September 7, 2023

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
5001 Campus Drive
College Park, MD 20740

Subject: GRAS Notification –
Docosahexaenoic Acid (DHA)-Rich Oil as a Food Ingredient for Use in Infant Formula and General Foods

To Whom It May Concern,

On behalf of Runke Bioengineering (Fujian) Co., Ltd. (Runke Bioengineering), we are submitting a GRAS notification for docosahexaenoic acid (DHA)-rich oil as a food ingredient. The enclosed document provides the notice of a claim that a food ingredient, the DHA-rich oil, described in the enclosed notification is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be Generally Recognized as Safe (GRAS), based on scientific procedures, as a food ingredient. We believe that this determination and notification are in compliance with Pursuant to 21 C.F.R. Part 170, subpart E.

Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,

September 5, 2023

Susan Cho, Ph.D.
Susanscho1@yahoo.com
Lead Expert Panel Member for Runke Bioengineering Biotechnology, Co., Ltd

THE GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION OF DOCOSAHEXAENOIC ACID (DHA)-RICH OIL AS A FOOD INGREDIENT FOR USE IN INFANT FORMULA AND GENERAL FOODS

Prepared for

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GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF DOCOSAHEXAENOIC ACID (DHA)-RICH OIL AS A FOOD INGREDIENT FOR USE IN INFANT FORMULA AND GERNERAL FOODS

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List of Abbreviations

ACAT2 = acyl-CoA:cholesterol acyltransferase 2
ADI = acceptable daily intake
ALP = alkaline phosphatase
ALT = alanine aminotransferase
AOCS = American Oil Chemists' Society
ARA = arachidonic acid
AST = aspartate aminotransferase
BAM = Bacteriological Analytical
bw = body weight
CAS = chemical abstract service
CCTCC = China Center for Type Culture Collection
CFR = Code of Federal Regulations
cGMP = current Good Manufacturing Practices
CHO = Chinese hamster ovary
COAs = Certificates of Analysis
DHA = docosahexaenoic acid
DIAMOND = DHA Intake and Measurement of Neural Development
DINO = DHA for the Improvement of Neurodevelopmental Outcome
DPA = docosapentaenoic acid
DRM = DHA-rich microalgae
EDI = estimated dietary intake
EFSA= European Food Safety Authority
EPA = eicosapentaenoic acid
FA = fatty acids
FAO = Food and Agriculture Organization
FCC = Food Chemicals Codex
FD&C = Federal Food, Drug, and Cosmetic
FDA = Food and Drug Administration
FR = Federal Register
GMO = genetically modified organisms
GOS = galactooligosaccharide
GRAS = Generally Recognized as Safe
HACCP = Hazard Analysis Critical Control Point
ISO = International Standardization Organization
IMCAS = Institute of Microbiology Chinese Academy of Sciences
LCPUFA = long-chain polyunsaturated fatty acid
LD_{50} = median lethal dose
LDL-C = low-density lipoproteins cholesterol
MCH = mean corpuscular hemoglobin

MCHC = mean corpuscular hemoglobin concentration
MCPD = monochloropropanediol
MCV = mean corpuscular volume
MN= micronucleated
MOBYDIck = Maternal Omega-3 Supplementation to Reduce Bronchopulmonary Dysplasia in Very Pre-term Infants
N3RO = N-3 (omega-3) Fatty Acids for Improvement in Respiratory Outcomes
NEC = necrotizing enterocolitis
NFMOA = National Fish Meal and Oil Association
NHANES = National Health and Nutrition Examination Survey
NOAEL = no-observed-adverse-effect-level
OECD = Organisation for Economic Co-operation and Development
PAH = polycyclic aromatic hydrocarbon
PCB = polychlorinated biphenyl
PCE = polychromatic erythrocyte
PUFA = polyunsaturated fatty acid
QC = quality control
RBC = red blood cell
rDNA = ribosomal deoxyribonucleic acid
rRNA = ribosomal ribonucleic acid
SCORAD = SCORing Atopic Dermatitis
TG = triglyceride
U.S. = United States
U.S.C. = United States Code
USDA = United States Department of Agriculture
VLBW = very low birth weight
WBC = white blood cell
WHO = World Health Organization

PART 1. SIGNED STATEMENTS AND A CERTIFICATION

1. A. Submission of GRAS Notice

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

1.B. Name and Address of the Notifier

Contact: Sunny Tsai
Company: Runke Bioengineering (Fujian) Co., Ltd.
Address: West of No. 552 Rd., Jindu Industrial Clusters Zone, Zhao'an, Zhangzhou, Fujian Province 363500, China
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1.C. Common or Trade Name

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

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

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

(1) Selected conventional foods

Runke Bioengineering intends for DHA-rich oil to be used in food categories currently listed in 21 CFR 184.1472(a)(3), except in egg, meat, poultry, and fish products (Table 1). These are the same food categories found in the GRAS notifications for algal oil derived from *Schizochytrium* sp. (GRNs 000137 and 000732) for which the Food and Drug Administration (FDA) did not raise any questions as to the safety when the intended uses included the food categories identified for menhaden oil. The only difference is that Runke Bioengineering does not intend to use its DHA-rich oil in egg, meat, poultry, and fish products.

(2) Infant formulas

Runke Bioengineering intends for the DHA-rich oil, produced from *Schizochytrium* sp., to be used as a food ingredient in cow milk-, goat milk-, soy-, amino acid-, extensively hydrolyzed protein-based, exempt and non-exempt formula for pre-term and/or low birth weight infants, and term infants in combination with a safe and suitable source of arachidonic acid (ARA). Runke Bioengineering's DHA-rich oil will be added to ready-to-drink or powder forms of infant formulas from which reconstituted infant formulas can be prepared. Exempt infant

formula refers to formulas for pre-term infants only and does not include use in other exempt formulas (e.g., hypoallergenic formulas, formulas for inborn errors of metabolism.

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

Selected Conventional Foods

As shown in Table 1, Runke Bioengineering intends for the DHA-rich oil (containing ≥35% DHA) to be used in the same food categories as those listed in GRN 000137 (future intended use levels listed on pages 22-23; stamped page 27-28), GRN 000732 (pages 4-5), GRN 000933 (page 7), GRN 000934 (page 25) and GRN 001008 (page 24), and in 21 CFR 184.1472(a)(3) (menhaden oil), except in egg, meat, poultry, and fish products, at maximum use levels that are 28.57% of those specified in 21 CFR 184.1472(a)(3), which was finalized in 2005 (FDA, 2005). Runke Bioengineering intends for the DHA-rich oil will be used as the sole added source of DHA in any given food category, or if blended with a source of EPA, the total dietary exposure to DHA will be not more than 1.5 g/person/day (g/p/d) and not more than 3.0 g/p/d of DHA and EPA combined.

Table 1. Maximum Intended Use Levels of DHA-Rich Oil from *Schizochytrium* sp.[1]

Food category	Maximum use levels, %	
	Menhaden oil 184.1472(a)(3)	Current notice
Baked goods and baking mixes (1)	5.0	1.43
Cereals (4)	4.0	1.14
Cheese products (5)	5.0	1.43
Chewing gum (6)	3.0	0.86
Condiments (8)	5.0	1.43
Confections and frostings (9)	5.0	1.43
Dairy products analog (10)	5.0	1.43
Fats and oils (12) (not including infant formula)	12.0	3.43
Frozen dairy products (20)	5.0	1.43
Gelatins and puddings (22)	1.0	0.286
Gravies and sauces (24)	5.0	1.43
Hard candy (25)	10.0	2.86
Jams and jellies (28)	7.0	2.00
Milk products (31)	5.0	1.43
Nonalcoholic beverages (3)	0.5	0.143
Nut products (32)	5.0	1.43
Pastas (23)	2.0	0.57
Plant protein products (33)	5.0	1.43
Processed fruit juices (35)	1.0	0.286
Processed vegetable juices (36)	1.0	0.286
Snack foods (37)	5.0	1.43
Soft candy (38)	4.0	1.14
Soup mixes (40)	3.0	0.86

Sugar substitutes (42)	10.0	2.86
Sweet sauces, toppings, and syrups (43)	5.0	1.43
White granulated sugar (41)	4.0	1.14

[1]The food categories correspond to those listed in 21 CFR 170.3(n). The number in parenthesis following each food category is the paragraph listing that food category in 21 CFR 170.3(n).
Intended use has been adopted from GRNs 137 and 732 with the exception of meat, poultry, and fish products.

Infant Formula

Runke Bioengineering's DHA-rich oil may be used at a maximum of 0.5% of total dietary fat as DHA. This level corresponds to 1.43% of total dietary fat providing 28-39 mg DHA/kg bw/day (or 80 to 111 mg DHA-rich oil/kg bw/day) in term infants and 39 mg/kg bw/day (or 111 mg DHA-rich oil/kg bw/day) in pre-term low-birth, very low-, and extremely low-birth weight infants (ages from birth to 12 months) with a safe and suitable source of ARA, because Runke Bioengineering's DHA-rich oil has ≥35% 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-rich oil in infant formula (GRN 000553 - stamped page 12 or page 6; GRN 000677 - page 6; GRN 000731 - page 5; GRN 000776 - page 3; GRN 000777 - page 3; GRN 933-page 8; GRN 000934-pages 24-25; GRN 001008- pages 1, 25, and amendment dated November 3, 2021- pages 12-14). Runke Bioengineering's DHA-rich oil will be added to ready-to-drink or powder forms of infant formulas from which reconstituted infant formulas can be prepared. The use in exempt infant formulas is for formulas for pre-term infants only, not for other exempt formulas (e.g., hypoallergenic formulas, formulas for inborn errors of metabolism).

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

The substance will be used as an ingredient in selected foods and in non-exempt and exempt infant formulas.

DHA-rich oil is a free flowing, yellow oil. The use of DHA-rich oil in the above-described food categories may also incidentally contribute its own color to the product. Its intended use would thus fall outside the definition of "color additive," in accordance with 21 CFR 70.3(f), "Substances capable of imparting a color to a container for foods----are not color additives unless the customary or reasonably foreseeable handling or use of the container may reasonably be expected to result in the transmittal of the color to the contents of the package or any part thereof. Food ingredients...which contribute their own natural color when mixed with other foods are not regarded as *color additives....".*

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

Selected general food applications: The population expected to consume the substance consists of members of the general population (aged 1 year or older) who consume at least one of the products described above.

Infant formula applications: Infants consuming formula (pre-term and/or low birth weight infants as well as full-term infants).

1.E. 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.F. 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 Tsai at Runke Bioengineering 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.G. Availability of Freedom of Information Act Exemption

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 United States Code (U.S.C.) §552.

1.H. Certification

We certify that, to the best of our knowledge, our 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 the substance.

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

Name: Sunny Tsai Date: August 31, 2023
Title: Export Manager

Address correspondence to
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Lead Expert Panel Member
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1.J. Food Safety and Inspection Service/United States Department of Agriculture (USDA) Statement

Runke Bioengineering 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).

Chemical Identity of Ingredients

DHA-rich oil is all-cis-4,7,10,13,16,19-docosahexaenoic acid (22:6) (Figure 1) esterified to glycerol. There are a number of common or usual names for oils extracted from closely related microalgae including but not limited to:

DHA Algal Oil
DHA Oil
DHA-rich Oil
DHA-rich Algal Oil
Oil from the micro-algae *Schizochytrium* sp.
Docosahexaenoic acid-rich single-cell oil
DHA single cell oil

2.A.1.3. CAS Registry Number

There is no chemical abstract service (CAS) number assigned for DHA-rich oil; however, DHA is assigned the CAS number 6217-54-5. Triglycerides (TGs) are assigned the CAS number 68424-59-9.

2.A.1.4. Empirical Formula

Molecular formula of DHA, $C_{22}H_{32}O_2$

2.A.1.5. Molecular Weight

DHA, 328.488

2.A.1.6. Structural Formula

Figure 1 shows the structure of DHA. DHA is a long chain, polyunsaturated fatty acid, with empirical formula $C_{22}H_{32}0_2$. The complete name is 4,7,10,13,16,19-docosahexaenoic acid. The numbers indicate the number of carbon atoms in the molecule (22), the number of double bonds (6), and the number of carbon atoms from the methyl terminus to the first double bond (3).

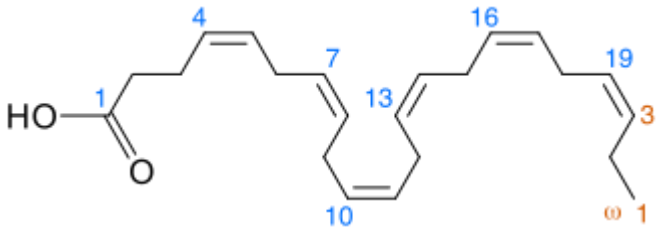

Figure 1. Structure of docosahexaenoic acid (DHA)

2.A.1.7. Physical Properties

Density of DHA, 0.943 g/cm^3

2.A.1.8. Background

DHA is a long-chain polyunsaturated fatty acids (LCPUFAs) that is a primary structural component of the human brain, retina, and other tissues. DHA's structure is a 22-carbon chain carboxylic acid with 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. It can be synthesized from alpha-linolenic acid or obtained directly from maternal milk, algal oil, or fish oil.

Runke Bioengineering's DHA-rich oil is derived from the heterotrophic fermentation of the marine alga, *Schizochytrium* sp. strain FJRK-SCH3.

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

Potential toxicants have not been identified in Runke Bioengineering's DHA-rich oil. Runke Bioengineering's DHA-rich oil is ≥35.0% pure with an average of 42%. The Certificates of Analysis (COAs) for DHA-rich oil are presented in Appendix A.

Shellfish Poison

No amnesic shellfish poison (domoic acid) was found in Runke Bioengineering's DHA-rich oil (Table 2; Appendix A).

Because the manufacturing process involves the fermentation of glucose with yeast extracts and mineral sources by *Schizochytrium* sp. and does not employ any organic solvents, it is not expected to have any significant amounts of dioxins and furans, polychlorinated biphenyls **(**PCBs), polycyclic aromatic hydrocarbons (PAHs), or organic solvent residues in the finished DHA-rich oil (Appendix A).

During industrial refining, monochloropropanediols (MCPDs) and glycidyl esters are processing contaminants that can form in edible oils: the oils are heated at very high temperatures to remove unwanted tastes, colors, or odors via acid-mediated hydrolysis and the

use of chlorinated solutions, including municipal water. Concerns regarding contamination of infant formula by MCPDs and glycidyl esters have been addressed by the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (EFSA, 2016). Due to the fact that the DHA-rich oil is not derived from vegetable sources and because there is no acid hydrolysis step or use of chlorinated solutions in their production, it is not expected to have significant amounts of MCPD and glycidyl esters in the DHA-rich oils. Analysis of 3 batches showed that the concentrations of MCPDs (2- and 3-MCPD; both free and ester forms) and glycidyl esters were near or below detection levels in the Runke Bioengineering's DHA-rich oil. Details are presented in Appendix A. In addition, Mioso et al. (2014) reported that *Schizochytrium* sp. did not produce any toxins. The bacterial endotoxin content is lower than the limit of quantitation (<0.109 EU/g) (Appendix A).

Overall, it is not expected to have a safety risk associated with potential contaminants such as shellfish poison, MCPD, glycidyl esters, and bacterial endotoxins.

Table 2. Analytical Results for Potential Contaminants

	Limit of Quantitation	11024713	11027715	11030717	Methods of Analysis
Domoic Acid*, mg/kg*	< 1.0	< 1.0	< 1.0	< 1.0	Eurofins internal validated method
2-MCPD, mg/kg	0.1	<0.10	<0.10	<0.10	AOCS Cd 29b-13
3-MCPD, mg/kg	0.1	0.14	0.14	0.14	
Glycidol, mg/kg	0.1	<0.10	<0.10	<0.10	
Bacterial endotoxins, EU/g		<0.109	<0.109	<0.109	USP 43<85>

*Analyzed by validated Eurofins' internal methods.

Abbreviations: AOCS, American Oil Chemists´ Society; MCPD, monochloropropanediol; USP, United States Pharmacopeia.

2.A.3. Particle Size

DHA-rich oil – Not applicable.

2.B. Method of Manufacture

DHA-Rich Oil Manufacturing Process

DHA-rich oil is a yellow to light, orange-colored oil derived from the heterotrophically grown marine alga, *Schizochytrium* sp., intended for use as a food ingredient. The *Schizochytrium* sp. FJRK-SCH3 is grown in a pure culture heterotrophic fermentation process, recovered from the fermentation broth. The resulting oil is subjected to centrifugation to separate cells from the oil. The crude oil is subsequently refined using processes and techniques common in the edible oil refining industry including alkali treatment, decolorizing, winterization, and

deodorization. Filtration is the last refining step after the addition of safe and suitable antioxidants to ensure stability. The product is packaged in airtight containers.

a. Fermentation

An oil rich in polyunsaturated fatty acids (PUFAs) is produced by a heterotrophic fermentation process with a single cell marine, micro-algae of the genus *Schizochytrium* sp. (FJRK-SCH3). This organism can be grown to a high cell density using a carbon-based substrate. Fermentation medium is composed of baker's yeast extract, glucose, corn syrup powder, sunflower seed oil, magnesium sulfate, potassium dihydrogen phosphate, calcium chloride, and sodium hydroxide. Operating parameters, such as temperature, aeration, agitation, and pH, are controlled throughout the process to ensure that results, in terms of cell growth and oil production, are reproducible.

b. Separation

Cells (biomass) from the liquid fermentation medium are separated from crude DHA-rich oil via centrifugation.

c. Refining

The crude oil is subsequently refined using processes and techniques common in the edible oil refining industry including alkali treatment (sodium hydroxide and sodium sulfate), decolorizing (activated carbon and activated clay), winterization, and deodorization (steaming at high temperature under vacuum). Filtration is the last refining step after the addition of safe and suitable antioxidants (vitamin E and ascorbyl palmitate) to ensure stability. The product is packaged in airtight containers. Figure 2 presents manufacturing process of DHA-rich oil.

DHA-rich oil is produced in accordance with Hazard Analysis Critical Control Point (HACCP) and current Good Manufacturing Practices (cGMP). All raw materials and processing aids used in the fermentation and manufacturing processes are food grade. Fermentation processing includes the sterilization of growth media and all vessels/containers/fermenters used to grow cells. The fermentation is carried out in the absence of light under axenic conditions. Organic solvents are not used in the manufacturing process. All these steps provide conditions that minimize the risk of contamination with foreign microorganisms. All processing aids and ingredients meet Food Chemicals Codex (FCC) and/or food grade specifications.

Critical control points are monitored to detect insufficient controls on the process (such as incorrect pH, temperature ranges, insufficient fatty acid composition, etc.). If any of the control characteristics fail to meet internal specifications, the fermentation is terminated, and the batch is rejected. Contamination checks also are conducted in the seed and production fermenters. All finished batches of DHA-rich oil undergo rigorous quality assurance testing to meet product specifications prior to release.

DHA (Docosahexaenoic Acid) Oil Manufacturing Process

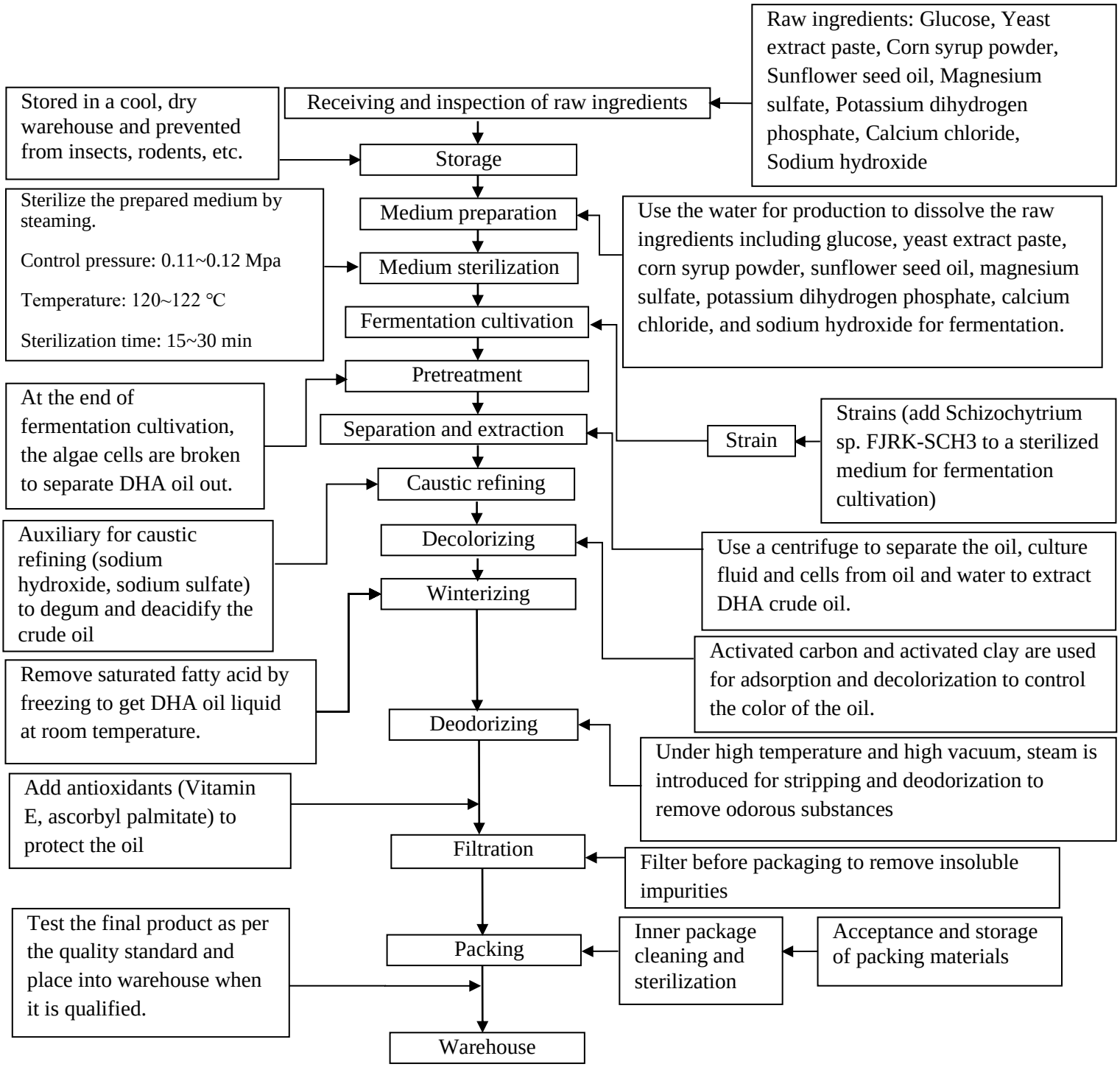

Figure 2. Manufacturing Flow Diagram of DHA-Rich Oil

Tables 3 and 4 present the regulatory status of raw materials used in fermentation and processing aids.

