Rat	DHA-rich oil[3]	No treatment-related adverse effects	M during premating, 3,421; M after mating, 2,339; F during mating, 3,558; F during gestation, 3,117; F during lactation, 7,464	Schmitt et al., 2012b
1, 2.5, or 5% in diet	Developmental and subchronic toxicity of second generation (diet)	106-111 d	Rat	DHA-rich oil[3]	No treatment-related adverse effects in the 5% group males; Higher food consumption and BW in the 5% group females	M, 3,526; F, 2,069	Schmitt et al., 2012b
0.6, 6.0, or	Single-generation	13 wk	Rat	DHA-rich	No treatment-related	DHA-rich	Hammond et

30% DRM in diet	reproduction toxicity (diet)			microalgae	adverse effects	microalgae M, 17,847; F, 21,000; DHA: M, 1500; F, 1800	al., 2001c

M=males; F=females

6.B.4. Human Clinical Studies of DHA

Studies of infant formula supplemented with DHA

It has been reported that the source of dietary long chain PUFAs do not affect their bioavailability and absorption when full-term infants were fed from birth to 3 months breast-milk, formula containing DHA in the form of egg phospholipids, or a formula supplemented with algal DHA (Sala-vila et al., 2006). Instead, bioavailability depends mainly on the lipid composition of the diet fed.

As shown in Tables 21 to 22, DHA supplementation up to 0.96% of total fat did not cause adverse effects in either preterm or term infants, regardless of DHA sources. Only one infant formula study (Chase et al., 2015) with DHA-rich oil derived from *Schizochytrium* sp. was identified in the literature published between January 2011 and July 2017. It is believed that DHA-rich oil derived from *Schizochytrium* sp. is bioequivalent to DHA from another type of algal oil (such as *C. cohnii*) or fish oil. A few studies reported no adverse effects of algal DHA from *C. cohnii* (preterm and term infants - Columbo et al., 2011; Drover et al., 2011; 2012). In addition, DHA from other sources such as egg yolk or fish oil also did not show adverse effects in term infants (DeJong et al., 2011). The data indicate that the source of DHA does not impact the safety of DHA or DHA-oils. Thus, we have focused on the studies of infant formulas supplemented with DHA from algal sources (*Schizochytrium* sp., *C. cohnii,* and unspecified sources) to make general conclusions about the safety of algal DHA-rich oil derived from *Schizochytrium* sp. Due to an abundance of papers on the topic, we focused on papers published between January 2011 and July 2017.

Term Infants

Chase et al. (2015) investigated the effect of supplementation of algal DHA derived from *Schizochytrium* sp. on stimulated inflammatory cytokine production in white blood cells (WBC) from infants with a high genetic risk for type 1 diabetes. This was a multicenter, two-arm, randomized, double-blind pilot trial of DHA-rich oil supplementation, beginning either in the last trimester of pregnancy (41 infants) or in the first 5 months after birth (57 infants). Levels of DHA in infant and maternal red blood cell (RBC) membranes and in breast milk, and inflammatory cytokines were assayed. This pilot trial showed that supplementation of infant diets with DHA-rich oil was safe. No adverse effects on measured outcomes were noted.

In the DHA Intake and Measurement of Neural Development (DIAMOND) studies of Colombo et al. (2011, 2013), Currie et al. (2015), and Drover et al. (2011, 2012), healthy, term infants were enrolled at 1-9 day of age and were randomly assigned to be fed one of the following 4 infant formulas containing equivalent nutrient amounts for 12 months: control (0% DHA), 0.32, 0.64, or 0.96% algal DHA derived from *C. cohnii*. All 3 DHA-supplemented formulas also provided 0.64% ARA derived from *M. alpina*. Algal DHA, up to 0.96% of total FAs, was well tolerated and no adverse effects were noted on measured outcomes including tolerance, adverse events, growth, red blood cell (RBC) concentrations of fatty acids, visual acuity, cognitive function, and school readiness.

Pre-term Infants

Recent studies on pre-term infants employed DHA of non-specified sources (Almaas et al., 2015; 2016; Kitamura et al., 2016; Van de Lagemaat 2011; Westerberg et al., 2011).

Regardless of its source, DHA up to 0.86% of total fatty acids (or 32 mg) was well tolerated. No treatment-related adverse events such as milk allergy, allergy-associated diarrhea, bloody stools, and anaphylaxis were reported. No adverse effects were found on measured outcomes including tolerance, adverse events, growth, anthropometric index, fatty acid composition of the erythrocyte membrane, cognition and child development in pre-term infants.