Table 3. Raw Materials

Ingredient	Regulatory status
Fermentation medium	
Baker's yeast extract	21 CFR 184.1983
Glucose	21 CFR 168.121 21 CFR 168.110
Corn syrup (powder)	21 CFR 184.1865
Sunflower seed oil	GRAS per 21 CFR 170.30
Magnesium sulfate (heptahydrate)	21 CFR 184.1443
Potassium dihydrogen phosphate	No CFR citation for intended use*
Calcium chloride	21 CFR 184.1193
Sodium hydroxide	21 CFR 184.1763
Auxiliary for caustic refining	
Sodium hydroxide	21 CFR 184.1763
Sodium sulfate	21 CFR 186.1797
Antioxidants	
Tocopherols	21 CFR 182.3890
Ascorbyl palmitate	21 CFR 182.3149

*CFR, Code of Federal Regulations

Table 4. Processing Aids

Processing Aids	Regulatory status
Decolorization agent	
Bentonite - Activated clay	21 CFR 184.1155
Activated carbon	Unpublished GRAS

Activated carbon – Unpublished GRAS
https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances&id=ACTIVATEDCARBON.

The production method (algal fermentation) is similar to those described by other companies whose production methods for DHA-rich oil that received 'no objections' letters from the FDA (GRN 000137 - FDA, 2004; GRN 000553- FDA, 2015; GRN 000677 - FDA, 2017; GRN 000731/000732 - FDA, 2018a, 2018b; GRN 000776/000777 - FDA, 2018c, 2018d; GRN 000836 -FDA 2019a; GRN 000843-FDA 2019b; GRN 000844-FDA 2019c; GRN 000862-FDA 2020a; GRN 000933 - FDA 2020b; GRN 000934 -FDA 2021; GRN 001008-FDA 2022) for use in both exempt pre-term and non-exempt term infant formulas and/or in selected conventional foods in the United States. DHA-rich algal oil ingredients are derived from the heterotrophic fermentation of the marine alga, a non-toxigenic and non-pathogenic strain of *Schizochytrium* sp. Based on the morphology and 18S ribosomal ribonucleic acid (rRNA) gene sequence analysis,

Institute of Microbiology Chinese Academy of Sciences (IMCAS) identified Runke Bioengineering's strain FJRK-SCH3 as *Schizochytrium* sp. (Appendix B).

Characterization of the Production Microorganism

DHA-rich oil is produced through a multi-step fermentation and refining process using a non-pathogenic, non-toxigenic, non-genetically modified, wild type marine microalgae, *Schizochytrium* sp. FJRK-SCH3. The production organism has been authenticated by morphological and ribosomal deoxyribonucleic acid (rDNA)18S sequence.

Schizochytrium sp. is a thraustochytrid and a member of the Chromista kingdom (Stramenopilia) which includes the golden algae, diatoms, yellow-green algae, haptophyte and cryptophyte algae, and oomycetes. *Schizochytrium* sp. microorganisms are widespread and are commonly found in marine environments throughout the world. The literature indicates that thraustochytrids, especially those of the genus *Schizochytrium* sp., are regularly consumed as food by a wide range of invertebrates. Based on existing published and unpublished scientific data, there have never been any reports of toxic compounds produced by these microorganisms. There are no reports of this organism producing toxic chemicals nor is it pathogenic. Field tests confirmed the widespread occurrence of thraustochytrids in a typical marine food chain. Consumption by man of thraustochytrids, especially those of the genus *Schizochytrium* sp., is primarily through consumption of mussels and clams. Indirect consumption, through the marine food chain (fish and shellfish), is more widespread. Strain identification report is shown in Appendix B. Bluegreen algae and dinoflagellates produce most of the toxic compounds produced by microalgae. *Schizochytrium* sp. is in a separate kingdom from both types of microalgae. As stated in Part 2.A.2., samples of oil from the micro-algae *Schizochytrium* sp. FJRK-SCH3 have been analyzed for algal toxin domoic acid (Appendix A). Chemical analysis of the finished DHA-rich oil ingredient confirmed the absence of common shellfish toxin, domoic acid. The taxonomic classification of *Schizochytrium* sp. is presented in Table 5.

Table 5. Taxonomic Classification of *Schizochytrium* sp.

Class	Scientific Classification
Kingdom	Chromista
Subkingdom	Harosa
Phylum	Bigyra
Subphylum	Sagenista
Class	Labyrinthulea
Order	Thraustochytrida
Family	Thraustochytriaceae
Genus	*Schizochytrium* sp.
Strain	*Schizochytrium* sp. FJRK-SCH3

2.C. Specifications and Composition

Product specifications for the DHA-rich oil are set for DHA content, acid value, free fatty acids, trans-fatty acids, unsaponifiable content, peroxide value, p-anisidine value, moisture, and heavy metals (arsenic, cadmium, mercury, and lead) (Table 6). Physical and chemical tests applied to the QC characterization of the oil are mostly adapted from American Oil Chemist's Society (AOCS), Association of Official Analytical Chemists (AOAC), or the Official Methods and Recommended Practices of International Standardization Organization (ISO).

Table 6 presents the specifications of Runke Bioengineering's DHA-rich oil in comparison with those described in GRNs 137 (page 21, stamped page 26), 553 (pages 17-18, stamped pages 23-24), 677 (page 15), 731 (page 18), and 933 (pages 17-18). The specifications of Runke Bioengineering's DHA-rich oil were also compared with the Food Chemicals Codex (FCC) standards for DHA-rich oils derived from *Schizochytrium* sp. and from *Crypthecodinium cohnii*. The bioequivalence of two algal sources of DHA-rich oils was established when administered in a blend with ARA oil to preweaning farm piglets and human infants (Fedorova-Dahms et al., 2014; Yeiser et al., 2016). Thus, it is reasonable to compare the specifications and fatty acid profiles of Runke Bioengineering's DHA-rich oil with FCC standards for DHA-rich oils derived from the two algal sources (FCC, 13th edition, 2022).

Table 7 summarizes the analytical values for Runke Bioengineering's DHA-rich oil. Three lots of DHA-rich oil were subjected to analytical testing for a wide variety of parameters. These data demonstrate a reproducible and representative process capable of meeting the proposed product specifications. Analytical parameters included DHA, acid value, free fatty acids, trans-fatty acids, unsaponifiable content, peroxide value, p-anisidine value, moisture, and heavy metals to ensure that Runke Bioengineering's DHA-rich oil meets the specifications.

The DHA content is comparable to those described in previous GRAS notices derived from *Schizochytrium* sp. sources. The DHA specification for Runke Bioengineering's DHA-rich oil meets the FCC specifications for DHA-rich oils: 30-40% and 35-47% DHA for DHA-rich oils derived from *Schizochytrium* sp. and from *Crypthecodinium cohnii*, respectively. The specification for free fatty acid (as % oleic acid), unsaponifiable matter, peroxide value, and p-anisidine value meet or exceed the FCC standards.

Fatty Acid Composition

The identified components present in DHA-rich oil have a demonstrated history of safe consumption. The lipid fraction of *Schizochytrium* sp. algae is comprised mainly of fatty acids and sterols. Fatty acids (Tables 8 and 9) are found esterified to glycerol (tri- and diacylglycerides).

Tables 8 and 9 show the fatty acid profile of Runke Bioengineering's DHA-rich oil and its comparison with those described in GRNs 000137 (page 24, stamped page 29), 000553 (pages

18-20, stamped pages 24 -26), 000677 (page 20), 000731 (pages 20-21), and 000933 (pages 20-23). The fatty acid profile of DHA-rich oil is substantially equivalent to DHA-rich oils previously concluded to be GRAS (GRNs 000137, 000677, 000731, and 000933); palmitic acid and DPA (n-6) are the predominant fatty acids next to DHA (Tables 8 and 9).

The analytical values for docosapentaenoic acid (DPA) are comparable between the subject of this GRAS notice (10-15%) and FCCs for algal DHA oil derived from *Schizochytrium* sp. (10.5-16.5%). The eicosapentaenoic acid (EPA) content of Runke Bioengineering's DHA-rich oil is comparable to the FCC specifications for the DHA oil from *Schizochytrium* sp. and from *C. cohnii* (Runke Bioengineering vs. FCC*Schizochytrium* sp. vs. FCC*C. cohnii*: DPA [n-6], 11.9 vs.10.5-16.5 vs. ≤0.1%; EPA, 0.42 vs.1.3-3.9 vs. ≤0.1%).

The specifications for Runke Bioengineering's DHA-rich oil do not include dihomo-γ-linolenic acid (DGLA; a 20-carbon ω−6 fatty acid) and ARA that are not present in large quantities. Compared to the specifications listed in the FCC monograph for algal oil, the levels of DGLA and ARA in Runke Bioengineering's DHA-rich oil are below the FCC specifications for corresponding fatty acids (Runke Bioengineering vs. FCC*Schizochytrium* sp.: DGLA, 0.25 vs. 1.7-2.8%; ARA, 0.21 vs. 0.6-1.3 %). Thus, the resulting exposure to DGLA, ARA, EPA, and DPA in the finished product will be less than oils that comply with the FCC specifications.

Overall, it is concluded that the major fatty acid profile of Runke Bioengineering's DHA-rich oil is comparable to those described in the above-mentioned GRAS notices and FCC specifications and that the presence of DGLA, ARA, EPA, and DPA in the Runke Bioengineering's DHA-rich oil (smaller amounts compared to FCC grade algal oil) would not impact the safety of the oil.

Table 6. Specifications of DHA-Rich Oil

Parameter	Current notice	GRN 137[a]	GRN 553[b]	GRN 677[b]	GRN 731[b]	GRN 933	FCC[c]	FCC[d]	Methods of Analysis for the Current Notice
DHA, %	≥35	32 – 45[f]	≥35[f]	≥35[f]	>45[e]	≥36[e]	30-40[f] ≥30	35-47[f] ≥35	AOAC 996.06 mod.
Acid value, mg potassium hydroxide (KOH)/g	≤0.8	≤0.5		≤0.5	< 0.5	≤0.8	NS		AOCS Cd 3d-63
Free fatty acid, as % oleic acid	≤0.4		≤0.4		< 0.1	≤0.4	≤0.4	≤0.4	AOCS Cd 3d-63
Trans fatty acids, relative area %	≤1.0	≤2.0	≤3.5	≤2.0	<1.0	≤1.0	NS		AOCS 996.06 mod.
Unsaponifiable matter, %	≤3.5	≤4.5	≤3.5	≤3.5	<1.0	≤3.0	≤4.5	≤3.5	AOCS Ca 6a-40
Peroxide value, meq/kg	≤5.0	≤5.0	≤5.0	≤5.0	<5.0	≤5.0	≤5.0	≤5.0	AOCS Cd 8b-90:2017
Moisture (direct drying method), wt%	≤0.1	≤0.1	≤0.02	≤0.05	<0.1	≤0.1	NS		AOCS Ca 2c-25
Lead, ppm	≤0.1	≤0.2	≤0.1	<0.1	<0.1	≤0.1	≤0.1	≤0.1	BS EN ISO 17294-2 2016 mod.
Arsenic, ppm	≤0.1	≤0.5	≤0.1	<0.1	<0.1	≤0.1	≤0.1	≤0.1	
Cadmium, ppm	≤0.1		≤0.1		<0.1	≤0.1			
Mercury, ppm	≤0.04	<0.2	≤0.04	<0.1	<0.01	≤0.04	≤0.1	≤0.1	BS EN 13806:2002
p-Anisidine value	NS						≤20.0		AOCS Cd 18-90
Total oxidation value	NS						≤26		
Docosapentaenoic acid (DPA, n-6), %	NS	10 - 20					10.5-16.5	0-0.1	AOAC 996.06 mod.
Arachidonic acid (ARA), %	NS						0.6-1.3		AOAC 996.06 mod.
Eicosapentaenoic acid (EPA), %	NS		≤10				1.3-3.9	0-0.1	AOAC 996.06 mod.

AOAC = Association of Official Analytical Chemists; AOCS = American Oil Chemist's Society; BS-EN = British adoption of a European (EN) standard; mod=modifications; meq = milliequivalents; NS = not specified.

[a]DHA-rich oil derived from *Schizochytrium* sp. for selected general food applications;

[b]DHA-rich oil derived from *Schizochytrium* sp. for infant formula applications;

[c]FCC specifications for DHA oil derived from *Schizochytrium* sp.;

[d]FCC specifications for DHA oil derived from *Crypthecodinium cohnii*.
[e] wt% (Eurofins' COAs have reported the DHA content in wt%);
[f]relative area%.

Table 7. Summary of Analytical Values for Runke Bioengineering's DHA-Rich Oil*

Parameter	Analytical Values			LOQ	Mean
	11024713	11027715	11030717		
DHA, wt%	43.01	41.71	42.76	0.02	42.49
Acid value, mg KOH/g	0.23	0.37	0.21	0.05	0.27
Free fatty acids as oleic acid, %	0.12	0.19	0.11	0.01	0.14
Free fatty acids, %	0.08	0.10	0.08	0.01	0.09
Total trans fatty acids, %	0.25	0.22	0.26	0.02	0.24
Unsaponifiable matter, %	1.19	1.28	1.33	0.05	1.27
Peroxide value, meq/kg	0.36	0.48	0.24	0.05	0.36
p-Anisidine value	8.8	7.8	9.6	1	8.7
Moisture and volatiles, %	<0.01	<0.01	<0.01	0.01	<0.01
Protein, μg/g	<25	<25	<25	<25	<25
Diglycerides, %	3.9	4.7	3.7	1	4.1
Glycerol, %	2.8	2.9	2.7	1	2.8
Monoglycerides, %	2.2	3.2	1.8	1	2.4
Triglycerides, %	94.2	92.1	94.5	1	93.6
Mercury, mg/kg	<0.005	<0.005	<0.005	0.005	<0.005
Lead, mg/kg	<0.05	<0.05	<0.05	0.05	<0.05
Arsenic, mg/kg	<0.005	<0.005	<0.005	0.005	<0.005
Cadmium, mg/kg	<0.005	<0.005	<0.005	0.005	<0.005

*Samples were taken from 3 non-consecutive batches. LOQ = limit of quantitation.

Table 8. Fatty Acid Profile and Glyceride Composition of Runke Bioengineering's DHA-Rich Oil

Parameters, wt%	Lot number			
	11024713	11027715	11030717	Mean
C16:4 (Hexadecatetraenoic acid)	<0.02	<0.02	<0.02	<0.02
C10:0 (Capric acid)	<0.02	<0.02	<0.02	<0.02
C11:0 (Undecanoic acid)	<0.02	<0.02	<0.02	<0.02
C12:0 (Lauric acid)	0.04	0.03	0.04	0.04
C14:0 (Myristic acid)	0.31	0.29	0.36	0.32
C14:1 (Myristoleic acid)	<0.02	<0.02	<0.02	<0.02
C15:0 (Pentadecanoic acid)	0.05	0.04	0.06	0.05
C15:1 (Pentadecenoic acid)	<0.02	<0.02	<0.02	<0.02
C16:0 (Palmitic acid)	15.93	15.53	16.36	15.94
C16:1 Omega 7	0.09	0.08	0.09	0.09
C16:1 Total (Palmitoleic acid + isomers)	0.26	0.23	0.26	0.25
C16:2 (Hexadecadienoic acid)	<0.02	<0.02	<0.02	<0.02
C16:3 (Hexadecatrienoic acid)	<0.02	<0.02	<0.02	<0.02
C17:0 (Margaric acid)	0.06	0.05	0.06	0.06
C17:1 (Heptadecenoic acid)	<0.02	<0.02	<0.02	<0.02
C18:0 (Stearic acid)	1.35	1.32	1.33	1.33
C18:1 (Vaccenic acid)	0.17	0.15	0.16	0.16
C18:1 Omega 9 (Oleic acid)	3.88	4.05	3.54	3.82
C18:1 Total (Oleic acid + isomers)	4.09	4.24	3.75	4.03
C18:2 Omega 6 (Linoleic acid)	8.24	9.13	7.50	8.29
C18:2 Total (Linoleic acid + isomers)	8.46	9.32	7.81	8.53
C18:3 Omega 3 (Alpha linolenic acid)	0.12	0.13	0.12	0.12
C18:3 Omega 6 (Gamma linolenic acid)	0.13	0.11	0.14	0.13
C18:3 Total (Linolenic acid + isomers)	0.25	0.25	0.26	0.25
C18:4 Omega 3 (Octadecatetraenoic acid)	0.19	0.19	0.21	0.20
C18:4 Total (Octadecatetraenoic acid)	0.19	0.19	0.21	0.20
C20:0 (Arachidic acid)	0.24	0.21	0.24	0.23
C20:1 Omega 9 (Gondoic acid or 11-eicosenoic acid)	0.02	0.03	0.03	0.03
C20:1 Total (Gondoic acid + isomers)	0.04	0.05	0.06	0.05
C20:2 Omega 6	<0.02	<0.02	0.03	<0.02
C20:2 Total (Eicosadienoic acid)	<0.02	<0.02	0.03	<0.02
C20:3 Omega 3	<0.02	<0.02	<0.02	<0.02
C20:3 Omega 6 (DGLA)	0.26	0.20	0.28	0.25
C20:3 Total (Eicosatrienoic acid)	0.26	0.20	0.28	0.25
C20:4 Omega 3	0.61	0.52	0.62	0.58
C20:4 Omega 6 (ARA)	0.19	0.22	0.23	0.21
C20:4 total (Eicosatetraenoic acid)	0.80	0.73	0.85	0.79
C20:5 Omega 3 (EPA)	0.42	0.46	0.37	0.42
C21:5 Omega 3 (Heneicosapentaenoic acid)	<0.02	<0.02	<0.02	<0.02

C22:0 (Behenic acid)	0.22	0.20	0.24	0.22
C22:1 Omega 9 (Erucic acid)	0.28	0.21	0.35	0.28
C22:1 Total (Erucic acid + isomers)	0.28	0.21	0.35	0.28
C22:2 Docosadienoic Omega 6	<0.02	<0.02	<0.02	<0.02
C22:3 Docosatrienoic, Omega 3	0.16	0.12	0.17	0.15
C22:4 Docosatetraenoic Omega 6	<0.02	<0.02	0.02	0.02
C22:5 Docosapentaenoic Omega 3	0.08	0.07	0.08	0.08
C22:5 Docosapentaenoic Omega 6	12.31	10.60	12.60	11.84
C22:5 Total Docosapentaenoic acid (DPA)	12.40	10.68	12.68	11.92
C22:6 Docosahexaenoic Omega 3	**43.01**	**41.71**	**42.76**	**42.49**
C24:0 (Lignoceric acid)	0.13	0.11	0.13	0.12
C24:1 Omega 9 (Nervonic acid)	<0.02	<0.02	<0.02	<0.02
C24:1 Total (Nervonic acid + isomers)	0.10	0.04	0.07	0.07
C4:0 (Butyric acid)	<0.02	<0.02	<0.02	<0.02
C6:0 (Caproic acid)	<0.02	<0.02	<0.02	<0.02
C8:0 (Caprylic acid)	<0.02	<0.02	<0.02	<0.02
Total Fat as Triglycerides	92.85	89.86	92.47	91.73
Total Fatty Acids	89.13	86.26	88.77	88.05
Glycerides composition				
Diglycerides	3.9	4.7	3.7	4.1
Glycerol	2.8	2.9	2.7	2.8
Monoglycerides	2.2	3.2	1.8	2.4
Triglycerides	94.2	92.1	94.5	93.6

LOQ: 0.02% for individual fatty acids and 0.1 wt% for total fat as triglycerides.

Table 9. Comparison of Fatty Acid Profiles of DHA-Rich Oils (wt% Unless Noted Otherwise)

	Current notice	GRN 137[a]	GRN 553[b,*]	GRN 677[b,*]	GRN 731[b]	GRN 933	FCC[c,*]	FCC[d,*]
DHA (Docosahexaenoic acid) specifications	≥35	32 - 45	≥35	≥35	>45	≥36	35-40; ≥30	35-47; ≥35
Actual content, %	42.5	35.0	43.3	40.22	50.7	38.87		
Fatty Acid Profile, g/100g								
C 6:0 (Caproic acid)	<0.02				< 0.02			
C 8:0 (Caprylic acid)	<0.02				< 0.02	<0.02		
C 10:0 (Capric acid)	<0.02				< 0.02	<0.02		
C 12:0 (Lauric acid)	0.04	0.4	<0.10	0.91	0.10	0.08		
C 14:0 (Myristic acid)	0.32	10.11	1.18	11.87	0.82	1.29		
C 14:1 (Myristoleic acid)	<0.02		<0.10	<0.10	< 0.02	<0.12		
C 15:0 (Pentadecanoic acid)	0.05		0.24	0.52	0.06	1.05		
C 15:1 (Pentadecenoic acid)	<0.02				0.07	<0.02		
C 16:0 (Palmitic acid)	15.94	23.68	13.78	25.43	20.96	26.20		
C 16:1 (Palmitoleic acid)	0.09	1.76	<0.10	3.42	0.51	0.19		
C 17:0 (Margaric acid or Heptadecanoic acid)	0.06		<0.10	<0.12	0.08	0.84		
C 18:0 (Stearic acid)	1.33	0.45	1.65	0.82	1.30	1.12		
C 18:1 (Oleic acid)	3.82	NA		4.77	0.27	1.83		
C 18:1n7 (Vaccenic acid)	0.16	Trace-1.36	0.26		0.51			
C 18:2n6 (Linoleic acid)	8.29		2.01	<0.33	< 0.02	3.85	NA	0-1.0
C 18:3n3 (alpha-Linolenic acid)	0.12		<0.10	NA	0.14	0.48		
C 18:3n6 (gamma-Linolenic acid)	0.13		NA	0.23	0.09	0.12		
C 20:0 (Arachidic acid)	0.23		0.32	<0.10	0.29	0.20		
C 20:1 (Eicosenoic acid)				<0.06	< 0.02	<0.03		
C 20:2n6 (Eicosodienoic acid)	<0.02		0.13		< 0.02	<0.03		
C 20:3n3 (Eicosatrienoic acid)	<0.02		<0.1		1.34	<0.03		
C 20:3n6 (homo-gamma-Linolenic acid)	0.25		<0.1	<0.11	0.21	0.19	1.7-2.8	0-0.1
C 20:4n6 (Arachidonic acid; ARA)	0.21	0.94	0.69	0.70	0.15	1.01	0.6-1.3	
C 20:5n3 (Eicosapentaenoic acid; EPA)	0.42	2.63	6.23	1.18	0.70	0.31	1.3-3.9	0-0.1

C 21:0 (Heneicosanoic acid)					0.04			
C 22:0 (Behenic acid)	0.22			<0.10	0.15	0.12		
C 22:1n9 (Erucic acid)	0.28				< 0.02			
C 22:2n6 (Docosadienoic acid)	<0.02		0.53		< 0.02	<0.02		
C 22-5n3 (Docosapentaenoic acid)	0.08		0.76		0.11	0.08		
C 22-5n6 (Docosapentaenoic acid; DPA)	11.84	13.5	2.53	7.81	10.33	8.76	10.5-16.5	0-0.1
C 23:0 (Tricosanoic acid)					< 0.02			
C 24:0 (Lignoceric acid)	0.12			<0.10	0.15	<0.054		
C 24:1 (Nervonic acid)	<0.02				0.41	<0.02		

NA= not available; [a]DHA-rich oil derived from *Schizochytrium* sp. for selected general food application; [b]DHA-rich oil derived from *Schizochytrium* sp. for infant formula application; [c]FCC specifications for DHA oil derived from *Schizochytrium* sp.; [d]FCC specifications for DHA oil derived from *Crypthecodinium cohnii*.