Table 21. Term Infants Studies

Objective	Subject	Test materials	Duration	Results	Reference
To investigate the effect of DHA supplementation on stimulated inflammatory cytokine production in white blood cells (WBC) from infants with a high genetic risk for type 1 diabetes	A. 41 mother and 57 infant pairs with first-degree relative with type 1 diabetes and to have HLA DR3 and/or DR4 genes	Mothers- 800 mg/d DHA (DHASCO-5 oil from *Schizochytrium* sp.) or corn/soy oil until delivery, and continued on this same dose after delivery if breast-feeding. Formula-fed infants – 3.4 or 10.2 mg DHA/oz. At 12 mo, all infants received 400 mg/d DHA or corn/soy oil until 36 mo.	Test 1. Last trimester of pregnancy until birth; formula fed until 5 mo of age; follow up – up to 36 mo of age	Infant WBC stimulated inflammatory cytokine production (IL-1β, TNFα, or IL-12p40); the inflammatory marker, high-sensitivity C-reactive protein (hsCRP); biochemical islet autoantibodies; maternal and infant levels of RBC DHA and infant levels of RBC DPA	Chase et al., 2015
To evaluate the effects of feeding DHA-ARA supplemented formula throughout infancy on growth from birth to 6 years	159 term infants	3 concentrations of DHA (from *C. cohnii* oil), 0.32%, 0.64%, or 0.96% with fixed conc. of ARA, 0.64% (34 mg/100 kcal, from *M. alpina*); or control-unsupplemented From DIAMOND study	Formula fed for 12 mo; follow-up from birth to 6 yr	Growth	Currie et al., 2015
To evaluate cognition beyond 18 mo and longitudinal cognitive change from 18 mo to 6 y in children who were fed variable amounts of DHA and ARA;	81 full term infants		Formula fed for 12 mo; re-enrolled at 18 mo and tested every 6 mo until 6 y	Performance on standardized tests of language and performance (Bayley Scales of Infant Development, version 2 and MacArthur-Bates Communicative Development Inventory; Delayed Response task; Bear-Dragon Go/No-Go Task; Stroop tasks;	Colombo et al., 2013

(0.64%) compared with children who were not fed LCPUFA as infants.				Dimensional Change Card Sort; Tower of Hanoi task; Peabody Picture Vocabulary Test, 3rd edition; The Weschler Preschool Primary Intelligence Scale, 3rd edition)	
To determine the optimal DHA concentration (with fixed ARA conc.) in term formula to support cognitive maturation.	181 term infants		First 12 mo of life, sole source of nutrition until < 4 mo of age; Follow up at 18 mo	Cognitive development as measured by Bayley Scales of Infant Development II including the Psychomotor Development Index and the Behavior Rating Scale	Drover et al., 2011
To determine the effects of different dietary ARA/DHA provided during the first 12 months of life on language development and school readiness	182 term infants at 1-9 days of age		Prospective, RCT; 12 m intervention; follow up until age 2-3.5 y	School readiness at age 2.5 and 3.5 yr; receptive vocabulary at age 2 and 3.5 yr	Drover et al., 2012
To evaluate the effects of DHA on visual habituation protocol that yielded both behavioral and psycho-physiological indices of attention at 3, 6, and 9 mo	122 term infants		RCT; from birth to 12 mo	A cognitive index derived from the convergence of behavioral and cardiac responses; a visual habituation protocol that yielded both behavioral and psychophysiological indices of attention	Colombo et al., 2011

DPA= docosapentaenoic acid**;** IL=interleukin; mo=months; RBC=red blood cell; RCT=randomized controlled trial; TNFα =tumor necrosis factor alpha; WBC=white blood cell; yr=years.

Table 22. Pre-term Infants Studies

Objective	Subject	Test materials	Duration	Measurements	Reference
To test the hypothesis that DHA/ARA supplementation of very low birth wt. infants would influence cerebral white matter measured by DTI and improve behavioral outcome at 8 years of age	129 very low birth wt. infants with birth weights of <1500 g	Human milk supplemented with 32 mg DHA (0.86% of total FA) and 31 mg ARA (0.91% of total FA); Source-NA	9 wk after birth; 8 yr follow-up	White matter measured by diffusion tensor imaging of brain; and behavioral outcome	Almaas et al., 2016
To test the hypothesis that DHA/ARA supplementation of very low birth wt. infants fed human milk would show persistent positive effects on cognition	129 very low birth wt. infants with birth weights of <1500 g	Human milk supplemented with 32 mg DHA (0.86% of total FA) and 31 mg ARA (0.91% of total FA); Source-NA	9 wk after birth; 8 yr follow-up	Cognitive testing, general intellectual abilities, short-term and working memory, learning and memory, MRI analysis;	Almaas et al., 2015
To investigate the safety and efficacy of an infant formula (H2025A) fortified with DHA and ARA	35 low birth wt. infants	Formula with DHA/ARA ratio of 2:1 or formula with DHA only; Source-NA Morinaga Milk Industry	1 mo	Adverse event such as milk allergy, allergy-associated diarrhea, bloody stools, and anaphylaxis; growth; Anthropometric Index; fatty acid composition of the erythrocyte membrane	Kitamura et al., 2016
To study associations between growth and erythrocyte (RBC) DHA and ARA	139 preterms (gestational age 30.3±1.5 wk, birth weight 1341±288 g)	Human milk with breast milk fortifier or preterm formula until term, followed by post-discharge formula (0.4% DHA, 0.4% ARA), term formula (0.2% DHA, 0.2% ARA), or human milk; Source-NA (Friso	6 mo	RBC concentrations of ARA, DHA, and EPA; and growth (weight gain, length, and head circumference)	Van de Lagemaat et al., 2011

		Prematuur, FrieslandCampina)			
To investigate the effect of DHA and ARA in early neonatal life on cognitive functions among human milk fed very low birth weight infants (<1500 g) at 20 mo of chronological age	92 very low birth wt. infants	Human milk with 0.5 mL oil (containing 32 mg DHA and 31 mg ARA or placebo) per 100 mL milk. Source-NA	1 wk after birth until discharge from hospital; 9 wk on average; follow up at 20 mo	Cognitive function tests at 20 months (free-play sessions, Bayley Scales of Infant Development the Ages and Stages Questionnaire); and plasma DHA and ARA concentration	Westerberg et al., 2011

Footnotes: DHA and ARA= Percentages in diet given as % of total fatty acids unless noted otherwise.
EPA=eicosapentaenoic acid; MRI-magnetic resonance imaging; NA=not available; RBC=red blood cell;
RCT=randomized controlled trial; TNF=tumor necrosis factor; wk=weeks; yr=year.

6.C. Potential Adverse Effects

It is noteworthy that the Institute of Medicine (IOM, 2005) has not established any Tolerable Upper Intake Levels (UL) for DHA and EPA while establishing Dietary Reference Intakes for Americans. No adverse effects of DHA-rich oil supplementation to infant formulas were reported.

6.D. Safety determination

Numerous human and animal studies have reported health benefits of DHA-rich oils with no major adverse effects. There is broad-based and widely disseminated knowledge concerning the chemistry of DHA. This GRAS determination is based on the data and information generally available about the safety of DHA-rich oil, in particular, those derived from *Schizochytrium* sp. The literature indicates that DHA-rich oil supplementation offers infants health benefits without serious adverse effects.

The following safety evaluations fully consider the composition, intake, nutritional, microbiological, and toxicological properties of DHA-rich oils as well as appropriate corroborative data.

1. Linyi Youkang Biology's DHA-rich oil ingredients (both oil and powder forms) are manufactured under cGMP using common food industry materials and processes. Linyi Youkang Biology uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications.

2. Analytical data from multiple lots indicate that DHA-rich oil ingredients reliably comply with established specifications and meets all applicable purity standards.

3. As the DHA-rich oil in this GRAS notice has similar specifications and composition compared to the DHA-rich oils in the previous FDA GRAS notices (GRN 553 and 677), it is concluded that Linyi Youkang Biology's DHA-rich oil is substantially equivalent to those described in GRNs 677 and 553. Thus, it is recognized that the information and data in the previous GRAS notices are pertinent to the safety of the DHA-rich oil in this GRAS notice. In response to GRAS notifications on DHA-rich oils derived from *Schizochytrium* sp. (GRN 677 and 553), the FDA did not question the safety of DHA-rich oils for the specified food uses.