*Fatty acid contents were reported as relative area%.

Microbiology

Analysis of 3 non-consecutive batches showed that *Escherichia coli* (absent in 25 g), *Cronobacter* sp. (absent in 10 g), and Salmonella (absent in 25 g) are not present in Runke Bioengineering's DHA-rich oil (Table 10). Total aerobic plate counts, yeast, molds, and Enterobacteriaceae counts are below the detection limit (<10 cfu/g). COAs are presented in Appendix A.

Table 10. Microbial Counts of Runke Bioengineering's DHA-rich oil

	Lot number			Method of Analysis
	11024713	11027715	11030717	
Aerobic plate counts, cfu/g	<10	<10	<10	US FDA BAM Ch 3, Jan 2001
Yeast, cfu/g	<10	<10	<10	US FDA BAM Ch 18, April 2001
Molds, cfu/g	<10	<10	<10	
Escherichia coli/25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	ISO 16649-3:2015
Cronobacter sp./10 g	ND	ND	ND	ISO 22964:2017
Salmonella/25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g	US FDA BAM Ch 5, April 2001
Enterobacteiaceae, cfu/g	<10	<10	<10	ISO 21528-2-2017

BAM, Bacteriological Analytical Manual; Ch, chapter; ND, not detected.

Sterols

As stated in GRN 000137 (stamped page 14), the lipid fraction of *Schizochytrium* sp. algae is comprised mainly of fatty acids and sterols and the non-saponifiable fraction of the DHA-rich oil consists primarily of squalene, sterols, and carotenoids. These components are all present in the food supply. Fatty acids (not just DHA) and sterols/stanols that are present in the algal oil (≥35% DHA) are also common to the diet from other food sources. The safety of dietary cholesterol and phytosterols is well documented in the scientific literature (Brownawell and Falk, 2010). The major sterols found in the DHA algal oil are found in human breast milk and commercially available infant formula (Mellies et al., 1976).

A few scientific papers reported that main sterols present in infant formulas are cholesterol (0.03-2.58 wt%/v) and demosterol (0.05-0.31 wt%/v) (Claumarchirant et al., 2015). These sterols are also present in human milk (cholesterol, 0.065-2.92 wt%/v). In infant formulas, total plant sterol (%wt/v) ranged from 0.31 to 0.50. β-Sitosterol, the most abundant phytosterol, ranged from 0.18 to 0.30, followed by campesterol (0.072−0 .115), stigmasterol (0.027−0.053), and brassicasterol (0.014−0.028) (Claumarchirant et al., 2015).

Total plant sterol and stanol (wt%/v) content in Runke Bioengineering's DHA-rich oil was 0.571 wt% (Table 11-1). Cholesterol was the most abundant sterol (0.32 wt%), followed by sitosterol (0.112), delta-7-stigmastenol (0.05), stigmasterol (0.031), brassicasterol (0.017), delta-7-avenasterol (0.01), and campesterol (0.01). Table 11-2 presents the sterol content of Runke Bioengineering's DHA-rich oil in comparison with those described in GRNs 000553 (pages 21-22, stamped pages 27-28), 000677 (page 21), and GRN 000137 (stamped page 30). This level is comparable to the average total sterol values calculated from the values reported in GRN 553 (0.54 wt%) and GRN 677 (0.15 wt%), but much lower than the value reported in GRN 137 (3.1 wt%). It is noteworthy that GRN 000137 reported much higher total sterol concentrations compared to other GRAS notices. These sterols are commonly present in infant formulas. However, all the sterols that are present in subject of this GRAS determination were not directly quantified to compared to the subject of GRNs 000553 and 000677. It is likely that the unidentified fraction could be 24-methylene cholesterol, clerosterol, delta-5,23-stigmastadienol, delta-5- avenasterol, delta-7-campesterol, ergosta-7,22-dien-3-ol, and ergosta-7,24-dien-3-ol (whose values were included in GRN 000137, 000553, and/or 000677). Some peaks were difficult to clearly identified, thus, were summed and reported as unidentified sterols in the COAs (Appendix A). Chen et al. (2014) reported that sterol extract from alga *Schizochytrium* sp included lathosterol, ergosterol, stigmasterol, 24-ethylcholesta-5,7,22-trienol, stigmasta-7,24(24(1))-dien-3β-ol, and cholesterol.

Table 11-1. Sterol composition in DHA-Rich Oils

Parameters, g/100 g	Lot #11024713	Lot #11027715	Lot #11030717	Mean
24-Methylenecycloartanol	0.002	0.004	0.003	0.003
Brassicasterol	0.018	0.016	0.018	0.017
Campestanol	0.002	0.002	0.002	0.002
Campesterol	0.009	0.011	0.009	0.010
Cholesterol	0.318	0.319	0.324	0.320
Citrostadienol	0.007	0.008	0.006	0.007
Cycloartenol	0.007	0.006	0.008	0.007
Delta-7-avenasterol	0.011	0.009	0.011	0.010
Delta-7-stigmastenol	0.054	0.043	0.054	0.050
Delta-5,24-stigmastadienol	0.020	0.014	0.020	0.018
Sitostanol + delta-5-avenasterol	0.006	0.007	0.005	0.006
Sitosterol	0.112	0.115	0.109	0.112
Stigmasterol	0.031	0.032	0.031	0.031
Unidentified sterols	0.328	0.286	0.326	0.313
Total plant sterols + stanols	0.591	0.537	0.584	0.571

* The values represent total sterols in fats (wt%). Like other DHA-rich oil (GRN 677), it is assumed that Runke Bioengineering's DHA oil is composed of 99-100% fats.
NR=not reported.

Table 11-2. Comparison of Plant Sterols/Stanols in DHA-Rich Oils

Parameters, wt%	Current Notice	GRN 553*	GRN 677*	GRN 137
24-Methylenecholesterol	NR	0.0080	0.0064	NR
24-Methylenecycloartanol	0.003	NR	NR	NR
Brassicasterol	0.017	0.0070	<0.0045	0.465
Campestanol	0.002	0.0005	<0.0002	NR
Campesterol	0.010	0.0097	0.0035	NR
Cholesterol	0.320	0.0664	0.0345	0.775
Citrostadienol	0.007	NR	NR	NR
Clerosterol	NR	0.0086	0.0188	NR
Cycloartenol	0.007	NR	NR	NR
Delta-7-avenasterol	0.010	0.0049	0.0065	NR
Delta-5-avenasterol	NR	0.0095	0.0045	NR
Delta-7-campesterol	NR	0.0022	<0.0044	NR
Delta-7-stigmastenol	0.050	0.0103	<0.0129	NR
Delta-5,23-stigmastadienol	NR	0.0045	<0.0069	NR
Delta-5,24-stigmastadienol	0.018	0.0022	0.0086	NR
Sitostanol	NR	0.0028	<0.0003	NR
Sitostanol + delta-5-avenasterol	0.006	NR	NR	NR
Sitosterol	0.112	NR	NR	NR
Stigmasterol	0.031	0.3413	<0.0204	0.589
Stigmastadien-3-ol	NR	NR	NR	0.248
Ergosta-7,22-dien-3-ol	NR	NR	NR	0.155-0.217
Ergosta-7,24-dien-3-ol	NR	NR	NR	0.155-0.186
Unidentified sterols	0.313	NR	NR	NR
Total plant sterols + stanols	0.571	0.54	0.15	3.1

* The values represent total sterols in fats (wt%).
NR=not reported.

2.D. Stability

Three non-consecutive lots of DHA-rich oil filled with nitrogen in tightly closed original aluminum container were stored at ≤ 25°C and -10°C for testing DHA content, acid value, peroxide value, and anisidine value every four months. As shown in Table 12, Runke Bioengineering's DHA-rich oil is stable for 12 months at ≤ 25°C and -10°C. Based on the stability data, the proposed shelf life of Runke Bioengineering's DHA-rich oil is 12 months.

Table 12. Stability Testing for DHA-Rich Oil

Batch Number	Parameters	Storage Time (months)			
		0	4	8	12
Storage at ≤ 25°C					
11024713	Acid value	0.14	0.14	0.15	0.1
	Peroxide value	<0.1	0.6	1.6	1.8
	Anisidine value	3.5	8.8	10.1	11.4
	DHA%	43.4	43.4	43.5	43.3
11027715	Acid value	0.37	0.29	0.31	0.29
	Peroxide value	<0.1	1.2	1.8	1.9
	Anisidine value	5.5	8.6	10.4	11.6
	DHA%	44.3	44.3	44.2	44.7
11030717	Acid value	0.17	0.18	0.18	0.16
	Peroxide value	<0.1	0.6	1.1	1.5
	Anisidine value	4.7	6.1	7.8	10.2
	DHA%	44.0	43.9	43.9	44.5
Storage at -10°C					
11024713	Acid value	0.14	0.14	0.14	0.13
	Peroxide value	<0.1	<0.1	<0.1	0.4
	Anisidine value	3.5	3.4	3.6	4.6
	DHA%	43.4	43.5	43.5	43.4
11027715	Acid value	0.37	0.32	0.31	0.31
	Peroxide value	<0.1	<0.1	<0.1	0.3
	Anisidine value	5.5	5.6	5.4	6.9
	DHA%	44.3	44.3	44.4	44.5
11030717	Acid value	0.17	0.18	0.17	0.16
	Peroxide value	<0.1	<0.1	<0.1	0.5
	Anisidine value	4.7	4.1	4.8	5.2
	DHA%	44.0	43.9	43.8	44.2

DHA = docosahexaenoic acid (test method = AOCS Ce 1-62-1989).
Acid value, unit, mg KOH/g; acid values meet the specification (≤ 0.8 mg KOH/g).
Peroxide value, unit, meq/kg oil; peroxide values meet the specification (≤ 5.0 meq/Kg oil).
Anisidine values meet the specification (≤ 20.0)

2.E. GMO Status

No genetically modified ingredients or genetic modification technology is used in the production of the DHA-rich oil and powder.

2.F. Allergens

Raw materials used in the production contained no allergenic substances. The manufacturing facility is free of potential allergens. In addition, the protein content in Runke

Bioengineering's DHA-rich oil is trivial (<25 μg/g), thus, it is not expected that the DHA-rich oil would be allergenic.

2.G. Intended Technical Effects

The DHA-rich oil will be used as a food ingredient in selected conventional foods and in non-exempt term and exempt pre-term infant formulas.

PART 3. EXPOSURE ESTIMATES

3.A. Exposure Estimates

Selected General Foods

In accordance with 21 CFR 184.1(b)(2), the ingredient may be used in food to ensure that the total intake of EPA or DHA does not exceed 3.0 grams/person/day (21 CFR 184.1472). The DHA-rich oil will be added to the same food categories, excluding egg, meat, poultry, and fish products, as those currently listed in 21 CFR 184.1472(a)(3) (menhaden oil) and GRN 000137 at maximum use levels that are 28.57% of those specified in that regulation. As discussed in GRN 000137, the proposed use levels of the DHA-rich oil are expected to result in a maximum dietary exposure of less than 1.5 g of DHA per day. Because the DHA-rich oil is intended to be used as an alternative to menhaden oil, there will be no increase in exposure to DHA from the intended use as described in Table 1. Runke Bioengineering's DHA-rich oil is not to be combined with any other added oil that is a significant source of DHA or EPA. It would be possible, however, to blend DHA-rich oil with other sources of DHA and/or EPA.

The 28.57% value was derived from the following factors:

1) Since menhaden oil is considered GRAS at a level providing no more than 3 grams of DHA and EPA per day, the use levels in each food category are decreased by 50% so that the total daily consumption of DHA from the DHA-rich oil will be no more than 1.5 grams per day.
2) The levels of use are based on the quantity of DHA-algal oil that can be added to each product. Additional adjustment is needed because the DHA-algal oil has a different concentration of DHA than that found in menhaden oil. DHA-algal oil contains approximately 35 wt% compared to about 20% of combined EPA and DHA in menhaden oil. An additional adjustment of 57.143% (20/35) is needed to accommodate the different concentrations of DHA in the two oils.
3) The 28.57% adjustment is calculated by multiplying the 50% adjustment that is needed in accordance with the first bullet point above by the 57.143% adjustment that is needed in accordance with the second bullet point above, i.e., (0.50) x (0.5714) x 100 = 28.57%.

These are the same food categories (except egg, meat, poultry, and fish products) found in the GRAS notifications for DHA-algal oils (GRN 137, stamped pages 10 to 12 and 27 to 28 - FDA, 2004; GRN 732, pages 4 to 5 - FDA, 2018b) for which the agency did not raise any objections to the companies' conclusion that DHA-algal oil derived from *Schizochytrium* sp. would be considered GRAS when used in the food categories identified for menhaden oil.

The estimated dietary intakes (EDIs) of DHA established in the early 2000s when the menhaden oil rule was finalized (FDA, 2005) and when DHA-rich oil derived from

Schizochytrium sp. (GRN 137 - FDA, 2004) received no question letters from the FDA are still applicable. Our comparative National Health and Nutrition Examination Survey (NHANES) analysis (2001-2002 vs. 2015-2016) revealed that the total number of food servings consumed was slightly decreased in the mid-2010s when compared to the early 2000s. For example, the mean and 90th percentile numbers of total food servings of the 26 food categories specified in Table 1 were 11.8 and 20.0 servings, respectively, in 2001-2002 and 11.0 and 18.9 servings, respectively, in 2015-2016 for individuals in the American population aged 1-99 years (detailed analytical data not shown).

In summary, when the subject of this notice (≥35% DHA) is used as an ingredient as the sole added source of DHA in any given food category, or if blended with a source of EPA, the total dietary exposure to DHA will be not more than 1.5 g/person/d and not more than 3.0 g/person/day of DHA and EPA combined for the U.S. population 2 years of age and older.

EDIs of DHA for Term Infants

According to tables of daily energy intake by formula-fed infants provided by Fomon (1993), the 90th percentile energy intakes were approximately 140 kcal/kg bw/day in infants aged 14-27 days (141.3 and 138.9 kcal/kg bw /day in boys and girls, respectively). Assuming that approximately 50% of calories in infant formula are provided by fats, this indicates intake of approximately 70 kcal from fat/kg bw/day, or 7.8 g fat/kg bw/day (1 g fat=9 kcal/g). In infant formulas for which DHA provides 0.5% of the fatty acids, the 90th percentile intake of DHA would be 39 mg DHA/kg bw/day (7,800 mg fat/kg bw/day x0.005=39 mg/kg bw/day). Since an average new infant (<1mo) weighs approximately 4 kg, an EDI of DHA would be ~156 mg/infant/day.

As the infant grows, formula intake increases, but more slowly than weight gain, so that consumption assessed as the amount of formula or calorie intake/kg bw decreases for infants older than 27 days. In infants aged 86 to 195 days, the 90th calorie intake/kg bw/day are decreased to approximately 110 kcal/kg bw/day. Using the same assumption that 50% of calories in infant formula are provided by fats, EDIs for fat would be approximately 6.11 g/kg bw/day. Because DHA provides 0.5% of the fatty acids, the 90th EDIs of DHA would be 30.5 mg/kg bw/day (6,111 mg fat/kg bw/day x0.005= 30.55 mg DHA/kg bw/day). The intake estimates are similar to those estimated in GRN 000041 (30 mg DHA/kg bw/day based on DHA addition at 0.5% of total fatty acids).

Assuming older infants consume approximately 100 kcal/kg bw/day (corresponding to 5.55 g fat/kg bw/day), the EDI of DHA would be 27.8 mg DHA/kg bw/day in older infants at around 11.5 months of age. Since an average older infant weighs approximately 10.2 kg, an EDI of DHA would be ~284 mg/infant/day.

Overall, daily intakes of DHA for term infants are estimated to be in the range of 28 to 39 mg/kg bw/day depending upon the age of the infant. After considering body weight of infants, daily intakes of DHA under the intended use are estimated to be 156, 214, and 284 mg/infant/day in infants aged 0.5, 4.5, and 11.5 months, respectively (as corresponding average body weights are 4, 7, and 10.2 kg, respectively). For example, 39 mg DHA/kg bw/day x 4 kg bw/infant = 156 mg DHA/person/day for infants aged 0.5 months.

Runke Bioengineering's DHA-rich oil may be used at a maximum use level of 1.428% of total dietary fat because it has ≥35% DHA (0.5% total fat/0.35=1.428% as DHA). Because the intended use will result in 27.8 to 39 mg DHA/kg bw/day, EDIs for DHA-rich oil would be 79 to 111 mg/kg bw/day. For example, 27.8 mg DHA/kg bw/day is divided by 0.35 to get 79.4 mg DHA-rich oil/kg bw/day.

These estimated DHA intakes of 28-39 mg/kg bw/day are consistent with current DHA recommendations for term infants of 18 to 60 mg/kg bw/day (Koletzko et al., 2014a, 2014b).

EDIs of DHA for Pre-term Infants

The dietary exposure of pre-term low-, very low-, and extremely low-birth weight infants to DHA via infant formulas containing DHA-rich oil was calculated using two calculation methods as shown below and summarized in Table 13.

The maximum amount of fat allowed in infant formula is 6 g/100 kcal according to 21 CFR 107.100. The recommended calorie intake for pre-term very low-birth weight infants is 110-130 kcal/kg bw/day (Koletzko et al., 2014a). Because DHA will be used at a maximum use level of 0.5% of total fatty acids (i.e., a maximum of 0.5% total fat as DHA), it is likely that practical maximum amount of DHA is expected to be 39 mg/kg bw/day based on the following formulas: 6,000 mg fat/100 kcal x 130 kcal/kg bw/day x 0.005 (0.5% fat as DHA)= 39 mg DHA/kg bw/day.

To calculate EDIs in terms of per infant, body weights were considered. It is expected that EDIs of DHA in terms of per person per day would be 97.5, 58.5, and 39 mg DHA/person/day in pre-term low- (2.5 kg bw), very low- (1.5 kg bw), and extremely low- (1 kg bw) birth weight infants, respectively. For example, daily DHA intake/person/day in pre-term low-birth weight infants would be 39 mg DHA/kg bw/day x 2.5 kg bw/person =97.5 mg DHA/person/day.

The maximum of 39 mg DHA/kg bw/day corresponds to 111.4 mg DHA-rich oil/kg bw/day as DHA-rich oil contains a minimum of 35%. Thus, EDIs of DHA-rich oil would be 278,

167, and 111 mg DHA-rich oil/person/day in low- (2.5 kg), very low- (1.5 kg), and extremely low- (1 kg) birth weight pre-term infants.

In summary, the daily intakes of DHA are estimated to be 28-39 mg/kg bw/day in term infants. In pre-term infants, the practical maximum EDI of DHA is expected to be 39 mg/kg bw/day. These EDIs are consistent with current DHA recommendations for pre-term infants of 18 to 60 mg/kg bw/day (Koletzko et al., 2014a, 2014b, 2020).

Table 13. Summary of EDIs of DHA and DHA-rich Oil

	mg DHA/kg bw/day	mg DHA/ person/day	mg DHA-rich oil/kg bw/day	mg DHA-rich oil/person/day
Term infants	28-39	156-284	79-111	446-811
Pre-term infants				
Low-birth weight (2.5 kg)	39	97.5	111	278
Very low-birth weight (1.5 kg)	39	58.5	111	167
Extremely low-birth weight (1 kg)	39	39	111	111
General population		1,500		4,286

bw, body weight; DHA, docosahexaenoic acid; EDI, estimated dietary intake.

Runke Bioengineering's DHA-rich oil is intended for use in infant formula in a similar manner as the currently approved oils. Runke Bioengineering's DHA-rich oil is expected to be used as an alternative to existing DHA-rich oils, thus, cumulative EDIs are not expected to be changed.

3.B. Food Sources of DHA

The DHA-rich oil is intended to provide DHA to term and pre-term infants and the general population. These formulas will either be the sole source of DHA, in combination with a suitable, safe source of ARA for the infants 0 – 12 months of age or will augment breast milk during that period. Because formula will be used as a substitute for breast milk and the levels of DHA in formula are similar to those in breast milk, the DHA exposure is not expected to change.

Human milk is a significant source of DHA. The worldwide mean DHA content of human milk is 0.32 -0.37% of total fatty acids and ranges from 0.06% to 1.4% (Brenna et al., 2007; Fu et al., 2016). Fish oil and egg yolks also are known to be excellent sources of DHA.

3.C. Estimated Daily Intakes (EDIs) of Naturally Occurring DHA from the Diet

A meta-analysis of human milk DHA concentrations (Brenna et al., 2007) found that the mean and standard deviation of DHA concentration as a percentage of total fatty acids was 0.32 ± 0.22% (range: 0.06-1.4%). The highest concentrations were observed in coastal regions, possibly due to the ingestion of sea foods (up to 1.4% of total fatty acids as DHA).

The average daily intake of DHA from food sources is about 160 mg in American juveniles aged 12-19 years (Zhou et al., 2023) and approximately 58 mg in American women aged 20–44 years (Wang et al., 2022).

3.D. EDI of Other Components Under the Intended Use

EDIs of Sterols Under the Intended Use

The EDIs of sterols under the intended use were calculated using the EDI values described in Part 3 of this GRAS determination and the ratio of total sterols to DHA present in Runke Bioengineering's DHA-rich oil. Daily intakes for total sterols were estimated to be 4.5, 1.6, and 24 mg/person/day for term infants, pre-term infants, and general population, respectively.

Infants

To calculate EDIs of sterols/person/day, EDIs of sterols/kg bw/day were calculated first. EDIs of sterols were calculated as 0.45-0.62 mg/kg bw/day for term infants and 0.58 mg/kg bw/day for pre-term infants using the following formulas: 1) total sterols and DHA content present in 1 gram of Runke Bioengineering's DHA-rich oil is 5.7 mg and 350 mg, respectively. Thus, the ratio of total sterols to DHA is 0.016:1. 2) EDIs of DHA were 28-39 mg DHA/kg bw/day for term infants and 39 mg/kg bw/day for pre-term infants (please see pages 33-35 for details). Thus, to calculate the EDIs of sterols for term infants, EDIs of DHA (28-39 mg/kg bw/day) were multiplied by 0.016 to get EDIs of sterols. For example, 28-39 mg DHA/kg bw/day was multiplied by 0.016 to get 0.45 -0.62 mg sterols/kg bw/day.

Then, after considering body weight of infants, daily intakes of DHA under the intended use are estimated to be up to 284 mg/infant/day in term infants aged 11.5 months weighing 10.2 kg (27.8 mg DHA/kg bw/day x 10.2 kg=284 mg/infant/day). These levels correspond to up to 4.5 mg sterols/infant/day for term infants (284 mg DHA x 0.016 sterols/DHA=4.5 mg sterols).

The EDI of DHA would be 97.5 mg DHA/person/day in pre-term low-weight infants weighing 2.5 kg; this level may correspond to the EDI of 1.56 mg sterols/infant/day.

These intakes are well below the amount of sterols already consumed as natural constituents in infant formulas because mean total sterol intake was estimated at 41−66 mg/day

in infants aged 0.5- to 5-month-old infants consuming infant formulas (Claumarchirant et al., 2015). Thus, the estimated intake of sterols through the proposed uses of DHA-rich oil would not have an impact on the relative amount of cholesterol and phytosterols already consumed via infant formulas.

General Population

Similarly, for the general population, the maximum EDI value of DHA (1,500 mg/person/day) was multiplied by 0.016 to get 24 mg sterols/person/day. This level (24 mg sterols/person/day) is well below the amount of sterols already consumed as natural constituents in the diet (up to 463 mg/person/day; Andersson et al., 2004), and thus, the estimated intake of sterols under the intended uses of DHA-rich oil would not have a significant impact on the relative amount of total sterols already consumed in the diet. Therefore, the dietary exposure to total sterols including cholesterol, sitosterol, delta-7-stigmastenol, delta-5,24-stigmastadienol, and others from the intended use of DHA-rich oil would not be expected to produce adverse effects on human health.

Taken together, the estimated intake of sterols through the proposed uses of DHA-rich oil would not pose a safety concern.

<u>EDIs of DPA Under the Intended Use</u>

All fatty acids present in DHA-rich oil are components of a normal diet or normal metabolites of fatty acids.

Infants

Analysis of 3 lots of DHA-rich oils indicates total DPA concentration of approximately 11.9% (Table 8). The ratio of total DPA to DHA is 11.9:35. The EDIs of DHA were 28-39 mg DHA/kg bw/day for term infants and 39 mg/kg bw/day for pre-term infants (please see pages 33-35 for details). Thus, to calculate the EDIs of DPA for term infants, EDIs of DHA (28-39 mg/kg bw/day) were multiplied by 11.9:35. to get EDIs of DPA. For example, 28-39 mg DHA/kg bw/day was multiplied by 11.9:35. to get 9.52 -13.3 mg DPA/kg bw/day.

General Population

Based on the fatty acid composition of DHA algal oil derived from *Schizochytrium* sp. algae, the estimated intake of DPA (n-3 and n-6) through the intended conditions of use of DHA-rich oils would amount to a maximum of 0.51 g/person/day assuming all foods listed in Table 1 containing the maximum use level of oil would be consumed daily by a consumer. The daily intake of 0.51 g DPA was calculated by the following formulas; the maximum daily intake for DHA is 1.5 g/person/day; Runke Bioengineering's DHA-rich oil contains at least 35% and 12% of DHA and DPA, respectively. Thus, 1.5 g x 12/35=0.51 g DPA/person/day. This intake is within the range of levels of DPA provided via seafood consumption. Thus, DPA intake under

the intended use is not expected to produce adverse effects in humans. The actual daily average intake of DPA[n-6] should be significantly less than 0.51 g/person/day for the general population because it is not likely that a consumer would choose all foods in the marketplace within the proposed food categories that contain DHA algal oil as a substitute for another edible oil.

Analysis of the fatty acid component of DHA-rich oil revealed the presence of 2 forms of DPA (22:5): n-6 DPA (11.8%), and n-3 DPA (0.08% total fatty acids). Both DPA isomers are component acids of fish oil (Byelashov et al., 2015). It is also known that n-6 DPA is β-oxidized to arachidonic acid, and that the deficiency of n-3 essential fatty acids in animals causes a compensatory rise in the n-6 DPA level in the brain/retina (Tam et al., 2000). Seafood is a good source of DPA: for instance, raw salmon provides up to 393 mg DPA per 100 g of edible portion (https://wicworks.fns.usda.gov/resources/usda-food-composition-databases). The consumption of 12 ounces of salmon alone would provide up to 191 mg DPA per day. Thus, estimated daily intake (EDI) of DPA was calculated to be 1.7 to 4.0 g DPA (Byelashov et al., 2015). On the other hand, the EFSA's review reported that the mean daily intakes of DPA from food only were between 25 -75 mg/day, and that the 95th percentile intakes of DPA from food only were between 100 mg/day (Belgium, women, 18-39 years) and 138 mg/day (France, men, 45 years).

Thus, DPA present in DHA-rich oil is not expected to produce adverse effects in humans under the intended use.

Summary of Exposure Estimates

For general food applications, DHA-rich oil will be added to the same food categories as those currently listed in 21 CFR 184.1472(a)(3) (menhaden oil) at the maximum use levels, with the exception of egg, meat, poultry, and fish products. The proposed use levels of the DHA-rich oil are expected to result in a maximum dietary exposure of 1.5 g of DHA per day. To ensure the safe use of the substance, the DHA-rich oil is intended to be the sole source of DHA in any given food category.

For infant formulas, the intended use will result in 28 - 39 mg DHA/kg bw/day (or 79 - 111 mg DHA-rich oil/kg bw/day) for term infants and 39 mg DHA/kg bw/day for pre-term infants, which are consistent with current DHA recommendations for term and pre-term infants of 18 - 60 mg/kg bw/day depending on the gestational age.

Sterols and DPA are naturally occurring substances in the diet and these components present in Runke Bioengineering's DHA-rich oil would not have an impact on the safety in pre-term and term infants as well as in general population.

PART 4. SELF-LIMITING USE LEVELS

No known self-limiting levels of use are associated with the DHA-rich oil.

PART 5. HISTORY OF CONSUMPTION

EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

The statutory basis for the conclusion of the GRAS status of the algal DHA-rich oil in this document is not based on common use in food before 1958. The GRAS determination is based on scientific procedures.

PART 6. NARRATIVE

6.A. Current Regulatory Status

Due to the compositional similarity and DHA content of fish-, marine algal-, and egg-derived oils to DHA-rich oil, the available scientific literature on the safety of these oils supports the safety of DHA-rich oil. Menhaden oil is a refined marine oil that is produced from the Brevoortia species of fish. In 1997, in response to the GRAS Petition (GRASP) 6G0316 submitted by the National Fish Meal and Oil Association (NFMOA), the FDA affirmed the GRAS status of menhaden oil and partially hydrogenated menhaden oil with an iodine number between 11 and 119, provided that under the conditions of intended use in foods, the total EPA plus DHA daily intake does not exceed 3 g/person/day (FDA, 1997). At that time, the FDA raised concerns about the consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of low-density lipoproteins cholesterol (LDL-C), and influence glycemic control in subjects with type 2 diabetes (menhaden oil final rule; 62 Federal Register [FR] 30751; June 5, 1997). Based on this review, the FDA concluded that a combined intake of EPA and DHA of up to 3 g/person/day would not result in any adverse health effects (FDA, 1997). NFMOA later submitted a petition to amend rule § 184.1472 (21 CFR 184.1472). In 2005, FDA issued a final rule on menhaden oil, reallocating the use levels and categories of use within the GRAS affirmation, but ensuring daily intakes of EPA and DHA do not exceed 3 g/person/day (FDA, 2005). As DHA represents approximately one half of the combined DHA plus EPA, it is reasonable to consider that the acceptable daily intake (ADI) of DHA is 1.5 g/person/day.

Subsequently, numerous algal and marine sources of DHA have been evaluated by the FDA over the past 20 years for the proposed incorporation in food for human consumption. GRAS notifications for DHA-rich oils (derived from algae, krill, and fish) have received “no questions” responses from the FDA. In this review, GRAS notices on DHA-rich oils derived from *Schizochytrium* sp. only are summarized.

As shown in Table 14, algal DHA-rich oil derived from *Schizochytrium* sp. received GRAS notice status within the United States (U.S.) These include FDA’s no question letters for infant formula applications (GRN 000553 - FDA, 2015; GRN 000677 - FDA, 2017; GRN 000731 - FDA, 2018a, GRNs 000776/000777 - FDA, 2018c, 2018d; GRN 000862 – FDA, 2020a; GRN 000933 – FDA, 2020b; GRN 000934 – FDA, 2021; GRN 001008-FDA, 2022) and selected conventional food applications (GRN 000137 - FDA, 2004; GRN 000732 - FDA, 2018b; GRN 000836 - FDA 2019a; GRN 000843/000844 – FDA, 2019b, 2019c; GRN 000862 – FDA, 2020a; GRN 000933 – FDA, 2020b; GRN 000934 – FDA, 2021; GRN 001008-FDA, 2022).

Table 14. Regulatory Approvals for Use of DHA-Rich Oil Derived from *Schizochytrium* sp. in Foods and Infant Formulas

Item	Year	DHA content; *Schizochytrium* sp. strain name	
Selected foods with intended uses as a direct food ingredient in the same categories as considered GRAS for menhaden oil [21CFR184.1472(a)(3)]			Intended use and EDI
GRN 000137	2004	32-45%; strain name, not disclosed	The same food categories as those listed in 21 CFR 184.1472(a)(3) (menhaden oil); EDI, <1.5 g DHA/person/day
GRN 000732	2018b	>45% DHA; strain LU310 (except products under USDA jurisdiction)	
GRN 000843	2019b	≥35% DHA; strain FCC-1324	
GRN 000844	2019c	≥55% DHA; strain FCC-3204	
GRN 000862	2020a	~40% DHA (oil) or ~10% (powder); strain ONC-T18 (except products under USDA jurisdiction)	
GRN 000933	2020b	≥36% DHA; strain DHF (except products under USDA jurisdiction)	
GRN 000934	2021	≥35% DHA; strain CABIO-A2	
GRN 001008	2022	≥45% DHA; *Schizochytrium limacinum* TKD-1	
Foods with intended uses in selected conventional foods			
GRN 000836	2019a	50-60% DHA; strain HS01	90th PCTL, 460 mg/p/d
Infant Formula applications			
GRN number, infant types	Year	DHA content; *Schizochytrium* sp. strain name	Intended use level and EDI
GRN 000553, pre-term and term	2015	≥35% DHA; strain name, not disclosed	0.5% of total fat as DHA in combination with a safe and suitable source of ARA (at a ratio 1:1 to 1:2 of DHA to ARA); EDI, 27-33 mg DHA/kg bw/day
GRN 000677, pre-term and term	2017	≥35% DHA; strain ONC-T18	
GRN 000731, pre-term and term	2018a	>45% DHA (oil) or >8% DHA (powder); strain LU310	
GRN 000776, pre-term and term	2018c	≥35% DHA; FCC-1324	
GRN 000777, pre-term and term	2018d	≥55% DHA; FCC-3204	
GRN 000862, pre-term and term	2020a	~40% DHA (oil) or ~10% (powder); strain ONC-T18	
GRN 000933, pre-term and term	2020b	≥36% DHA; strain DHF	
GRN 000934, term	2021	≥35% DHA; strain CABIO-A2	
GRN 001008,	2022	≥45% DHA;	Amendment to

pre-term and term		*Schizochytrium limacinum* TKD-1	45 and 30-40 mg/kg bw/day for pre-term and term infants, respectively

bw, body weight, d=day; DHA, docosahexaenoic acid; EDI, estimated daily intake; PCTL, percentile.

6.B. Review of Safety Data

The safety of Runke Bioengineering's DHA-rich oil derived from *Schizochytrium* sp. FJRK-SCH3 was evaluated in a battery of toxicity studies including a bacterial reverse mutation test, an *in vitro* chromosomal aberration test using human blood peripheral lymphocyte, and a mammalian erythrocyte micronucleus test as well as in acute toxicity studies, including, a 28-day subacute toxicity study, and a 90-day subchronic toxicity study (Lewis et al., 2016), and developmental and reproductive toxicity studies (Falk et al., 2017).

As the DHA-rich oil in this GRAS notice has similar specifications and chemical composition compared to those described in the previous FDA GRAS notices involving algal DHA-rich oil (Table 6), it is recognized that the information and data in those GRAS notices are pertinent to the safety evaluation of the DHA-rich oil in this GRAS notice. The safety of DHA-rich oil derived from *Schizochytrium* sp. was evaluated in animal toxicity studies and/or mutagenicity/genotoxicity studies by many research groups, and the data are presented in the published papers (Fedorova-Dahms et al., 2011a, 2011b; Schmitt et al., 2012a, 2012b) and previous GRAS notices. Therefore, this notice incorporates by reference the safety and metabolic studies discussed in those GRAS notices and will not discuss previously reviewed references in detail. Additionally, this notice discusses human studies that have been published between January 2021 and May 2023 in addition to key human clinical studies of DHA-rich oil ingredients related to gastrointestinal tolerance and allergy.

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

The DHA content varies considerably among organs, being particularly abundant in neural tissue, such as brain and retina. DHA is obtained directly in the diet or biosynthetically produced via desaturation and elongation of dietary precursor essential fatty acids. DHA is mainly found in the form of triglycerides, although they also occur in phospholipids in breast milk (Martin et al., 1993).

Available evidence indicates that the absorption, distribution, and metabolism of DHA are similar to other dietary fatty acids. The digestive process for the triglyceride form of DHA, the form present in DHA-rich oil from *Schizochytrium* sp., is complex and requires lipase

activities of lingual, gastric, intestinal, biliary, and pancreatic sources. Gastric lipase and pancreatic lipase, the quantitatively most important enzymes in humans, are primarily specific to the sn-1 and sn-3 positions of triglycerides to produce predominately sn-2 monoglycerides and free fatty acids.

This facilitates the absorption of PUFAs at the sn-2 position and the transfer to tissues. 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 triglycerides (Kroes et al., 2003). These reconstructed triglycerides enter the lymph in the form of chylomicrons for transport to the blood, which allows distribution and incorporation into plasma lipids, erythrocyte membranes, platelets, and adipose tissue. The chylomicron-containing triglycerides are hydrolyzed by lipoprotein lipase during the passage through the capillaries of adipose tissue and the liver to release free fatty acids to the tissues for metabolism or for cellular uptake with subsequent re-esterification into triglycerides and phospholipids for storage as energy or as structural components of cell membranes. The metabolism of fatty acids 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 shortening of the fatty acid carbon chain and the production of acetic acid and acetyl coenzyme A, which combines with oxaloacetic acid and enters the citric acid cycle for energy production. The degree of transport of fatty acids across the mitochondrial membrane is contingent upon the length of the carbon chain; fatty acids of 20 carbons or more are transported into the mitochondria to a lesser degree than shorter chain fatty acids. Therefore, long chain fatty acids, such as DHA, may not undergo mitochondrial beta-oxidation to the same extent (Kroes et al., 2003). Instead, they are preferentially channeled into the phospholipid pool where they are rapidly incorporated into the cell membranes of the developing brain and retina. These fatty acids may be conditionally essential depending on the essential fatty acid availability.

<u>Bioequivalence of two types of algal DHA-rich oils</u>

Numerous GRAS notices have considered that DHA from algal sources is equivalent to that of fish oil. In addition, the bioequivalence of two types of algal DHA-rich oils (derived from either *Crypthecodinium cohnii* [DHASCO®] or *Schizochytrium* sp. [DHASCO-B®]) has been demonstrated in preweaning farm piglets and in humans when administered in a blend with ARA oil (Fedorova-Dahms et al., 2014; Yeiser et al., 2016).

In the study by Fedorova-Dahms et al. (2014), blends of DHA- and ARA-rich oils were tested for both types of DHA-rich algal oils; a lower dose provided 0.32% and 0.64% of total fatty acids as DHA and ARA, respectively, and a higher dose provided 0.96% and 1.92% of total fatty acids as DHA and ARA, respectively. The high doses of DHA correspond to 283.9 and

305.4 mg/kg bw/day for males and females, respectively, in the DHASCO-B® groups and 288.4 and 294.4 mg/kg bw/day, respectively, in the DHASCO® group. There were no treatment-related effects of DHA/ARA on piglet growth and development, hematology, clinical chemistry, urinalysis, and terminal necropsy parameters. No significant group differences were noted in the DHA concentrations in plasma, red blood cell (RBC), heart, liver, and brain, but showed dose-related accumulation. The authors concluded that the dietary exposure to the two types of DHA-rich algal oils was well tolerated by the neonatal piglets during the 3-week dosing period right after birth, and both DHA-rich algal oils were bioequivalent.

In addition, the study by Yeiser et al. (2016) demonstrated that DHASCO® (derived from *C. cohnii*) and DHASCO-B® (derived from *Schizochytrium* sp.) were equivalent sources of DHA as measured by circulating RBC DHA in infants. Healthy term infants were randomized to receive one of the study formulas (17 mg DHA/100 kcal), either DHASCO® (n=140) or DHASCO-B® (n=127) from 14 to 120 days of age. The study formulas were provided as ready-to-use liquids (20 kcal/fluid ounce) with ARA (34 mg/100 kcal) and a prebiotic blend of polydextrose and galactooligosaccharide (GOS) at 4 g/L (1:1 ratio). Compared to the control formula (DHASCO®), the 90% confidence interval for the group mean (geometric) total RBC DHA ratio for the DHASCO-B® group was 91-104%. These values fell within the pre-specified equivalence limit of 80 to 125%. In addition, no significant group differences were noted in growth rates, RBC concentrations of total or individual saturated and monounsaturated fatty acid concentrations, and tolerance. This study demonstrated that both types of DHA-rich oils were safe, well-tolerated, and associated with normal growth. The results from this study indicate that both types of algal DHA-rich oils are bioequivalent when circulating RBC DHA is used as a biomarker.

The results from these studies indicate that the data obtained from studies of the two types of DHA-oils can be interchangeable.

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

Due to the abundance of literature, the review of mutagenicity and genotoxicity studies focused on studies of DHA-rich oil derived from *Schizochytrium* sp. only instead of DHA-rich oil from various sources.

Bacterial Reverse Mutation Assays for Runke Bioengineering's DHA-Rich Oil

The safety of Runke Bioengineering's DHA-rich oil from *Schizochytrium* sp. strain was evaluated in mutagenicity and genotoxicity studies (Lewis et al., 2016).

To test for mutagenicity, *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, and *E. coli* strain WP2 uvrA were exposed to 0.062, 0.185, 0.556, 1.667, 2.5, 3.75, or 5 mg/plate using the plate incorporation and preincubation methods in the absence and presence of S9. In the absence of S9, the positive controls were 2-nitrofluorene (TA98), sodium azide (TA100 and TA1535), 4-nitroquinoline 1-oxide (*E. coli* WP2 uvrA), and 9-aminoacridine (TA1537). The positive control in the presence of S9 was 2-aminoanthracene for all bacteria. No revertant colonies that exceeded three times the mean of the solvent control and no dose-related increases were observed at any DHA-rich oil dose regardless of S9 (Table 15). Thus, it was concluded that the DHA-rich oil was not mutagenic under the test conditions.

In Vitro Chromosomal Aberration Test Using Human Blood Peripheral Lymphocyte with Runke Bioengineering's DHA-Rich Oils

The potential of Runke Bioengineering's DHA-rich oil to induce chromosomal aberrations was evaluated in human peripheral blood lymphocyte cultures (Lewis et al., 2016; Table 15). The chromosomal aberration tests consisted of two phases. For phase I in the absence and presence of S9, the exposure period was 4 hours, the recovery period was approximately 20 hours, and the harvesting period was after 25 hours. For phase II, the exposure period was 4 hours and the harvesting period was 24 hours with no recovery period in the absence of S9. In the presence of S9, the conditions were the same as in the absence of S9 with an addition of a recovery period of 20.5 h. The peripheral blood lymphocyte cultures were exposed to 1.25, 2.5, or 5.0 mg/mL DHA-rich oil and controls. The positive controls were ethyl methanesulfonate in the absence of S9 and cyclophosphamide in the presence of S9. The mean percentage of aberrant cells was determined. The DHA-rich oil doses did not induce a significant increase in the number of chromosomal aberrations in the absence or presence of S9, while treatment with positive controls resulted in a significant increase in percent aberrant cells. The increased frequency of aberrations observed in the concurrent positive control groups (Phase I and II) demonstrated the sensitivity of the test system and the suitability of the methods and conditions. It was concluded that the DHA-rich oil doses up to 5 mg/mL were not mutagenic or clastogenic under the experimental conditions.

In vivo Mammalian Erythrocyte Micronucleus Test for Runke Bioengineering's DHA-Rich Oil

The potential of Runke Bioengineering's DHA-rich oil to induce micronuclei in polychromatic erythrocytes (PCEs) of the bone marrow was evaluated in Wistar rats (Lewis et al., 2016). Wistar rats received 1,000, 2,500, or 5,000 mg/kg bw/day DHA-rich oil or vehicle corn oil for two days (5 male and 5 female rats/group). The positive control, cyclophosphamide, was administered on the second dosing day. All doses were well tolerated, and no adverse clinical signs were observed. There was no effect of treatment on the body weight of animals, and there was no evidence of toxicity and no mortalities. The bone marrow of each animal was collected 24 h after the final dose of control or DHA and bone marrow smears were prepared. Mean frequencies of PCE to normochromatic erythrocytes (%PCE) and individual frequencies of

micronucleated (MN) PCE were assessed. These parameters were not significantly different among the DHA-rich oil and control groups. Compared with the rats treated with the negative control, rats that were treated with the positive control had significantly elevated numbers of MN PCE. The data indicated that the assay system was considered valid. It was concluded that DHA-rich oil showed no evidence of genotoxicity when administered to rats at doses of up to 5000 mg/kg bw/day under the experimental conditions.