4. As noted in GRNs 677 and 553, Linyi Youkang Biology's DHA-rich oil may be used at a maximum use level of 0.5% of total fat as DHA (U.S. FDA, 2001a) in infant formulas for term and pre-term infants. This level corresponds to a maximum of 1.11% of dietary fat as DHA-rich oil (U.S. FDA, 2001a). DHA-rich oil powder may be used at a maximum use level of 6.24% to provide DHA at 0.5% of total fat. The intended use level is the same as another approved use for incorporation of DHA-rich oils in infant formula for term and pre-term infants (GRNs 553 and 677).

5. It is assumed that Linyi Youkang Biology's DHA-rich oil products will replace currently marketed DHA or other DHA sources. Thus, cumulative exposures are not expected to change.

6. In the previous GRAS notices to the FDA, the safety of DHA has been established in toxicological studies in animals, mutagenicity and genotoxicity studies, and is further supported by clinical studies in human. In a recent animal toxicity study (Lewis et al., 2016), the NOAEL was determined to be 5,000 mg/kg bw/day in a subchronic toxicity study in rats. After applying a safety margin of 100, it can be concluded that doses up to 50 mg/kg bw/day is safe for human use. The EDIs under the intended use are far less than the estimated safe intake levels in infants.

7. Furthermore, historical consumption of DHA-rich oils supports the safety of DHA.

6.E. Conclusions and General Recognition of the Safety of DHA-Rich Oil

Several sources of DHA and DHA-rich oils have been evaluated by the FDA and other global regulatory agencies over the past 15 years for proposed incorporation of algal DHA-rich oil products, in particular, derived from *Schizochytrium* sp., in infant formulas for consumption by term and pre-term infants. Relevant U.S. GRAS notifications include GRNs 677 and 553 (FDA, 2017; 2014). All the GRAS notices provided information/clinical study data that supported the safety of the proposed DHA ingredients for use in infant formulas. In all the studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues attributable to DHA or DHA-rich oils. Because this safety evaluation was based on generally available and widely accepted data and information, it satisfies the so-called "common knowledge" element of a GRAS determination.

In addition, the intended uses of DHA-rich oil have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. The specifications of the proposed GRAS substance, Linyi Youkang Biology's DHA-rich oil ingredients, are substantially equivalent to those that have received FDA "no question" letters.

The DHA-rich oil ingredients that are the subject of this GRAS determination are produced by non-toxigenic algae, *Schizochytrium* sp., and their purity is over 45% for DHA-rich oil and over 8% for DHA-rich oil powder. The DHA-rich oil ingredients are manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes. Linyi Youkang Biology's DHA-rich oil powder has similar specifications and composition although its DHA content is diluted. No toxicants have been detected from Linyi Youkang Biology's DHA-rich oil ingredients.

Literature searches did not identify safety/toxicity concerns related to DHA-rich oil ingredients. Toxicity studies of DHA-rich oils include acute, subacute, and subchronic toxicity, a battery of genotoxicity studies, and developmental and reproductive toxicity studies. In these reports, no evidence of toxicity was noted at up to 5,000 mg/kg bw/day, the highest dose levels

tested. The publicly available scientific literature on the consumption and the safety of DHA-rich oils in clinical studies with both term and pre-term infants is extensive and sufficient to support the safety and GRAS status of the proposed DHA-rich oil ingredients.

Linyi Youkang Biology also has concluded that its DHA-rich oil ingredients are GRAS under the intended conditions of use on the basis of scientific procedures. Therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

Linyi Youkang Biology is not aware of any information that would be inconsistent with a finding that the proposed use of DHA-rich oil products meets appropriate specifications, and its use according to cGMP, is GRAS.

6.F. Discussion of Information Inconsistent with GRAS Determination

We are not aware of information that would be inconsistent with a finding that the proposed use of DHA-rich oil ingredients in infant formula, meeting appropriate specifications and used according to cGMP, is GRAS.

PART 7. REFERENCES

7.A. A List of the Data and Information that are Generally Available References

Abril R, Garrett J, Zeller SG, Sander WJ, Mast RW. Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. Part V: target animal safety/toxicity study in growing swine. Regul Toxicol Pharmacol. 2003;37:73-82.

Almaas AN, Tamnes CK, Nakstad B, Henriksen C, Walhovd KB, Fjell AM, Due-Tønnessen P, Drevon CA, Iversen PO. Long-chain polyunsaturated fatty acids and cognition in VLBW infants at 8 years: an RCT. Pediatrics. 2015;135:972-80.