Table 15. Summary of Studies Showing No Mutagenicity and/or Genotoxicity of Runke Bioengineering's DHA-Rich Oil

Test	Test system	Concentration/dose of DHA-Rich Oil	Results
Bacterial reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. coli* WP2 uvrA	Up to 5.0 mg/plate, plate incorporation and preincubation ± S9	No mutagenicity
In vitro chromosomal aberration test using human blood peripheral lymphocyte	Human blood peripheral lymphocytes	Phase I: Concentration of 0.0, 1.25, 2.5, and 5 mg/mL culture ± S9; Phase II: 1.25, 2.5, and 5.0 g mg/mL culture ± S9 (2%)	No mutagenicity
Mammalian erythrocyte micronucleus test	Polychromatic erythrocytes in bone marrow of treated rats	0, 1,000, 2,500, and 5,000 mg/kg bw/day	No evidence of genotoxicity

Adapted from Lewis et al. (2016), Table 8.
ARA= arachidonic acid; bw= body weight; DHA= docosahexaenoic acid.

Studies of Other Sources of DHA-Rich Oil Reviewed in Previous GRAS Notices

In GRNs 000553 (stamped pages 36-38, 47-50, 54-55), 000677 (pages 35-36, 39-40), 000731 (pages 28-30), 000732 (pages 31-33), 000776 (pages 17-23), 000777 (pages 15-21), 000836 (pages 32-37, 42), 000843 (pages 19-24), 000844 (pages 18-24), 000933 (page 34), 000934 (pages 32-34), and 001008 (pages 39-41), it was summarized that no studies found mutagenicity or genotoxicity of DHA-rich oil or DHA-rich microalgae (DRM) from *Schizochytrium* sp. The studies reviewed in these GRAS notices include bacterial reverse mutation assays (Hammond et al., 2002; Fedorova-Dahms et al., 2011a, 2011b; Schmitt et al., 2012a), chromosome aberration assays (Fedorova-Dahms et al., 2011a, 2011b; Hammond et al., 2002; Schmitt et al., 2012a), *in vivo* micronucleus tests in mice and rats (Fedorova-Dahms et al., 2011a, 2011b; Hammond et al., 2002; Schmitt et al., 2012a), and *in vitro* Chinese hamster ovary (CHO) AS52/XPRT gene mutation assay (Hammond et al., 2002). These studies reported that the DHA-rich oils were not mutagenic or genotoxic under the test conditions.

6.B.3. Animal Toxicity Studies of DHA-Rich Oil and DRM Derived from *Schizochytrium* sp.

Due to the abundance of literature, the review of animal toxicity studies focused on studies of DHA-rich oil derived from *Schizochytrium* sp. instead of DHA-rich oil from various sources. The results of various animal toxicity studies are summarized in Table 16.

Table 16. Animal Toxicity Studies of DHA-Rich Oil or DRM from *Schizochytrium* sp.

Study Design	Dose (purity)	Duration	Species	Primary Observations	NOAEL mg/kg bw/d unless noted otherwise	Reference
Toxicity Studies of Runke Bioengineering's DHA-Rich Oil						
Acute toxicity	5,000 mg/kg bw (41.37% DHA of total FAs in DHA-rich oil)	Single dose; observed for 14 d	Rats	No treatment-related adverse effects	LD_{50} > 5,000 mg/kg bw	Lewis et al., 2016
28-day toxicity	1,000, 2,500, or 5,000 mg/kg bw/d (41.37% DHA of total FAs in DHA-rich oil)	28 d	Rats	No treatment-related adverse effects	5,000	Lewis et al., 2016
Subchronic toxicity (gavage)	1,000, 2,500, or 5,000 mg/kg bw/d (41.37% DHA of total FAs in DHA-rich oil)	90 d	Rat	No treatment-related adverse effects	5,000 (M) 5,000 (F)	Lewis et al., 2016
Maternal/ paternal reproductive and develop-mental toxicity (oral gavage)	1,000, 2,500, or 5,000 mg/kg bw/d (41.37% DHA of total FAs in DHA-rich oil)	M - 98 d (84 d premating + 14 d mating; F - 71 d (14 d premating + 14 d mating+ 22 d pregnancy + 21 d lactation)	Rat	No treatment-related adverse effects	5,000 for maternal toxicity and embryo/fetal development; 5,000 for paternal or maternal treatment-related reproductive toxicity	Falk et al., 2017
DHA-Rich Oil Studies Reviewed in Previous GRAS Notices						

Acute oral toxicity (gavage)	5,000 mg/kg (40.23 area% DHA in DHA-rich oil)	Single dose; observed for 14 d	Rat	No treatment-related adverse effects	LD_{50}>5 g/kg	Schmitt et al., 2012a
Subchronic toxicity (diet)	0.5, 1.5, or 5 wt% in diet (37% DHA of total FAs in DHA-rich oil)	90 d	Rat	Reduced food consumption in all treatment and fish oil control groups; attributed to high fat content rather than treatment.	3,149 (M) 3,343 (F)	Fedorova-Dahms et al., 2011a
Subchronic toxicity (diet)	1, 2.5, or 5% in diet (40.23 area% in DHA-rich oil)	90 d	Rat	No treatment-related adverse effects	3,305 (M) 3,679 (F)	Schmitt et al., 2012a
Reproductive and develop-mental toxicity	0.5, 1.5, or 5 wt% in diet (43% DHA of total FAs in DHA-rich oil)	F_0: M & F-28 d premating and ≤14 d mating periods; F-followed by gestation and lactation period; F_1: 90 d with an *in utero* phase, followed by a 4 wk recovery phase	Rat	No treatment-related adverse effects	F_0 premating: 3,466 (M), 4,013 (F); F_0 gestation: 3,469 (F); F_0 lactation: 8,322 (F). F_1 90 day with *in utero* exposure phase: 4,122 (M), 4,399 (F)	Fedorova-Dahms et al., 2011b
Prenatal develop-mental toxicity (gavage)	400, 1,000, or 2,000 mg/kg bw/d (~42% DHA in DHA-rich oil)	Gestation days 6 to 19	Rat	No treatment-related adverse effects	2,000 for both maternal and embryo/fetal development toxicity	Schmitt et al., 2012b

Reproductive and develop-mental toxicity	0, 1.0, 2.5, or 5% in diet (42% DHA in DHA-rich oil)	F_0 M - 89-91 d; F_0 F - 75-77 d	Rat	No treatment-related adverse effects	F_0: 5% (both M and F) in diet; F_0 during premating, 3,421 (M), 3,558 (F); after mating, 2,339 (M); F_0 during gestation, 3,117 (F); F_0 during lactation, 7,464 (F)	Schmitt et al., 2012b
		F_1 M- 106-107 d with an *in utero* phase; F_1 F -110-111 d with an *in utero* phase	Rat	Developmental toxicity-5% in diet for both M and F. Systematic toxicity-No treatment-related adverse effects in the 5% group males; Higher food consumption, body weight, and body weight gain in the 5% F_1 female group	F_1: 5% in diet (both M and F); F_1: 3,526 (M), 4,138 (F); Systematic toxicity-3,526 (M) and 2,069 (F).	
DRM Studies Reviewed in Previous GRAS Notices						
Sub-chronic toxicity (diet)	1.169, 2.680, 3.391, or 5.746 kg DRM per pig (22.3% DHA on a dry wt basis)	2.680 kg DRM/pig-120 d, a whole-life exposure; 1.169, 3.391, or 5.746 kg DRM/pig during the last 42 d	Pig (M)	No treatment-related adverse effects for low-, mid-, and high-dose groups (261, 756, and 1,281 g DHA per pig during expt. period)	No feed consumption data on a mg/kg bw basis; no NOAEL was reported	Abril et al., 2003
Subchronic toxicity (diet)	400, 1,500, or 4,000 mg/kg bw/d (8.7% DHA on a dry	13 wk	Rat	No treatment-related adverse effects	4,000 DRM (corresponding to 348 DHA**)	Hammond et al., 2001a

	wt basis)					
Reproductive and develop-mental toxicity (diet)	0.6, 6.0, or 30% DRM in diet (8.7% DHA on a dry wt. basis)	Gestation days 6 to15	Rat	No treatment-related adverse effects	Both maternal and developmental toxicity - 22,000 DRM (corresponding to 1,914 DHA**)	Hammond et al., 2001b
Single-generation reproduction toxicity (diet)		M-15 wk; F-2 weeks prior to mating, during mating, and throughout gestation and lactation (10 wk)	Rat	No treatment-related adverse effects	17,847 DRM (corresponding to 1,512 DHA**) (M); 20,669 DRM (corresponding to 1,680 DHA**) (F)	Hammond et al., 2001c
Reproductive and develop-mental toxicity (gavage)	180, 600, or 1,800 mg DRM/kg/d (8.7% DHA on a dry wt basis)	F_0 mother-13 d (gestation days 6 to 18)	Rabbit	High-dose (1,800) DHA oil and fish oil groups: F_0 mothers had reduced food consumption and body weight and a slightly higher abortion rate (but within the historical limits for the laboratory). NS effect on post-implantation loss, mean foetal body weight/litter, or morphological developments.	F_0: 600 DRM (corresponding to 52 DHA**) (F); F_1: Developmental, 1,800 DRM (corresponding to 157 DHA**) (both M and F)	Hammond et al., 2001b

bw = body weight; d = day; DHA = docosahexaenoic acid; DRM = DHA-rich microalgae; F = females; FAs = fatty acids; LD_{50} = median lethal dose; M = males; NOAEL = no-observed-adverse-effect-level; wt = weight.

*Conversion from DHA to DHA-rich oil quantity was based on the assumption that a typical DHA-rich oil used in various studies would contain 40% DHA.

**DHA values for DRM are on a dry weight basis.

Toxicity Studies of Runke Bioengineering's DHA-Rich Oil

Acute Toxicity Study of Runke Bioengineering's DHA-Rich Oil

The acute toxicity of Runke Bioengineering's DHA-rich oil was evaluated in rats (Lewis et al., 2016). The study was completed in compliance with "Guidelines for Toxicity, FDA, Chapter IV C.2: Acute Oral Toxicity Tests".

Five female Wistar rats aged 8-10 weeks (180-189 g prior to dosing) were fasted for 16–18 h and then were orally administered 5000 mg/ kg bw of DHA-rich oil (41.37% DHA) at a maximum dose volume of 10 mL/kg body weight. The rats were starved for 3 to 4 h after dosing and were observed for clinical signs at 30 min, 1, 2, 3, and 4 h post dosing. From days 2 through 14, the rats were observed in the morning and evening for mortality and clinical signs. Body weight was determined on days 0 (prior to dosing), 7, and 14. When the observation period ended, the surviving rats were sacrificed, and gross pathological examinations were performed. No unscheduled deaths occurring during the 14-day observation period. Thus, an additional group of 5 rats received 5,000 mg/kg bw/day DHA-rich oil and was observed for 14 days to get similar results from the first group of rats. Morbidity, mortality, and body weight were monitored. During the observation period, no mortality and no clinical signs were observed as well as no internal or external abnormalities. Body weights of all rats increased normally and were within the typical ranges.

Therefore, the acute oral median lethal dose (LD_{50}) of the DHA-rich oil was above 5,000 mg/kg bw for both male and female rats. The data indicate that the DHA-rich oil is 'practically non-toxic' (Altug, 2003).

28-Day Oral Toxicity Study of Runke Bioengineering's DHA-Rich Oil

Lewis et al. (2016) conducted a 28-day oral toxicity study in compliance with "Toxicological Principles for the Safety Assessment of Food Ingredients. Redbook 2000 Chapter IV.C.3.a. Short term Toxicity Studies with Rodents" and OECD Principles of Good Laboratory Practice as revised in 1997 and adopted on November 26, 1997 by decision of the OECD Council [C(97)186/Final].

Male and female Wistar rats aged 6-8 weeks old were randomly assigned to one of 5 treatment groups: 1,000, 2,500, or 5,000 mg/kg bw/day DHA-rich oil (purity, 41.37%), distilled water (control), or corn oil (vehicle control) by gavage for 28 days. Morbidity and mortality were monitored. Detailed clinical observations included changes in skin, fur, eyes, or mucous membranes, occurrence of secretions and excretions, autonomic activity, changes in gait, posture, and response to handling, and presence of clonic or tonic movements, stereotypy, and bizarre behaviors. Body weight and food and water consumption levels were measured. Surviving animals completed clinical pathology examinations.

Hematology included white blood cells (WBCs), RBCs, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets. Clinical biochemistry parameters were albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, creatinine, glucose, total protein, triglycerides, alkaline phosphatase (ALP), chloride, sodium, and potassium. Urinalysis analyzed urine output, color, appearance, specific gravity, pH, protein, glucose, bilirubin, blood cells, leukocytes, urobilinogen, ketones, and water intake. Necropsy was completed after the animals were fasted overnight. Macroscopical examination was done for the cranial, thoracic, and visceral cavities. Histopathological examinations were also completed.

No mortality was observed. There were no differences in body weight in the DHA groups, and the mean body weights were similar among all groups. No treatment-related clinical signs or symptoms were found. In the control and high-dose groups, the ophthalmological examinations were normal. No treatment-related abnormalities were found in feed consumption, hematology, urinalysis, and mean body weights. There were no significant adverse effects at DHA doses up to 5,000 mg/kg bw/day. The NOAEL of the DHA-rich oil was 5,000 mg/kg bw/day (Lewis et al., 2016).

90-Day Oral Toxicity Study of Runke Bioengineering's DHA-Rich Oil

Male and female Wistar rats aged 6-8 weeks old were randomly assigned to one of 5 treatment groups (n = 20 males and 20 females per group): 1,000, 2,500, or 5,000 mg/kg bw/day DHA-rich oil (purity, 41.37%), distilled water (control), or corn oil (vehicle control) by oral gavage for 90 days after which they were sacrificed (Lewis et al., 2016). Two additional groups of animals (20/group/sex) were treated with vehicle control (corn oil) or 5,000 mg/kg bw/day DHA-rich oil for additional 14 days. At day 105, rats in recovery groups were sacrificed after fasting overnight.

Body weight and water and feed consumption were measured. Hematology and coagulation parameters, clinical biochemistry analysis, and urinalysis results were assessed. On day 91, necropsy and detailed gross pathological evaluation were completed for all surviving animals except the control and high recovery groups, which completed the analyses at day 105. Histopathological examination was completed.

No unscheduled deaths were observed. No abnormal effects were found in the ophthalmological examination, detailed neurobiological examination, physical examination, home cage observation, handheld examination, open field observation, and sensory reactivity tests. However, paper biting was observed on all study days.

Body weight and body weight changes in the DHA groups were comparable to the water and vehicle controls during the 90-day treatment and the recovery periods. Food consumption

was increased in the corn oil and male DHA groups compared to the water control with no difference between the corn oil and male DHA groups. In females, transient differences in food consumption were observed in the corn and DHA groups compared to the water control. The differences in food consumption were resolved by week 9. Compared to the vehicle (corn oil) control, the difference in feed consumption was sporadic and observed only in the low-dose group at week 6.

No biologically significant differences among groups were observed in hematological measurements including WBC, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, platelets, mean platelet volume, prothrombin time, activated partial thromboplastin time, or in neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts (Table 17). Statistically significant hematological changes included small changes in RBC counts, hematocrit, neutrophil counts; however, these changes were not considered to be adverse because they were observed in one sex, resolved during the recovery period, and were not dose dependent.

No biologically significant differences among the groups were observed for clinical chemistry measurements including albumin, cholesterol, creatinine, glucose, total protein, triglycerides, chloride, sodium, potassium, gamma-glutamyl transferase, sorbitol dehydrogenase, calcium, urea, phosphorous, total bilirubin, globulin, and lactate dehydrogenase (Table 18). The small increases were noted in cholesterol and triglycerides in all DHA-rich oil doses for both sexes. Triglycerides for the female ARA-rich oil treated group remained slightly elevated after discontinuation of the treatment compared to the water control but equivalent to the corn oil control group (data not shown). These changes were considered to be related to the consumption of a high-fat diet and non-adverse, and were resolved by the end of the recovery period.

Small increases in ALP, ALT, and AST were reported (corn oil control vs. mid- vs. high-dose: males, ALP, 144 vs. 147 vs. 151 IU/L; ALT, 60 vs. 74 vs. 76 IU/L; AST, 106 vs. 113 vs. 115 IU/L; females, ALP, 142 vs/ 148 vs. 151 IU/L; ALT, 62 vs. 70 vs. 71 IU/L; AST, 108 vs. 115 vs. 112 IU/L; P values of all high- and mid-doses, <0.05 relative to corn control; Table18). However, the differences were small in magnitude, were resolved by the end of the recovery, and were not accompanied by histopathology. Increases in the concentrations of bilirubin, albumin, total protein, phosphorus, globulin, and lactate dehydrogenase were small in magnitude (corn oil control vs. high-dose: bilirubin, males, 0.31 vs 0.41, females, 0.26 vs. 0.34 mg/dL; albumin, males and females, 4.2-4.3 vs. 4.5; total protein, females, 6.5 vs. 6.8 mg/dL; phosphorus, males and females, 6.0-6.1 vs. 6.7-6.8 mg/dL; globulin, females, 3.8 vs. 3.9 g/dL; and lactate dehydrogenase, females, 76 vs. 83 IU/L). These differences were small in magnitude, occurred mostly in one sex, and were resolved during the recovery period. Thus, these increases were considered non-adverse.

No significant differences were found in most urine chemistry parameters compared to the controls. Differences in volume and specific gravity were observed in the DHA groups, and decreased pH was observed in the low-dose group compared to the water control (data not shown). These changes were resolved during the recovery period, not dose dependent, and were comparable to those found in the vehicle control group. Thus, the changes in urine chemistry were considered as non-adverse.

DHA treated animals had significant differences in the absolute weight of the adrenal gland and the absolute weight of the pituitary gland (Table 19). However, gross pathological analyses, physical parameters, microscopic examination, and organ weights were not different among the groups. No treatment-related gross pathological lesions were found. Histopathology analyzed the brain, thymus, spinal cord, sternum, heart, aorta, lungs, trachea, esophagus, liver, kidneys, adrenals, spleen, stomach, caecum, colon, duodenum, ileum, jejunum, rectum, epididymis, and ovary/testis. Non-specific histopathological changes were observed in some organs and were irrespective of the doses. Thus, the authors concluded that the DHA-rich oil did not induce pathological changes.

Taken together, the authors concluded that the NOAEL of Runke Bioengineering's DHA-rich oil was 5,000 mg/kg bw/day, the highest level tested.

Table 17. Hematology and Coagulation Parameters for Wistar Rats Administered DHA-Rich Oil for 90 Days

	Dose (mg/kg bw/day)				
	0 (water)	0 (corn)	1,000	2,500	5,000
Males					
RBC x 10^6 µL	7.7±0.4[b]	7.4±0.3[a]	7.5±0.4[a]	7.6±0.4[a]	7.6±0.4
HCT, %	41±3	43±4	45±5	45±3[a]	44±3
MCV, μm^3	54±3	54±3	56±2	55±3	56±3
Hgb, g/dL	15±1	15±1	15±1	15±1	15±1
MCH, pg	18±1	18±1	18±1	18±1	18±1
MCHC, g/dL	35±5	36±1	36±2	36±1	36±1
Platelets	952±50	963±69	972±73	980±75	985±57
MPV	54±2	55±2	55±2	55±2	55±2
WBC x 10^3 µL	8.6±1.1	8.5±1	8.7±1	8.8±0.9	8.9±0.9
Neutrophil	13±2[b]	12±2[a]	13±2[b]	14±2[b]	14±2[b]
Lymphocyte	84±2	83±2	83±2	84±2	84±2
Monocyte	2.2±1.0	2.7±0.9	2.4±0.9	2.5±1.0	2.6±1.0
Eosinophil	1.4±0.9	1.6±0.8	1.7±0.7	1.3±0.9	1.6±0.7
Basophil	0±0	0±0	0±0	0±0	0±0
PT	11±1	11±1	11±1	11±1	11±1
aPTT	16±1	16±1	16±1	16±1	16±1
Females					
RBC x 10^6 µL	7.5±0.3[b]	7.7±0.4[a]	7.5±0.4	7.6±0.3	7.5±0.4
HCT, %	44±3	44±3	45±3[a]	46±4[a]	46±4
MCV, μm^3	53±2	53±2	53±1	53±1	53±2
Hgb, g/dL	15±1	15±1	15±1	16±1	16±1
MCH, pg	18±1	18±1	18±1	18±1	18±1
MCHC, g/dL	35±1	36±2	36±2	37±2	37±1
Platelets	944±48	936±60	973±58	963±62	957±58
MPV	55±2	54±3	54±2	54±3	54±2
WBC x 10^3 µL	8.0±0.9	7.9±1.0	7.8±0.9	7.7±1.1	8.0±1.1
Neutrophil	11±3	12±2[a]	13±2[a]	12±2[a]	14±2[a]
Lymphocyte	83±2	82±2	83±2	83±1	84±2
Monocyte	2.5±0.9	2.2±1.1	2.2±1.0	2.1±1.0	2.2±1.0
Eosinophil	1.5±0.7	1.4±0.8	1.4±0.8	1.2±0.7	1.5±0.9
Basophil	0±0	0±0	0±0	0±0	0±0
PT	11±1	12±1	11±1	11±1	12±1
aPTT	16±1	16±1	16±1	16±1	16±1

Adopted from Lewis et al. (2016) Table 2. Values are mean±SD for group of 20 rats treated for 90 days prior to sacrifice. [a]$p<0.05$ vs water control; [b]$p<0.05$ vs vehicle control.
aPTT = activated partial thromboplastin time; bw = body weight; HCT = hematocrit; Hgb = hemoglobin; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; PT = prothrombin time; RBC = red blood cell; WBC = white blood cell.