Almaas AN, Tamnes CK, Nakstad B, Henriksen C, Grydeland H, Walhovd KB, Fjell AM, Iversen PO, Drevon CA. Diffusion tensor imaging and behavior in premature infants at 8 years of age, a randomized controlled trial with long-chain polyunsaturated fatty acids. Early Hum Dev. 2016;95:41-6.

Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr. 2007;85:1457-64.

Chase HP, Boulware D, Rodriguez H, Donaldson D, Chritton S, Rafkin-Mervis L, Krischer J, Skyler JS, Clare-Salzler M; Type 1 Diabetes TrialNet Nutritional Intervention to Prevent (NIP) Type 1 Diabetes Study Group. Effect of docosahexaenoic acid supplementation on inflammatory cytokine levels in infants at high genetic risk for type 1 diabetes. Pediatr Diabetes. 2015;16:271-9.

Colombo J, Carlson SE, Cheatham CL, Fitzgerald-Gustafson KM, Kepler A, Doty T. Long-chain polyunsaturated fatty acid supplementation in infancy reduces heart rate and positively affects distribution of attention. Pediatr Res. 2011;70:406-10.

Colombo J, Carlson SE, Cheatham CL, Shaddy DJ, Kerling EH, Thodosoff JM, Gustafson KM, Brez C. Long-term effects of LCPUFA supplementation on childhood cognitive outcomes. Am J Clin Nutr. 2013;98:403-12.

Currie LM, Tolley EA, Thodosoff JM, Kerling EH, Sullivan DK, Colombo J, Carlson SE. Long chain polyunsaturated fatty acid supplementation in infancy increases length- and weight-for-age but not BMI to 6 years when controlling for effects of maternal smoking. Prostaglandins Leukot Essent Fatty Acids. 2015;98:1-6.

de Jong C, Boehm G, Kikkert HK, Hadders-Algra M. The Groningen LCPUFA study: No effect of short-term postnatal long-chain polyunsaturated fatty acids in healthy term infants on cardiovascular and anthropometric development at 9 years. Pediatr Res. 2011;70:411-6.

Drover JR, Felius J, Hoffman DR, Castañeda YS, Garfield S, Wheaton DH, Birch EE. A randomized trial of DHA intake during infancy: school readiness and receptive vocabulary at 2-3.5 years of age. Early Hum Dev. 2012;88:885-91.

Drover JR, Hoffman DR, Castañeda YS, Morale SE, Garfield S, Wheaton DH, Birch EE. Cognitive function in 18-month-old term infants of the DIAMOND study: a randomized, controlled clinical trial with multiple dietary concentrations of docosahexaenoic acid. Early Hum Dev. 2011;87:223-30.

Falk MC, Zheng X, Chen D, Jiang Y, Liu Z, Lewis KD. Developmental and reproductive toxicological evaluation of arachidonic acid (ARA)-Rich oil and docosahexaenoic acid (DHA)-Rich oil. Food Chem Toxicol. 2017;103:270-8.

FDA (U.S. Food and Drug Administration). 2001a. Agency Response Letter. GRAS Notice No. GRN000041. DHASCO (docosahexaenoic acid-rich single-cell oil) and ARASCO (arachidonic acid-rich single-cell oil). May 17, 2001. http://www.fda.gov/Food/FoodIngredientsPackaaina/GenerallyRecognizedasSafeGRAS/GRASListingducm 154 126.htm.

FDA (U.S. Food and Drug Administration). 2006. Agency Response Letter. GRAS Notice No. GRN000094. Docosahexaenoic acid-rich oil from tuna (DHA-rich tuna oil) and arachidonic acid-rich oil from *Mortierella alpina (AA-rich fungal oil).* April 18, 2006. http://www.fda.gov/Food/FoodIngredientsPackanina/GenerallyRecognizedasSafeGRAS/GRASListingducm 154630.htm

FDA (U.S. Food and Drug Administration). 2011. Agency Response Letter. GRAS Notice No. GRN000379. Tuna oil. November 8, 2011. http://www.fda.gov/Food/FoodIngredientsPackanina/GenerallyRecognizedasSafeGRAS/GRASListingducm 282570.htm.

FDA. 2015. Agency Response Letter. GRAS Notice No. GRN000553. Algal oil (40% docosahexaenoic acid) derived from *Schizochytrium* sp. June 19, 2015. https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm462744.htm.

FDA. 2017. Agency Response Letter. GRAS Notice No. GRN000677. Docosahexaenoic acid oil produced in *Schizochytrium* sp. May 2, 2017. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM566347.pdf.