Table 18. Blood Biochemistry for Wistar Rats Administered DHA-Rich Oil for 90 Days

	Dose (mg/kg bw/day)				
	0 (water)	0 (corn)	1,000	2,500	5,000
Males					
Glucose, mg/dL	113±6.6	114±7.9	113±6.3	114±5.8	114±6.2
Cholesterol, mg/dL	61±3.9	60±3.4	67±4.2[a,b]	70±3.7[a,b]	70±3.3[a,b]
Triglyceride, mg/dL	64±3.4[b]	60±4.5[a]	73±2.7[a,b]	76±2.8[a,b]	76±3.0[a,b]
ALT, IU/L	60±3.9	60±4.8	71±3.5[a,b]	74±3.1[a,b]	76±3.6[a,b]
AST, IU/L	107±3.6	106±4.2	109±5.7	113±6.1[a,b]	115±5.9[a,b]
ALP, IU/L	144±4.0	144±3.7	148±3.9[a,b]	147±4.6[b]	151±5.0[a,b]
SDHIU, /L	18±3.8	17±3.5	17±3.2	17±3.7	17±3.2
Calcium, mg/dL	14±1.2	14±1.3	14±1.6	14±0.9	15±1.1
Urea, mg/dL	16±1.4	15±1.0	16±1.8	17±1.7b	17±1.6[b]
Phosphorus, mg/dL	5.9±0.8	6.1±0.9	6.4±0.8	6.5±0.8[a]	6.8±0.6[a,b]
Albumin, g/dL	4.2±0.3	4.3±0.3	4.4±0.2	4.4±0.2	4.5±0.3[a]
Total protein, g/dL	6.8±0.4	6.7±0.4	6.6±0.3	7.0±0.4	7.0±0.5
Total bilirubin, mg/dL	0.33±0.10	0.31±0.10	0.40±0.20[b]	0.34±0.09	0.41±0.13[b]
Creatinine, mg/dL	0.46±0.2	0.40±0.2	0.46±0.16	0.38±0.15	0.39±0.18
Globulin, g/dL	3.9±0.7	4.2±0.6	3.7±0.6	3.9±0.7	4.2±0.60
LDH, IU/L	79±7.1	80±7.0	82±8.4	83±11.1	85±10.1
GGT, IU/L	0.16±0.06	0.16±0.06	0.14±0.07	0.14±0.07	0.15±0.06
Sodium, mmol/L	146±3.3	146±3.5	146±3.3	147±3.2	146±3.9
Potassium mmol/L	5.7±0.77	5.9±0.48	6.2±0.52	5.9±0.6	6.2±0.6
Chloride, mmol/L	104±1.6	104±1.3	105±1.2	104±1.7	104±1.4
Females					
Glucose, mg/dL	109±5.2	109±6.4	110±6.8	112±6.7	112±7.8
Cholesterol, mg/dL	58±5.3	60±2.8	67±3.6[a,b]	71±6.6[a,b]	70±3.3[a,b]
Triglyceride, mg/dL	61±3.7	62±3.4	72±2.1[a,b]	72±3.7[a,b]	73±4.2[a,b]
ALT, IU/L	57±4.6[b]	62±3.7[a]	66±3.6[a,b]	70±3.1[a,b]	71±4.2[a,b]
AST, IU/L	106±3.4	108±5.1	112±6.0[a]	115±7.3[a,b]	112±5.7[a]
ALP, IU/L	144±4.4	142±4.4	149±5.3[a,b]	148±5.9[a,b]	151±5.4[a,b]
SDHIU, /L	16±2.5	16±2.9	18±3.1	17±2.8	17±3.6
Calcium, mg/dL	13±1.2	13±1.3	13±1.5	13±1.4	15±0.8[a,b]
Urea, mg/dL	13±1.5	14±0.9	14±1.1	14±1.4	15±1.0[a]
Phosphorus, mg/dL	5.4±0.4	6.0±0.5	5.8±0.6	6.4±0.9[a]	6.7±0.8[a,b]
Albumin, g/dL	4.2±0.3	4.2±0.2	4.4±0.2[a]	4.2±0.3	4.5±0.2[a,b]
T. protein, g/dL	6.6±0.3	6.5±0.3	6.8±0.3[b]	6.7±0.3	6.8±0.5b
T. bilirubin, mg/dL	0.24±0.09	0.26±0.06	0.27±0.12	0.32±0.12	0.34±0.12[a,b]
Creatinine, mg/dL	0.40±0.13	0.36±0.12	0.42±0.15	0.44±0.15	0.39±0.14
Globulin, g/dL	4.3±0.4[b]	3.8±0.7[a]	4.6±0.4[b]	4.34±0.4[b]	3.9±0.8[b]
LDH, IU/L	74±7.6	76±9.0	82±7.6[a,b]	80±11	83±9.9[a]
GGT, IU/L	0.13±0.05	0.13±0.06	0.17±0.06	0.13±0.07	0.16±0.06
Sodium, mmol/L	145±3.4	146±3.3	147±3.7	147±3.2	146±3.4
Potassium mmol/L	5.7±0.5	5.7±0.4	5.9±0.4	5.9±0.4	5.7±0.4

Chloride, mmol/L	103±1.7	103±1.3	103±1.5	104±1.1	104±1.3

Adopted from Lewis et al. (2016) Table 4. Values are mean±SD. [a]p<0.05 vs water control; [b]p<0.05 vs. vehicle control.
ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; SDH = sorbitol dehydrogenase.

Table 19. Organ Weights for Wistar Rats Administered DHA-Rich Oil for 90 Days

	Dose (mg/kg bw/day)				
	0 (water)	0 (corn)	1,000	2,500	5,000
Males					
Brain	2.65±0.12	2.67±0.15	2.63±0.13	2.65±0.11	2.73±0.12
Adrenals	0.094±0.01	0.094±0.01	0.093±0.01	0.095±0.01	0.096±0.01
Pituitary	0.013±0.001	0.012±0.001	0.013±0.001	0.013±0.002	0.013±0.002
Prostate/S.V	1.78±0.10	1.79±0.10	1.51±0.08	1.50±0.08	1.48±0.08
Prostate/uterus	0.74±0.06	0.75±0.07	0.52±0.09	0.54±0.08	0.56±0.08
Testes/ovaries	4.24±0.14	4.20±0.11	4.20±0.12	4.20±0.13	4.19±0.13
Epididymis	1.96±0.09	1.93±0.06	1.90±0.06	1.9±0.06	1.93±0.05
Heart	1.56±0.11	1.49±0.14	1.28±0.11	1.30±0.10	1.39±0.11
Liver	12.7±0.50	12.7±0.88	12.3±0.73	11.9±1.12	12.33±0.98
Kidneys	2.75±0.17	2.76±0.13	2.66±0.19	2.56±0.18	2.52±0.26
Spleen	0.74±0.08	0.75±0.06	0.75±0.10	0.72±0.11	0.73±0.09
Thymus	0.48±0.19	0.49±0.10	0.33±0.08	0.32±0.08	0.45±0.09
Females					
Brain	2.21±0.12	2.18±0.13	2.16±0.12	2.16±0.17	2.12±0.15
Adrenals	0.057±0.01	0.068±0.01	0.064±0.01	0.067±0.01	0.069±0.009
Pituitary	0.012±0.001	0.012±0.001	0.12±0.002	0.012±0.001	0.012±0.001
Prostate/S.V.	-	-	-	-	-
Prostate/uterus	0.783±0.04	0.781±0.05	0.800±0.06	0.792±0.05	0.811±0.04
Testes/ovaries	0.279±0.02	0.288±0.01	0.289±0.01	0.284±0.02	0.280±0.02
Epididymis	-	-	-	-	-
Heart	0.92±0.29	0.98±0.07	0.85±0.39	1.00±0.09	1.00±0.233
Liver	9.2±0.78	9.4±0.70	9.5±0.56	9.6±0.51	9.6±0.51
Kidneys	1.53±0.08	1.56±0.06	1.56±0.06	1.55±0.05	1.58±0.09
Spleen	0.51±0.06	0.55±0.05	0.56±0.05	0.54±0.06	0.54±0.06
Thymus	0.51±0.05	0.49±0.05	0.50±0.05	0.50±0.05	0.51±0.05

Adopted from Lewis et al. (2016) Table 6. Values are mean±SD.
bw = body weight; S.V. = seminal vesicle.

Developmental Toxicity Study of Runke Bioengineering's DHA-Rich Oil

The developmental toxicity of a DHA-rich oil from *Schizochytrium* sp. was evaluated in rats (Falk et al., 2017). In the prenatal developmental toxicity study, healthy female Wistar rats (aged 6-7 weeks old) were randomly assigned to one of 5 dose groups: control (untreated), vehicle control (corn oil), 1,000, 2,500, or 5,000 mg/kg bw/day DHA-rich oil via oral gavage from day 6 to day 20 of gestation. Body weight was measured at 3-day intervals. Dosing of animals occurred sequentially in group order at close to the same time of day. There were no premature deaths of dams, clinical signs that were indicative of toxicity treatment-related changes in body weight, or differences in premating or lactation periods. There were no differences in food consumption, treatment-related lesions, or the weight of the reproductive organs among the DHA-rich oil and control groups.

Fetal Data

There were no significant differences between any DHA-rich oil dose groups and the control group for mean litter size, sex ratio, live birth index, weaning index, number of implantation sites, corpora lutea, and pre- and post-implantation loss (data not shown). No significant or dose dependent differences compared to the control were found for the external observations including fetal size, generalized arrested development, kinked tail, bent tail, bulged eyelid, microphthalmia, subcutaneous hemorrhage, or malformed head (Table 20).

Minor visceral anomalies observed in the high-dose group included dilated lateral ventricles in the brain, hemorrhagic foci in the liver, brownish discoloration of the lung, and small or absent renal papillae. The mid-dose group had dilated lateral brain ventricles, brownish discoloration around the cerebral hemisphere, small or absent dilated renal papillae, dilated renal pelvis, and brownish discoloration in the lung. The low-dose group exhibited Grade 2 dilated lateral ventricles in the brain with fragile and ruptured cerebral hemisphere, small or absent renal papillae, and dilated renal pelvis. The observed malformations in the DHA-rich oil groups were also found in the vehicle control with comparable frequencies (Table 20).

The DHA-rich oil groups showed no dose-dependent changes in the skeleton. In all DHA-rich oil and control groups, the incidences of supernumerary ribs (14^{th} pair, 14^{th} unilateral), rudimentary rib, wavy and bent ribs, few detached ribs, absent hyoid, ischium pubis, tympanic ring, widen fontanellae with holes in the parietal and inter parietal, misshapen and misaligned sternebrae, biolobed centra, and incomplete or delayed ossification in the cranial bones were all within historical control ranges. These changes were considered as spontaneous and incidental (Table 21).

Reproductive Toxicity Study of Runke Bioengineering's DHA-Rich Oil

Healthy Wistar rats (aged 6-7 weeks old) were randomly assigned to one of 5 dose groups (n=24/group): control (untreated), vehicle control (corn oil), 1,000, 2,500, or 5,000 mg/kg bw/day DHA-rich oil. The effect of DHA-rich oil on spermatogenesis were investigated by dosing male rats during the growth period and for a minimum of one complete spermatogenic cycle (84 days). To study the effects of treatment with DHA on the estrus cycle, female rats in the parent generation were dosed for two complete estrus cycles (14 days). One male per 2 female rats were cohabitated until all females became pregnant as evidenced by a sperm positive (E+) vaginal smear. Once a female rat gave a sperm positive smear, it was housed individually and the day on which this occurred was designated as gestation day 0. Dosing occurred for rats of both sexes during the mating period, during pregnancy for 22 days, and during the nursing and lactation period which lasted for 21 days.

Female rats were observed for signs of difficult or prolonged parturition. For each litter, the pups were examined for the number and sex of pups, the number of still and live births, and the presence of gross observations such as ear opening, eye opening, hair growth, tooth eruption, and gross anomalies. Physical and behavioral abnormalities in the dams were noted. In order to determine the length and pattern of the estrus cycle and to confirm sperm positive (E+ females), vaginal smears were performed for two weeks including before mating, during the gestation period, with care being taken to avoid disturbing the mucosa while acquiring vaginal/cervical cells. Clinical pathological analyses of animals were performed on day 15 and day 45 and before necropsy. The animals were fasted overnight for approximately 16 to 18 hours before being sacrificed. Blood samples were collected for clinical chemistry tests. Morbidity, mortality, body weight, food consumption, gross pathological examination, histopathological examination, clinical signs and symptoms, detailed clinical examination, and parturition were analyzed. Fetuses were examined for weight, sex, external malformations, abnormalities in soft tissues, and anatomical changes.

F0 generation

No treatment-related mortality was observed in the parental or pup generation during the course of the study. For the F0 generation, no significant differences in mean body weight were observed between control group and groups treated with DHA-rich oil. A slight increase in the body weight gain of male rats was observed from day 1 to day 64 (30-37%) for the mid-and high-dose groups. Gross necropsy of the animals in all treatment groups in the F0 generation revealed no external or internal abnormalities. No differences between the groups were observed during the pre-mating, mating, and lactation period.

Histopathological analysis of the corn oil and high-dose groups included testes, epididymides, seminal vesicles, prostate, and pituitary in males and uterus, ovary, cervix and

vagina, and pituitary in females. The only abnormality observed was polymorphonuclear cell infiltration of the uterus in one female in the high-dose group. There were no significant differences in absolute and relative organ weights as well as eye opening, ear opening, hair growth, or tooth eruptions between any of the experimental groups. No significant differences were observed among the groups for female fertility index, gestation index, fecundity index, estrus cycle length, or gestation period (Table 22) as well as mean litter size, sex ratio, live birth index, weaning index, number of implantation sites, corpora lutea, and pre- and post-implantation loss (data not shown).

F_1 Generation

For the pups, no treatment-related clinical signs were found (Table 23). In addition, no differences were noted among the groups for mortality, clinical signs, body weight or body weight gain. Male rats in the low-dose group had higher food consumption during weeks 5, 9, and 10 compared with the control group. During gestation, female rats in the low-and mid-dose groups had higher mean food consumption during days 4–6 and females in the high-dose group had higher mean food consumption during day 4–6 and days 13–15.

In addition, gross necropsy of the animals in all F1 generation groups revealed no abnormalities in external or internal changes. Pups that died prematurely had weakened body condition, cannibalized injuries on the neck, thoracic cavity, shoulder region, and neck and empty stomach (no milk). Red discoloration of the brain was associated with hemorrhage. Congestion, hemorrhage, and atelactasis were observed in the lungs. Injuries on the brain, thoracic cavity, and neck were associated with cannibalization. Liver pallor was noted in one animal in the low-dose group. None of these findings had a dose-related pattern and the number of findings was sparse. There were no significant differences in absolute and relative organ weights.

Taken together, the authors concluded that the NOAEL for maternal toxicity and embryo or fetal development and for paternal and maternal treatment-related reproductive toxicity was 5,000 mg/kg bw/day, the highest level tested.

Table 20. Changes in Fetal Development in the Prenatal Developmental Toxicity Study

	Untreated	Corn Oil	DHA LD	DHA MD	DHA HD
No. of fetuses (litters)	203 ± 22	186±22	269 ± 24	279 (24)	242 (24)
General external observations – Number (% of total)					
Smaller in size	2 (1.0%)	6 (3.2%)	2 (0.7%)	8 (2.9%)	-
Larger in size	3 (1.5%)	4 (2.2)	4 (1.5%)	-	9 (3.7%)
Generalized arrested development	1 (0.5%)	-	-	-	1 (0.4%)
Subcutaneous hemorrhage	-	-	3 (1.1%)	7 (2.5%)	4 (1.7%)
Number of fetuses	100	96	83	102	107
Minor Visceral Anomalies – Number (% of total)					
Dilated lateral ventricles brain	1 (1.0%)	2 (2.1%)	1 (1.2%)	6 (5.9%)	7 (6.5%)
Dilated and fragile ventricles brain	-	3 (3.1%)	-	-	1 (0.9%)
Dilated and fragile ventricles brain with dilated neural canal, small spinal cord	-	3 (3.1%)	-	-	-
Dilated lateral ventricles brain with fragile and ruptured cerebral hemisphere	-	-	3 (3.6%)	-	-
Brownish discoloration around cerebral hemisphere	-	-	1 (1.2%)	4 (4.0%)	-
Hemorrhagic foci – liver	1 (1.0%)	1 (1.1%)	1 (1.2%)	2 (1.9%)	4 (3.7%)
Subcutaneous hemorrhage	-	-	-	-	-
Yellowish perivascular areas liver	-	-	-	-	-
Small or absent renal papillae	4 (4.0%)	4 (4.4%)	5 (6.0%)	4 (4.0%)	4 (3.7%)
Brownish discoloration lung	3 (3.0%)	1 (1.1%)	1 (1.2%)	4 (3.9%)	2 (1.9%)
Common Variants					
Dilated renal pelvis	2 (2.0%)	6 (1.0%)	2 (1.2%)	2 (1.9%)	1 (0.9%)

Adopted from Falk et al. (2017).
HD = high-dose; LD = low-dose; MD = mid-dose.

Table 21. Summary of Major Malformations and Minor Skeletal Variations in the Prenatal Developmental Toxicity Study

	Untreated	Corn Oil	DHA LD	DHA MD	DHA HD
Number of pups	100	96	83	102	107
Major Malformations – Number (% of total)					
Cranial skeletal	15 (15%)	11 (11%)	12 (14%)	17 (17%)	14 (13%)
Ribs	5 (5%)	7 (7%)	6 (5%)	4 (4%)	4 (4%)
Vertebral	12 (12%)	26 (28%)	24 (21%)	18 (16%)	18 (16%)
Sternebrae	12 (12%)	26 (28%)	24 (21%)	18 (16%)	16 (16%)
Limbs	7 (7%)	7 (7%)	5 (4%)	8 (7%)	4 (4%)
Malformed head	1 (0.5%)	-	-	-	1 (0.4%)
Kinked tail	-	2 (1.1%)	3 (1.1%)	5 (1.8%)	-
Bent tail	1 (0.5%)	1 (0.5%)	2 (0.7%)	-	-
Bulged eyelid	2 (1.0%)	2 (1.1%)	-	6 (2.2%)	6 (2.5%)
Microphthalmia	-	1 (0.5%)	5 (1.9%)	1 (0.4%)	8 (3.3%)
Minor Skeletal Anomalies Delayed/Incomplete Ossification – Number (% of total)					
Cranial	38 (39%)	12 (13%)	27 (24%)	39 (35%)	27 (27%)
Sternebrae	2 (5%)	5 (5%)	1 (1%)	2 (2%)	4 (4%)
Ribs	1 (1%)	-	2 (2%)	2 (2%)	2 (2%)

Adopted from Falk et al. (2017).
HD = high-dose; LD = low-dose; MD = mid-dose.

Table 22. F_0 Fertility and Reproductive Performance in the Reproductive Toxicity Study

Fertility Indices	Corn Oil	DHA LD	DHA MD	DHA HD
No. of females	24	24	24	24
No. of mated females	24	24	24	24
No. of females littered (pregnant)	24	24	24	24
Female fertility index, %	100	100	100	100
Gestation index, %	100	100	100	100
Pregnancy/fecundity index, %	100	100	100	100
Premating group estrus cycle*	3.89±0.54	3.93±0.40	4.05±0.55	3.98±0.61
Gestation period*	21.67±0.56	21.17±0.82	21.58±0.72	21.33±0.76
Percent males	59.5	58.2	56.1	52.2
Pups delivered	245	219	255	232
Mean male pup weight day 0	5.74 ± 0.64	5.74 ± 0.60	5.63 ± 0.35	5.74 ± 0.55
Mean male pup weight day 22	34.58 ± 5.84	35.34 ± 5.30	33.47 ± 4.47	35.27 ± 5.08
Mean female pup weight day 0	5.45 ± 0.61	5.55 ±0.49	5.43 ± 0.29	5.50 ± 0.45
Mean female pup weight day 22	33.63 ± 5.71	35.36 ± 4.47	32.37 ± 5.59	34.76 ± 5.08

Adopted from Falk et al. (2017). *Mean days±SD
HD = high-dose; LD = low-dose; MD = mid-dose.

Table 23. Physical Observations and Gross Necropsy Findings of F_1 Newborn Pups in the Reproductive Toxicity Study

Physical Observations – Mean days ± SD	Corn Oil	DHA LD	DHA MD	DHA HD
Males				
Eye opening	13.90±0.89	13.52±1.13	13.24±1.05	13.08±0.95
Ear opening	15.68±1.36	15.83±0.88	15.69±1.01	15.46±1.05
Hair growth	6.04±0.97	6.04±1.14	5.49±1.09	5.43±1.08
Tooth eruption	11.75±1.04	11.86±0.94	12.04±0.90	11.79±0.82
Females				
Eye opening	14.36±0.89	13.56±1.08	13.50±1.27	13.46±0.90
Ear opening	16.1±0.94	15.09±0.85	15.93±1.76	16.02±0.85
Hair growth	6.37±0.96	6.30±1.2	5.88±1.16	5.85±0.98
Tooth eruption	11.96±1.12	11.65±0.92	12.07±1.0	12.04±0.87
Gross Necropsy Findings – Number of animals				
Pups	245	219	255	232
Dead	8	17	22	12
Cannibalism	19	13	14	12
Weak animal	0	2	0	0
Stomach: Empty, no milk	9	10	4	4
Lung: Atelactasis	0	4	0	0
Lung discoloration	0	2	0	0
Liver: Pallor	0	1	0	0
Brain: Red discoloration/ hemorrhage	0	0	3	0
Thoracic and shoulder region hemorrhage	0	0	1	0
Thoracic cavity blood clot	0	0	0	1
Neck region hemorrhage	0	0	0	0

Adopted from Falk et al. (2017). Mean days±SD
HD = high-dose; LD = low-dose; MD = mid-dose.

Studies of Other DHA-Rich Oil Ingredients from *Schizochytrium* sp.

In GRNs 000553 (stamped pages 37-47, 40-54), 000677 (pages 33-41), 000731 (pages 30-34), 000732 (pages 33-37), 000776 (pages 17-24), 000777 (pages 15-22), 000836 (pages 32-34, 38-45), 000843 (pages19-25), 000844 (pages 18-25), 000862 (pages 29-38), 000933 (page 34-40), 000934 (pages 35-44), and 001008 (pages 42-45), the safety of DHA-rich oil or DHA-rich microalgae (DRM) from *Schizochytrium* sp. was extensively reviewed. Therefore, this notice incorporates by reference the safety studies discussed in those GRAS notices and will not discuss previously reviewed references in detail.

Briefly, the NOAELs of other sources of DHA-rich oil and DRM are summarized as follows:

1) For DHA-rich oils, the NOAELs, established from subchronic toxicity studies, ranged from 3,149 to 5,000 mg/kg bw/day in rats (Fedorova-Dahms et al., 2011a; Lewis et al., 2016; Schmitt et al., 2012a). The LD_{50} was determined to be over 5 g/kg bw, the highest dose tested, in rats (Schmitt et al., 2012a).

2) From reproductive and developmental toxicity studies of DHA-rich oils, the NOAELs for F_0 were found to range from 2,000 (Schmitt et al., 2012b) to 8,322 mg/kg bw/day (F_0 females during lactation) in rats (Fedorova-Dahms et al., 2011b).

3) In subchronic toxicity studies with an *in utero* phase, the NOAELs for F_1 ranged from 2,069 (females - Schmitt et al., 2012b) to 4,399 mg/kg bw/day (females - Fedorova-Dahms et al., 2011b) in rats.

Studies of DRM from *Schizochytrium* sp.

1) For DRM, the highest dose tested was 5.746 kg DRM per pig, corresponding to 1.281 kg DHA per pig (DRM contained 22.3% DHA) (Abril et al., 2003). The DHA supplementation at all doses did not result in treatment-related adverse effects on measured outcomes such as clinical observations, body weights, food consumption, mortality, hematologic values, gross necropsy findings, organ weights, or histopathology in pigs. However, the authors did not provide the feed consumption or NOAEL on a kg bw/day basis. This level may correspond to roughly 297 mg DHA/kg bw/day.

   For a very rough estimate of DHA intake in mg/kg bw/day, the following calculation method was used. The abstract and page 79 stated that the total DHA administered during the last 42-day period was 1,281 g of DHA for pigs in the high-dose DRM groups. To calculate the average daily intake of DHA, we divided the total DHA administered to each pig (mg/pig) by 42. For T4, the high-dose group, we got 30,500 mg DHA/day. In the absence of average body weight during the last 42-day period, we assumed that the body weight gain was constant during the 120-day period. Based on the initial and final body weight values listed on Tables 5 to 6 in the article and the daily body weight gain shown in Table 7 in the article, we calculated the average body weight at day 79 for the T4 group. For example, body weight of T4 at day 79 was calculated using the following formula: (122.32 kg bw at day 120) – (42 days x 0.943 kg body weight gain/day) = 122.32 - 39.61 = 82.71 kg at day 79. To calculate the average body weight during the last 42 days, we took an average value between 82.71 and 122.32 kg, which is 102.515 kg bw. Then, we divided the average daily intake value of 30,500 mg DHA/day by 102.515 kg bw to derive 297.5 mg DHA/kg bw/day for the T4 group, the high-dose group.

However, because the authors did not provide feed consumption or NOAEL on a mg/kg bw basis, we did not present such a roughly estimated value in Table 16.

2) In the study by Hammond et al. (2001a), the authors reported that the NOAEL as 4,000 mg DRM/kg bw/day in rats and that DRM contained 8.7% DHA on a dry weight basis (page 193). The corresponding DHA level was calculated based on the following formula: *x* mg DRM x 0.087 (% DHA on a dry wt. basis) = *y* mg DHA. Thus, the corresponding DHA level is 348 mg/kg bw/day (4,000 x 0.087 = 348 mg DHA) on a dry weight basis.

3) In a subchronic toxicity study on another source of DRM, which contains 8.7% DHA on a dry weight basis (page 193), the authors reported the NOAEL as 4,000 mg DRM/kg bw/day in rats (Hammond et al., 2001a). The corresponding DHA level was calculated based on the following formula: *x* mg DRM x 0.087 (% DHA on a dry wt. basis) = *y* mg DHA. Thus, the corresponding DHA level is 348 mg/kg bw/day (4,000 x 0.087 = 348 mg DHA). Assuming a typical DHA-rich oil contains an average of 40% DHA, the corresponding DHA-rich oil level was obtained by dividing the DHA level by 0.4, which corresponds to 870 mg/kg bw/day of DHA-rich oil (*y* mg DHA/0.4 = *z* mg DHA-rich oil or 348 mg/0.4= 870 mg DHA-rich oil).

4) However, in a reproductive and developmental toxicity study in rabbits by Hammond et al. (2001b), both the high-dose (1,800 mg/kg/day) DRM and the fish oil control groups experienced marked and sustained reduction in food consumption during the prenatal period and a slight increase in abortions. In this the developmental toxicity of DRM in rabbits study, DRM was provided at levels of 180, 600, and 1800 mg/kg bw/day by oral gavage on GD 6–19. One female in the fish oil group and two females in the high-dose DRM group aborted on gestational days 23 and 25/26, respectively. The authors suggested that the presence of higher levels of dietary fat may have contributed to food intake reductions, leading to disruption of normal development and/or maintenance of pregnancy and abortions in these groups. Two of the three rabbits that aborted also had lower numbers of implantation sites (one to three per dam), although corpora lutea counts, which have an inverse association with an increased risk of abortion, were within normal limits. No other treatment-related abnormalities were observed in intrauterine growth, survival, or other developmental toxicity parameters at all dose levels. In summary, the NOAELs were determined to be 600 mg/kg bw/day for maternal toxicity and 1,800 mg/kg bw/day, the highest level tested, for developmental toxicity in rabbits. These levels correspond to 130 mg DHA-rich oil/kg bw/day for maternal toxicity and 392 mg DHA-rich oil/kg bw/day for developmental toxicity in rabbits. However, the authors noted that abortions occur spontaneously more frequently in rabbits than in other commonly used laboratory species and that the incidences of abortions in both the

high-dose DRM and the fish oil control groups fall within the historical limits for the laboratory.

It is noteworthy that the same DRM substance was well tolerated with no adverse effects in a reproductive and developmental toxicity study in rats conducted by the same research group (Hammond et al., 2001b). In this developmental toxicity of DRM in Sprague–Dawley rats, DRM was provided in the diet at 0.6, 6, and 30% on GD 6–15. In rats, the NOAEL was estimated to be 22,000 mg DRM/kg bw/day for both maternal and development toxicity. This level corresponds to 1,914 mg DHA/kg bw/day, assuming the DHA content in DRM was 8.7%.

5) In a single generation reproductive toxicity study, the NOAEL was estimated to be 17,847 and 20,669 mg DRM/kg bw/day for males and females, respectively (Hammond et al., 2001c). The authors stated that these levels of DRM intake correspond to an intake of approximately 1,512 and 1,680 mg/kg bw/day for DHA (page 358 of Hammond et al., 2001c).

Conclusion

The NOAEL of Runke Bioengineering's DHA-rich oil was determined to be 5,000 mg/kg bw/day from a single generation subchronic toxicity study in rats. However, for the purpose of the safety evaluation, the NOAEL was determined to be 2,069 mg/kg bw/day which was found in females from a subchronic systematic toxicity study with an *in utero* exposure in rats (Schmitt et al., 2012b).

6.B.4. Human Clinical Studies of DHA

All previous GRAS notices provided information/clinical study data that supported the safety of the proposed DHA ingredients for use in infant formula. In all the studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues in infants attributable to DHA-supplemented formulas when compared to the control infant formula group. Although these human clinical trials were not designed as safety studies, the absence of adverse effects provide some evidence of the safe use of DHA-rich oils.

A key concept in evaluating the safety of a substance is related to substantial equivalence. The 1996 joint consultation by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended that "if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (i.e., the food or food component can be concluded to be as safe as the conventional food or food component)" (Joint FAO/WHO, 1996).

Numerous GRAS notices have considered that DHA derived from algal oil is equivalent to that of fish oil. Thus, the GRAS panel convened by Runke Bioengineering also has considered that the FDA's 1997 final rule on menhaden oil is applicable to DHA-rich oils derived from *Schizochytrium* sp.

In addition, because DHA-rich oils derived from *C. cohnii* and *Schizochytrium* sp. have similar compositions and that the two types of algal DHA-rich oils were demonstrated to be bioequivalent (Fedorova-Dahms et al., 2014; Yeiser et al., 2016), the findings from the study of DHA-rich oils derived from *C. cohnii* may be pertinent when evaluating the safety of those derived from *Schizochytrium* sp. Thus, our review included the studies of DHA-rich oil derived from *C. cohnii* as corroborative data to support the safety of algal oil derived from *Schizochytrium* sp. for infant formula applications. In this review, it was assumed that unknown sources of algal DHA manufactured by Martek/DSM were derived from either *Schizochytrium* sp. or *C. cohnii.*

All the studies of algal DHA-rich oil reported no adverse events/effects on the measured outcomes (Tables 24 to 26). The DHA-rich oil in this GRAS determination has similar specifications compared to the those in the previous GRAS notices (Table 6), it is recognized that the information and data in those GRAS notices are pertinent to the safety of the DHA-rich oil in this GRAS determination. Therefore, this notice incorporates, by reference, the safety and metabolic studies discussed in the previous GRAS notices and will not discuss previously reviewed references in detail.

Studies of DHA in Adults

Since January 2021, no new studies of DHA from *Schizochytrium* sp. or algal sources have been published in adults. Previous GRAS notices reported that daily doses of up to 2 g DHA from algal sources were not associated with treatment-related adverse effects (Molfino et al., 2017, 2019; MacDonald and Sieving, 2018; Sanders et al., 2006; Smith et al., 2018) (GRN 933 pages 41 and 44; GRN 1008, pages 61-62).

The studies by Molfino et al. (2017, 2019) employed a daily dose of 2 g DHA derived from *Schizochytrium* sp. to assess DHA incorporation in RBC membranes and serum concentrations of epoxy-DHA, metabolites of the DHA in breast cancer patients and in healthy controls.

MacDonald and Sieving (2018) employed a daily dose of 2 g algal DHA for 3 months to assess measures of retina function, visual acuity, serum DHA concentrations, and adverse events. There were eight adverse events reported by four participants, and all eight events were considered not related to the DHA supplementation.

Overall, doses up to 2 g DHA/day were well tolerated with no side effects in adults (Molfino et al., 2017, 2019; MacDonald and Sieving, 2018).

Studies in Children

Since January 2021, no new studies of DHA were published in children. GRN 1008 included the study by Ingol et al. which was published in June 2019 (Table 24).

Briefly, Ingol et al. (2019) examined the effects of DHA and ARA on growth and adiposity in toddlers born pre-term. In a randomized, placebo-controlled trial, 377 children born at <35 weeks of gestation who were 10-16 months in corrected age (mean unadjusted age for prematurity of 17.3-17.4 months; mean adjusted age for prematurity of 15.6-15.7 months) were orally administered 200 mg/day algal DHA from *Schizochytrium* sp. and 200 mg/day fungal ARA from *Mortierella alpina* (Maretek Biosciences Corporation/DSM), or placebo (corn oil) for 180 days. Growth, adiposity, adherence, and adverse events were measured. A total of 683 adverse events were reported by 256 children; most reported adverse events were minor gastrointestinal illness and respiratory infections. The authors concluded that DHA supplementation had no effect on short-term growth or adiposity if it is implemented after the first year of life.

Studies of DHA in Pregnant Women and Offspring

Since January 2021, a few new studies of DHA derived from *Schizochytrium* sp. in pregnant women were published (Fougère et al., 2021; Garmendia et al., 2021) (Table 24).

Fougère et al. (2021) characterized the breast milk fatty acid profile among mothers who delivered very prematurely. From the Maternal Omega-3 Supplementation to Reduce Bronchopulmonary Dysplasia in Very Pre-term Infants (MOBYDIck) trial in neonatal intensive care units in Canada, 461 mothers (mean age of 31 years) of pre-term infants (before 29 weeks of gestation) were randomized within 72 h of delivery, to receive DHA rich-algae oil providing 1.2 g/day of DHA or a placebo (a mix of corn and soy oils) until their infant reached 36 weeks of postmenstrual age. Algal oil derived from *Schizochytrium* sp. contained 45% DHA, 19% n-6 DPA, and 17% palmitic acid while the major fatty acids in the placebo were 52% linoleic acid, 26% oleic acid, and 11% palmitic acid. Breast milk fatty acid composition was analyzed. No adverse effects were reported on the measured outcomes. The results demonstrated that DHA supplementation increased the DHA content of breast milk.

From the Maternal obesIty/overweight control throuGh Healthy nuTrition (MIGHT) study, Garmendia et al. (2021) evaluated the effects of DHA supplementation among 1002 obese and overweight pregnant women on metabolic control in mothers (18 years of age or older) and their offspring. Pregnant women were randomly allocated to one of the four parallel arms: 1)

Home-based dietary counseling +800 mg/day DHA (source, DHA-S: *Schizochytrium* sp., DSM); 2) 800 mg/day DHA only; 3) Home-based dietary counseling +200 mg/day DHA; 4) 200 mg/day DHA only. Intervention started from < 15 weeks of gestation until delivery. Measurements included the overall incidence of gestational diabetes mellitus, the incidence of macrosomia (birthweight >4000 g), and neonatal insulin resistance (cord blood Homeostasis Model Assessment for Insulin Resistance) and glucose concentrations. No adverse effects of DHA supplementation were reported on measured outcomes.

Overall, the review of recent human clinical trials is consistent with the conclusions of the previous GRAS notices (GRNs 000137, 000732, 000862, 000933, 000934, and 001008) that intake of DHA is safe as long as the daily intake does not exceed 1.5 g/person/day.

Table 24. Human Studies of DHA from Algal Sources in Children and Women during Pregnancy and/or through Postpartum*

Objective	Subject	Dose	Duration	Measurements	Reference
To examine the effects of supplementing toddlers born pre-term with DHA and ARA on growth and adiposity	377 children born pre-term (at <35 gestation) who were 10-16 mo in corrected age	2 groups: DHA (200 mg/d) (*Schizochy-trium* sp. source; Martek Biosciences Corp/DSM,) plus ARA (200 mg/d) or corn oil placebo	180 d	Growth and adiposity; adherence and adverse events Adverse events: Mainly minor gastrointestinal illness and respiratory infections; not treatment-related	Ingol et al., 2019
To characterize the breast milk fatty acid profile among mothers who delivered very prematurely after a neonatal DHA-rich algae oil supplementation	461 mothers who delivered before 29 wk of gestation from the MOBYDIck trial; mean age 30.9-31.1 y	2 groups: DHA rich-algae oil (1.2 g/d DHA; algal DHA from *Schizochytrium* sp., composed of 45% DHA, 19% n-6 DPA, and 17% palmitic acid) or placebo (corn and soy oils)	From <72 h after delivery until their infant reached 36 wk of postmenstrual age	Breast milk fatty acid composition	Fougère et al., 2021
To evaluate the effects of DHA supplementation among obese and overweight pregnant women on metabolic control in mothers and their offspring	100 obese or overweight pregnant women; a subsample of 226 newborns; Maternal obesIty/over-wei ght control throuGh Healthy nuTrition (MIGHT) study	4 groups: 1) Home-based dietary counseling + 800 mg/day DHA (source, *Schizochytrium* sp., DSM); 2) 800 mg/day DHA only; 3) Home-based dietary counseling +200 mg/day DHA; 4) 200 mg/day DHA only	From < 15 weeks of gestation until delivery	The overall incidence of gestational diabetes mellitus, the incidence of macrosomia, and cord blood Homeostasis Model Assessment for Insulin Resistance and glucose concentrations.	Garmendia et al., 2021

*Excluding studies of DHA from fish oil source or DHA-ethyl ether; ARA = arachidonic acid; d = day; DHA = docosahexaenoic acid; mo = months; MOBYDIck = Maternal Omega-3 Supplementation to Reduce Bronchopulmonary Dysplasia in Very Pre-term Infants.
None of these studies reported adverse effects of DHA on measured outcomes.

Studies of DHA in Term Infants

No studies published since January 2021 have been identified from the literature. However, this review includes a key term infant study as well as the published papers related to gastrointestinal tolerance and allergenicity of DHA-rich oils in term infants (Table 25).

A few studies employed DHA-rich oil from *C. cohnii* or fish oil sources to evaluate the efficacy and safety in term infants. Because it is not expected that safety profiles of DHA derived from fish oil and algal oil would be different, the findings from studies employing DHA from fish oil sources or *C. cohnii* are pertinent when evaluating the safety of DHA from algal oil. Thus, the findings from these studies of DHA from fish oils or *C. cohnii* were included as corroborative data to support the safety of DHA-rich oil derived from *Schizochytrium* sp.

Gastrointestinal Tolerance and Potential Allergy

Studies of term infants have not reported adverse events or adverse effects on allergies associated with DHA-supplemented infant formulas.

From the DHA Intake and Measurement of Neural Development (DIAMOND) study, Birch et al. (2010a) determined the effect of varying amounts of DHA supplementation on the visual acuity as well as visual acuity maturation, RBC fatty acids, tolerance, anthropometric measures, and adverse events of formula fed term infants at 12 months of age. In this study, 343 healthy term infants were randomized to 1 of 4 infant formulas with varying amounts of DHA (source, algal DHA oil derived from *Crypthecodinium cohnii*): 0% (control), 0.32%, 0.64%, or 0.96% of total fatty acids with the fixed amount of ARA (*M. alpina* source) at 0.64% of total FAs. The assigned formulas were fed from the time of enrollment (1 to 9 days of life) through age 52 weeks. Two hundred forty-four infants completed the study. The DHA levels correspond to daily intakes of up to 51 - 61 mg DHA/kg bw/day. The daily intake values of DHA were obtained based on the following assumptions: 1) infants consume about 100-120 kcal/kg bw/day; 2) 51 mg DHA/100 kcal was provided by the formula containing 0.96% DHA-rich oil (Colombo et al., 2017, page 3); and 3) infants consuming 100 kcal/kg bw/day will consume 51 mg DHA/kg bw/day (51 mg DHA/100 kcal x 100 kcal/kg bw/day=51 mg/kg bw/day), and those consuming 120 kcal/kg bw/day will consume 61 mg DHA/kg bw/day (51 mg DHA/100 kcal x 120 kcal/kg bw/day=61.2 mg/kg bw/day). DHA/ARA supplementation in the first year of life had no adverse effects on developmental outcome. No differences were observed in the proportions of infants with at least 1 adverse event or in the numbers with at least 1 serious adverse event in any of the 86 symptoms assessed, with the exception of watery eyes (increased only in the 0.64% DHA group; 0.64% DHA group vs. other 3 groups: 5% vs. 0 to 1%; $P<0.05$). The association between 1 case of sepsis in an infant in the 0.64% DHA group and the formula was not determined. The authors stated that infants tolerated all formulas well and had normal growth throughout the first 12 months of life.

From the same DIAMOND study, Birch et al. (2010b) investigated the effects of DHA (0.32-0.36% of total FAs) and ARA (0.64-0.72% of total FAs) the incidence of allergic and respiratory diseases through age 3 years in children fed DHA- and ARA-supplemented formula during the first 12 months of life. Blinded study nurses reviewed medical charts for upper respiratory infection, wheezing, asthma, bronchiolitis, bronchitis, allergic rhinitis, allergic conjunctivitis, otitis media, sinusitis, atopic dermatitis (AD), and urticaria. The authors concluded that DHA/ARA supplementation was not associated with incidence of upper respiratory infection and common allergic diseases up to 3 years of age.

The study by Burks et al. (2008) evaluated the DHA and ARA supplementation to an amino acid-based formula on overall growth, tolerance, and safety in 164 healthy term infants. Study 1 compared the effects on growth, tolerance, and safety in healthy infants of an amino acid-based formula (Nutramigen, Mead Johnson) to a control extensively hydrolyzed formula (casein based). Amino acid–based formulas are fed to infants who are highly sensitive to cow's milk and cannot be managed using extensively hydrolyzed formula. Both formulas were supplemented with DHA (0.32% of total fatty acids; 17 mg/100 kcal, source was not specified) and ARA (0.64% of total fatty acids; 34 mg/100 kcal). These levels were similar to those in human milk worldwide. The formulas were fed from 14 ± 2 through 120 ± 4 days of age. No differences were observed between the groups in the overall growth, formula acceptance, tolerance, and adverse events, in particular, the number of subjects who experienced at least 1 adverse event or the incidence of serious adverse events. However, two exceptions were noted: 1) parent-reported fussiness was lower in the control group ($P<0.05$) at age 90 days (data not shown) and 2) the incidence of diarrhea was significantly higher in the control group (control vs. test groups, 9 vs. 0 infants, $P<0.001$). The authors concluded that the amino acid-based formula supplemented with DHA and ARA at levels similar to those in human milk worldwide was hypoallergenic and safe in healthy term infants. The results of the same study were briefly reported in Vanderhoof (2008).

Study 2 (Burks et al., 2008) evaluated the hypo-allergenicity of the amino acid-based formula containing DHA and ARA in 32 infants and children (8 months to 10 years of age) with hypersensitivity to cow's milk. Any indication of allergy (extent and severity of rash, pruritus, or urticaria/angioedema; upper or lower respiratory symptoms; or gastrointestinal symptoms) and adverse events were assessed throughout the double-blind, placebo-controlled food challenge and open challenges. If the open challenge response was also negative, an extended observation was followed in a 7-day home feeding period during which the child's parent or guardian kept a daily diary of acceptance and tolerance measures and any adverse events were monitored. Of the 32 subjects, 29 completed both double-blind, placebo-controlled food challenge and open challenge. Ongoing allergic manifestations were noted in 24 of 29 subjects at study entry. Allergic gastrointestinal manifestations included allergic enterocolitis, esophagitis, and gastroesophageal reflux. All the 29 children were fed formulas in randomized order after a

pre-challenge elimination period, followed by an open challenge if the response to the food challenge was negative. As determined by daily parental record, acceptance and tolerance of the new amino acid-based formula were generally good. No serious adverse events occurred during the double-blind food challenge, open challenge, or extended 7-day feeding period on the amino acid-based formula and the subsequent open challenge reported no serious adverse events demonstrating the hypo-allergenicity of the formula containing DHA.

In a study by Hoffman et al. (2008), 244 healthy term infants received one of 2 formulas: (1) control, soy formula without supplementation (Enfamil ProSobee1, Mead Johnson & Company, IN) or (2) DHA + ARA, soy formula supplemented with a minimum of 17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal from fungal oil (Enfamil ProSobee1 LIPIL1, Mead Johnson & Company, IN), from 14 to 120 days of age. These levels correspond to approximately 0.3% of total fatty acids as DHA and 0.6% of total fatty acids as ARA. Of the 244 infants enrolled, 182 infants completed the study. Measurements included anthropometric measurements, atopic dermatitis, tolerance, and adverse events. The incidence of adverse events, formula intake, stool frequency and characteristics, and parental assessment of fussiness, diarrhea, and constipation were comparable between the groups. In addition, no statistically significant difference was noted in the atopic dermatitis scores, as assessed by mean SCORing Atopic Dermatitis (SCORAD) indices at 120 days of age between the 2 groups (control, 2.9 ± 0.76; test, 2.3 ± 0.72), indicating a very low occurrence of atopic dermatitis. The only differences noted were higher gastrointestinal reflux (control vs. test: 12 vs. 3 infants, $P = 0.009$) and the incidence of excessive gas (15% vs. 5%, $P = 0.026$) which were noted more in the control group than in the test group at 60 days of age. In the subset infants, no statistically significant differences were noted in blood chemistry profiles (total RBC lipids and plasma phospholipids, glucose, and kidney, liver, and pancreas function markers) between the 2 groups at 14 or 120 days of age (data not shown). The authors concluded that both formulas were well tolerated and supported normal growth.