Fedorova-Dahms I, Marone PA, Bailey-Hall E, Ryan AS. Safety evaluation of DHA-rich algal oil from *Schizochytrium* sp. Food Chem Toxicol. 2011a;49:70-7.

Fedorova-Dahms I, Marone PA, Bauter M, Ryan AS. Safety evaluation of DHA-rich algal oil from *Schizochytrium* sp. Food Chem Toxicol. 2011b;49:3310-8.

Hammond BG, Mayhew DA, Kier LD, Mast RW, Sander WJ. Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. Regul Toxicol Pharmacol. 2002;35:255-65.

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

Hammond BG, Mayhew DA, Holson JF, Nemec MD, Mast RW, Sander WJ. Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. II. Developmental toxicity evaluation in rats and rabbits. Regul Toxicol Pharmacol. 2001b;33:205-17.

Hammond BG, Mayhew DA, Robinson K, Mast RW, Sander WJ. Safety assessment of DHA-rich microalgae from *Schizochytrium* sp. III. Single-generation rat reproduction study. Regul Toxicol Pharmacol. 2001c;33:356-62.

Institute of Medicine (IOM). 2005. The Dietary Reference Intake for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. The National Academies Press. Washington D.C.

Kitamura T, Kitamura Y, Hamano H, Shoji H, Shimizu T, Shimizu T. The Ratio of docosahexaenoic acid and arachidonic acid in infant formula influences the fatty acid composition of the erythrocyte membrane in low-birth-weight infants. Ann Nutr Metab. 2016;68:103-12.

Koletzko B, Boey CC, Campoy C, Carlson SE, Chang N, Guillermo-Tuazon MA, Joshi S, Prell C, Quak SH, Sjarif DR, Su Y, Supapannachart S, Yamashiro Y, Osendarp SJ. Current information and Asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation, and infancy: systematic review and practice recommendations from an early nutrition academy workshop. Ann Nutr Metab. 2014;65:49-80.

Kremmyda LS, Tvrzicka E, Stankova B, Zak A. Fatty acids as biocompounds: their role in human metabolism, health and disease: a review. part 2: fatty acid physiological roles and applications in human health and disease. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2011;155:195-218.

Kroes R, Schaefer EJ, Squire RA, Williams GM. A review of the safety of DHA45-oil. Food Chem Toxicol. 2003;41:1433-46.

Lewis KD, Huang W, Zheng X, Jiang Y, Feldman RS, Falk MC. Toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. Food Chem Toxicol. 2016;96:133-44.

Martin JC, Bougnoux P, Antoine JM, Lanson M, Couet C. Triacylglycerol structure of human colostrum and mature milk. Lipids. 1993;28:637-43.

Schmitt D, Tran N, Peach J, Bauter M, Marone P. Toxicologic evaluation of DHA-rich algal oil: Genotoxicity, acute and subchronic toxicity in rats. Food Chem Toxicol. 2012a;50:3567-76.

Schmitt D, Tran N, Peach J, Edwards T, Greeley M. Toxicologic evaluations of DHA-rich algal oil in rats: developmental toxicity study and 3-month dietary toxicity study with an in utero exposure phase. Food Chem Toxicol. 2012b;50:4149-57.

Sala-Vila A, Campoy C, Castellote AI, Garrido FJ, Rivero M, Rodriguez-Palmer M, Lopez-Sabater MC. Influence of dietary source of docosahexaenoic and arachidonic acids on their incorporation into membrane phospholipids of red blood cells in term infants. Prostaglandins Leukot Essent Fatty Acids. 2006;74: 143-148.

Van de Lagematt M, Rotteveel J, Muskiet FA, Schaafsma A, Lafeber HN. Post-term dietary-induced changes in DHA and AA status related to gains in weight, length, and head circumference in preterm infants. Prostaglandins Leukot Essent Fatty Acids.2011;85:311-16.

Westerberg AC, Schei R, Henriksen C, Smith L, Veierød MB, Drevon CA, Iversen PO. Attention among very low birth weight infants following early supplementation with docosahexaenoic and arachidonic acid. Acta Paediatr. 2011;100;47-52.

7.B. References That Are Not Generally Available References

Gao Y. 2017. Oral acute toxicity study of DHA and ARA in rats. A report submitted to Linyi Youkang Biology.

APPENDIX. CERTIFICATE OF ANALYSIS FOR DHA-RICH OIL

APPENDIX. CERTIFICATE OF ANALYSIS FOR DHA POWDER