In the study by Fleddermann et al. (2014), 213 healthy term infants were randomized to receive 1 of 2 isoenergetic formulas: a test formula containing DHA (10.7 mg/100 kcal from egg and fish oil), ARA (10.7 mg/100 kcal), and alpha-lactalbumin, or a control formula with standard whey and no DHA and ARA from less than the first 28 days to 120 days of life. Breast-fed infants served as the reference group. Both formulas were well-accepted, and no differences were reported for stool consistency and color, colic, flatulence, and regurgitation or vomiting. The number of serious adverse events was higher in the test group than in the control group (10.2 vs. 3.3%), with 1 serious adverse event in each formula group considered as a potential association to the study formula (test formula: vomiting, blood in stool, and reflux; control formula: vomiting and blood in stool). However, the total number of adverse events (adverse event plus serious adverse event) was much lower in the test formula and reference groups than the control formula group (test vs. reference vs. control: 24% vs. 24% vs. 45%). The types of

adverse events were similarly distributed across the test and control groups. The authors concluded that all infants accepted the test formula supplemented with DHA and ARA well and that no adverse effects were found for all parameters tested.

In the Infant Fish Oil Supplementation study, healthy term infants of 420 allergic women were randomized to daily fish oil capsules (providing 0.280 g DHA + 0.110 g EPA) or placebo capsules (olive oil) from birth to 6 months (D'Vaz et al., 2012). Because of the supply issue, the final 27 children received similar capsules of fish oil (250 mg DHA and 60 mg EPA) or olive oil. A clinical follow-up was completed in 323 infants at 12 months of age. Measurements included PUFA concentrations in erythrocytes and plasma in infants at 6 months of age and those in their mothers' breast milk at 3 and 6 months. In addition, clinical outcomes, such as eczema, food allergy, asthma, and sensitization, were monitored in 323 infants at the 12 month-follow up. No statistically significant differences were noted in the prevalence of allergic outcomes (any allergic disease, overall sensitization, specific sensitization, eczema, or food allergy) between the 2 study groups at 12 months of age. None of the children had a diagnosis of asthma by 12 months of age. There were no significant differences in recurrent wheeze or persistent coughing between the study groups at 6 or 12 months. The supplementation of DHA from fish oil did not impact the allergy parameters at 6 and/or12 months in term infants.

Taken together, DHA supplementation did not result in any serious or non-serious adverse events, tolerance, food allergies, or other allergies in term infants consuming non-exempt infant formula. In addition, GRNs 000553 (pages 55-57; FDA, 2015), 000677 (pages 29-33; FDA, 2017), 000731 (pages 35, 37-38; FDA, 2018a), 000776 (pages 24-25; FDA, 2018c), 000777 (pages 22-24; FDA, 2018d), 000862 (pages 40-43; FDA, 2020a), 000933 (pages 42-43, 47; FDA, 2020b), 000934 (pages 45-53; FDA, 2021), and 001008 (pages 59-60; FDA, 2022) presented comprehensive summaries of clinical study literature regarding supplementation of DHA from algal oil sources to term infant formula. These GRAS notices concluded that supplementation of DHA (from algal sources), in combination with ARA, to infant formula was safe in term infants. Overall, algal DHA, up to 0.96% of total fatty acids (or up to 51-61 mg DHA/kg bw/day), in combination with ARA (0.64% of fatty acids) was well tolerated with no side effects in term infants.

Overall Conclusion for Infant Formula Applications for Term Infants

In summary, algal DHA, up to 0.96% of total fatty acids (or up to 51-61 mg DHA/kg bw/day), in combination with ARA was well tolerated, and no adverse effects were noted on the measured outcomes including gastrointestinal tolerance, adverse events, growth, RBC concentrations of fatty acids, visual acuity, cognitive function, and/or school readiness in both pre-term and term infants. Thus, it is concluded that the literature supports the intended use of DHA at 0.5% of total fatty acids in term infants.

Table 25. Human Studies of DHA from Algal Sources in Term Infants*

Objective	Subject	Dose	Duration	Measurements	Reference
To determine the effect of varying amounts of DHA supplementation on visual acuity, growth, safety, and clinical chemistry parameters	343 term infants	DIAMOND study: 3 concentrations of DHA (derived from *C. cohnii*): 0.32, 0.64, or 0.96% of fatty acids as DHA (or 0, 17, 34, or 51 mg DHA/100 kcal) with a fixed conc. of 0.64% ARA (or 34 mg ARA/100 kcal; from *M. alpina*); or control – unsupplemented cow-based formula	From the time of enrollment (1 to 9 days of life) through age 52 weeks	Visual acuity, visual acuity maturation, red blood cell fatty acids, tolerance, anthropometric measures over the 52-week period	Birch et al. (2010a)
To determine the effect of varying amounts of DHA supplementation on allergic reactions	179 term infants	DIAMOND study; DHA/ARA supplemented formula (DHA, 0.32-0.34% DHA/ARA, 0.64-0.72% of FAs) vs. unsupplemented formula	From the time of enrollment (1 to 9 days of life) through age 52 weeks; follow-up up to 3 y of age	Incidence of upper respiratory infection and common allergic diseases up to 3 years of age	Birch et al. (2010b)
To determine the effects on growth, tolerance, and safety in healthy infants of an amino acid-based formula	164 healthy term infants	DHA (0.32% of total FAs; 17 mg/100 kcal, source was not specified) and ARA (0.64% of total FAs; 34 mg/100 kcal)	From 14 ± 2 through 120 ± 4 days of age	Growth, formula acceptance, tolerance, and adverse events	Burks et al. (2008)
To evaluate the hypo-allergenicity of the	32 infants and children		Double-blind and open	Allergy (extent and severity of rash, pruritus, or	

amino acid-based formula containing DHA and ARA	with hyper-sensitivity to cow's milk		challenges, followed by a 7-day home feeding period	urticaria/angioedema; upper or lower respiratory symptoms; or gastrointestinal symptoms); and adverse events	
To evaluate the DHA+ARA supplementation on growth, atopic dermatitis, tolerance, and adverse events in term infants	244 term infants	Control, soy formula with without supplementation of DHA + ARA (17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal from fungal oil)	From 14 to 120 days of age	Anthropometric measurements, atopic dermatitis, gastrointestinal tolerance, and adverse events in all infants; clinical chemistry parameters in subset infants	Hoffman et al. (2008)
To assess the effect of a modified infant formula on growth and safety	213 healthy term infants	A test formula containing DHA (10.7 mg/100 kcal from egg and fish oil), ARA (10.7 mg/100 kcal), and alpha-lactalbumin, or a control formula	From less than the first 28 days to 120 days of life	Growth, gastrointestinal tolerance, and adverse events	Fledder-mann et al. (2014)
To assess the effect of fish oil supplementation on PUFA concentrations in erythrocytes and plasma in infants 6 months of age and allergy parameters	Healthy term infants of 420 allergic women	Fish oil capsules (providing 25 - 28 mg DHA + 60 - 110 mg EPA) or placebo capsules (olive oil)	From birth to 6 months; follow-up at 12 mo of age	PUFA concentrations in erythrocytes and plasma in infants 6 months of age; clinical outcomes such as eczema, food allergy, asthma, and sensitization	D'Vaz et al. (2012)

*Excluding studies of DHA from fish oil source or DHA-ethyl ether;
ARA = arachidonic acid; DHA = docosahexaenoic acid; DIAMOND study =DHA Intake And Measurement of Neural Development study
IQ = intelligence quotient; mo = months; y = years.
None of these studies reported adverse effects of DHA on measured outcomes.

Studies of DHA in Pre-term Infants

This review includes studies published until May 2023 and the papers related to gastrointestinal tolerance and allergenicity of DHA-rich oils in pre-term infants (Table 26).

Recently Published Studies (January 2021 – May 2023)

Frost et al. (2021) determined the feasibility of providing a concentrated emulsified DHA-ARA supplement to 30 very low birth weight infants and evaluated blood LCPUFA concentrations at 2 and 8 weeks. In this prospective, randomized, double-blind, placebo-controlled trial in neonatal intensive care units, 192 very low birth weight infants with a mean birth weight of 1,040 g (mean gestational age of 28 weeks) received 1 of the following 3 treatments for 8 weeks or until discharged, whichever came first: a placebo control supplement containing sunflower oil, supplements containing 40 mg/kg bw/day DHA (source, manufacturer, and country not specified) and 80 mg/kg bw/day ARA, or supplements providing 120 mg/kg bw/day DHA and 240 mg/kg bw/day ARA. Whole blood LCPUFA levels were measured. No adverse effects were reported on the measured outcomes.

Hewawasam et al. (2021) determined whether DHA supplementation in pre-term infants improves attention at 18 months' corrected age. This follow-up study was conducted from the N-3 (omega-3) Fatty Acids for Improvement in Respiratory Outcomes (N3RO) trial (Collins et al., 2017) conducted at neonatal centers in Australia, New Zealand, and Singapore. A total of 192 pre-term infants with 15-30 months' corrected age from the trial in South Australia (mean age of 3.0-3.5 days) received an enteral emulsion of 60 mg/kg bw/day DHA from tuna oil (manufacturer and country not specified) or control (soya oil) from within the first days of birth until 36 weeks postmenstrual age. Assessments of attention, cognition, language, and motor development were completed. No adverse effects were reported on the measured outcomes.

In a double-blind parallel clinical trial by Bernabe-García et al. (2021), 225 pre-term newborns (birth weight 1000- 1500 g) with an expected functional gastrointestinal tract were recruited and received an enteral dose of 75 mg of DHA/kg bw (source, DSM, algal type, not specified) diluted in high-oleic sunflower oil as a vehicle or high-oleic sunflower oil (control) daily for 14 days from the first enteral feed after birth. Primary endpoint was the incidence of necrotizing enterocolitis (NEC), an inflammatory bowel disease based on Bell's scale from stage IIa and IIb. No adverse effects of DHA on the measured outcome were reported. In addition, adverse events (apart from the incidence of NEC; including death, median platelet counts, bleeding events such as periventricular /intraventricular hemorrhage grade≥II and upper gastrointestinal tract and /or pulmonary bleeding) and fatty acid profile of erythrocyte membranes from pre-term infants were not different between groups although alpha-linolenic acid was higher in the DHA-group. Thus, it is concluded that supplementation of DHA at a daily dose of 75 mg of DHA/kg bw did not result in adverse effects on the incidence of NEC and fatty acid profile of erythrocyte membranes from pre-term infants.

Effects of DHA on Gastrointestinal Adverse Events or Food Allergy

A few pre-term infant studies specifically discussed the effects of DHA supplementation on gastrointestinal adverse events or food allergy. These studies did not report adverse effects or events associated with DHA supplementation in pre-term infants (Clandinin et al., 2005; Manley et al., 2011). The studies by Gunaratne et al. (2019) and Manley et al. (2011) employed DHA from fish oil sources to evaluate allergy parameters in pre-term infants. As it is not expected that safety profiles of DHA derived from fish oil and algal oil would be different, the findings from studies employing DHA from fish oil sources are pertinent when evaluating the safety of DHA from algal oil. Thus, the findings from these 2 studies of DHA from fish oils were included as corroborative data to support the safety of algal DHA oil derived from *Schizochytrium* sp.

In an Australian DHA for the Improvement of Neurodevelopmental Outcome (DINO) trial, Manley et al. (2011) evaluated the effect of DHA (fish oil source) supplementation on long-term atopic and respiratory outcomes in 657 pre-term infants of <33 weeks of gestation. They consumed expressed breast milk from mothers taking either tuna oil with high-DHA (tuna oil) or standard-DHA (soy oil) capsules. Lactating women with their infants were randomly assigned to the high-DHA group (3 g tuna oil per day) or the standard-DHA group (3 g soy oil per day) to achieve a breast milk DHA concentration that was 1% or 0.35% of total fatty acids without altering the naturally occurring concentration of ARA in breast milk. If supplementary formula was required, infants were given a high-DHA pre-term formula (1% fatty acids [FAs] as DHA and 0.6% FAs as ARA) or a standard pre-term infant formula (0.35% DHA and 0.6% ARA). The intervention in both groups continued until infants reached their expected date of delivery. Median duration of treatment was 9.4 weeks. The primary objective of the DINO trial was to determine the effect of meeting the estimated DHA requirement of pre-term infants on neurodevelopment. However, this study reported secondary outcomes, such as allergic (hay fever, eczema, asthma, or food allergy) and respiratory parameters (including the incidence of bronchopulmonary dysplasia) over the first 18 months' corrected age. No adverse effects of high-DHA supplementation (1% of total fatty acids) were noted on the measured outcomes including requirement for special diet for food allergy in pre-term infants of <33 weeks of gestation.

From the DINO study described above, Gunaratne et al. (2019) tested the efficacy and the safety of DHA from fish oil on allergy parameters. Primary endpoints were parent-reported incidence of respiratory allergic disease symptoms including wheeze and rhinitis at 7 years corrected age and other outcomes included the incidence of eczema symptoms, severity of any symptoms, and the incidence of wheeze, rhinitis, rhinoconjunctivitis, and eczema from birth to 7 years corrected age. Data were available for 569 of 657 children originally randomized. No adverse effects were reported on the measured outcomes.

In a study by Clandinin et al. (2005), 361 pre-term infants of < 35 postmenstrual age were randomly assigned to 3 study formula groups: 1) control, formula with no added DHA or ARA; (2) algal-DHA, formula with 17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal from fungal oil (Martek Biosciences, algal type was not specified); or (3) fish-DHA, formula with 17 mg DHA/100 kcal from tuna fish and 34 mg ARA/100 kcal from fungal oil. These levels of DHA and ARA were similar to those present in a typical mature human milk (approximately 0.3 wt% of fatty acids as DHA and 0.6 wt% as ARA). The study formulas were the sole source of nutrition for the pre-term subjects until 57 weeks postmenstrual age (or 4 months after term) and the primary source of nutrition until 92 weeks postmenstrual age. DHA supplementation was stopped at 92 weeks postmenstrual age, and the subjects were monitored until 118 weeks postmenstrual age (18 months after term). Term infants breast-fed for 4 months or longer were the reference group. All infants were assessed at birth and at 40, 44, 48, 53, 57, 66, 79, 92, and 118 weeks postmenstrual age. Measurement endpoints included growth, tolerance, adverse events, and Bayley development scores. There were no differences in caloric intake from the formula, daily gastric residuals, stool frequency and consistency, or abdominal distention among the pre-term groups during hospitalization (data not shown). In addition, there were no differences in parents reporting fussiness, diarrhea, or constipation (data not shown), although infants in the algal DHA and fish DHA-supplemented groups had more gas than usual at 40 and 44 weeks postmenstrual age ($p<0.05$), which reached no differences at 53 or 57 weeks. Overall, the authors concluded that DHA supplementation (either algal oil or fish oil source) did not increase morbidity or adverse events in pre-term infants. In addition, no adverse effects of DHA supplementation were reported on the measured outcomes.

In addition, GRNs 000553 (stamped pages 55-57), 000677 (pages 29-32), 000731 (pages 35-36, 39-40), 000862 (pages 42-43), 000933 (page 43), and 001008 (pages 46-58) presented comprehensive summaries of clinical study literature regarding supplementation of DHA from algal oil sources to pre-term infant formula. These GRAS notices concluded that supplementation of DHA (from *Schizochytrium* sp.), in combination with ARA, to infant formula was safe in pre-term infants. In particular, previous GRAS notices reviewed the studies by Almaas et al. (2015, 2016) that tested the hypothesis that DHA/ARA supplementation in very low birth weight infants would influence cerebral white matter measured by diffusion tensor imaging and behavioral and cognitive outcomes at 8 years of age. In these studies, human milk supplemented with 32 mg DHA (0.86% of total fatty acids as DHA; source not specified) and 31 mg ARA (0.91% of total fatty acids) per 100 mL was fed to pre-term infants each day for 9 weeks after birth with an 8-year follow-up. A recently published study (Bernabe-García et al., 2021) confirmed that supplementation of algal DHA (algae type, not specified) at 75 mg of DHA/kg bw/day (may correspond to 1.3% of total fatty acids as DHA) did not result in adverse effects in pre-term newborns with an expected functional gastrointestinal tract.

From a meta-analysis of 4 RCTs from five reports (1,966 neonates), Tanaka et al. (2022) reported that DHA supplementation did not increase the risk of BPD at 36 weeks of postmenstrual age among pre-term infants and the risk of other neonatal morbidities including death, necrotizing enterocols, intraventricular hemorrhage, severe retinopathy of prematurity, or sepsis.

In summary, DHA-rich oil derived from *Schizochytrium* sp. at the use level of up to 1.0 -1.3% of total fatty acids as DHA is not expected to adversely impact the pre-term infants who would be consuming these exempt infant formulas.

Table 26. Human Studies of DHA in Pre-Term Infants*

Objective	Subject	Dose	Duration	Measurements	Reference
To determine feasibility of providing a concentrated emulsified LCPUFA supplement to very low birthweight infants and to evaluate blood LCPUFA concentrations at 2 and 8 weeks of study supplementation	30 very low birthweight infants; mean birthweight 1,040 g; mean gestational age 28 wk	LCPUFA-120 (40 mg/kg bw/d DHA + 80 mg/kg bw/d ARA); LCPUFA-360 (120 mg/kg bw/d DHA + 240 mg/kg bw/d ARA) (DHA source not specified); placebo (sunflower oil)	8 wk or until discharge	Whole blood LCPUFA levels	Frost et al., 2021
To determine whether DHA supplementation in infants born pre-term improves attention at 18 months' corrected age	192 infants born <29 gestational wk within 3 d of first enteral feeding who participated in the N3RO trial; mean birthweight 905.3-927.8 g; mean age at randomization 3.0-3.5 d	DHA (60 mg/kg bw/d DHA) (DHA source not specified); control (soya-oil)	Until 36 wk of postmenstrual age	Attention assessment; assessments of cognition, language, and motor development	Hewawasam et al., 2021
To evaluate the efficacy of the enteral DHA to prevent NEC in pre-term infants	225 Pre-term infants with birth weight of 1,000 to 1,499 g	75 mg of algal DHA/kg/d or high oleic sunflower oil (control)	14 days	The incidence of NEC, adverse events, erythrocytes fatty acid profile	Bernabe-García et al., 2021
To determine the effect of meeting the estimated DHA requirement of pre-term	DINO trial, 657 pre-term infants of <33 weeks of gestation	High-DHA pre-term formula (1% DHA from fish oil and 0.6%	Until infants reached their expected date of delivery; FU at	Allergic (hay fever, eczema, asthma, or food allergy) and respiratory parameters (including the incidence of	Manley et al., 2011

infants on allergic and/or respiratory parameters		ARA) or a standard pre-term infant formula (0.35% DHA and 0.6% ARA).	12 and 18 mo	bronchopulmonary dysplasia)	
			Until infants reached their expected date of delivery; FU at 7 y CA	Incidence of eczema symptoms, severity of any symptoms, and the incidence of wheeze, rhinitis, rhinoconjunctivitis, and eczema	Gunaratne et al., 2019
To evaluate safety and benefits of feeding pre-term infants formulas containing docosahexaenoic acid (DHA) and arachidonic acid (ARA) until 92 weeks postmenstrual age (PMA), with follow-up to 118 weeks PMA	361 pre-term infants of < 35 postmenstrual age	Control formula with no added DHA or ARA; (2) algal-DHA formula with 17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal, or (3) fish-DHA formula with 17 mg DHA/100 kcal from tuna fish and 34 mg ARA/100 kcal. Reference group-term infant breast milk fed (~ 0.3 wt% of FAs as DHA and 0.6 wt% as ARA)	Intervention until 92 weeks postmenstrual age; FU until 118 weeks postmenstrual age; Reference group for ≥4 months starting between birth and 4 weeks of age	Growth, gastrointestinal tolerance, adverse events, and Bayley development scores	Clandinin et al., 2005

*Recently published studies or the studies related to gastrointestinal adverse events or food allergy only are summarized.
ARA = arachidonic acid; CA= corrected age; d = days; DHA = docosahexaenoic acid; DINO = DHA for the Improvement of Neurodevelopmental Outcome trial, EPA = eicosapentaenoic acid; FU = follow up; LCPUFA = long-chain polyunsaturated fatty acid; N3RO = N-3 (omega-3) Fatty Acids for Improvement in Respiratory Outcomes; wk = weeks; y = year.

6.B.5. Potential Adverse Effects

The FDA raised concerns about the consumption of high levels of EPA and DHA, which may increase bleeding time, increase levels of LDL-C, and influence glycemic control in participants with type 2 diabetes (menhaden oil final rule; 62 FR 30751; June 5, 1997). To assure that the combined exposure to EPA and DHA would not exceed 3 g/person/day, the FDA established the maximum levels of use for menhaden oil that would be permitted in specified food categories [21 CFR 184.1472(a)(3)]. No studies on type 2 diabetics have reported increased glucose levels in plasma when higher amounts (4.5 to 6.9 g/person/day) of omega-3 fatty acids were ingested (Bucher et al., 2002; Buckley et al., 2004). Overall, our review of human clinical trials supports the ADI of 1.5 g/person/day for DHA in adults.

No adverse effects of DHA in infant formula up to 0.96% of total fatty acids (51-61 mg DHA/kg bw/day) were reported.

Safety of Sterols

Safety of sterols present in Runke Bioengineering's DHA-rich oil can be justified from the two aspects: 1) animal safety studies and 2) EDIs of sterols under the intended use relative to total sterols already consumed via the diet.

Animal Safety Studies

Chen et al. (2014) reported that supplementation of sterol extract from *Schizochytrium* sp. source at a dose of 0.30 g/kg diet for 5 weeks did not result in adverse effects on lipid metabolism as measured by plasma total cholesterol as well as activities of intestinal acyl-CoA:cholesterol acyltransferase 2 (ACAT2) and hepatic 3-hydroxy-3-methylglutaryl -CoA (HMG-CoA) reductase in male golden hamsters. In other words, no adverse effects of sterol extract derived from *Schizochytrium* sp. were reported on measured outcomes. More importantly, a subchronic 90-day oral toxicity and a developmental and reproductive toxicity study of Runke Bioengineering's DHA-rich oil did not find any adverse effects on safety parameters in rats and the no observed adverse effect level (NOAEL) was determined to be 5,000 mg/kg bw/day, the highest level tested (Falk et al., 2017; Lewis et al., 2016). Thus, the sterols present in the Runke Bioengineering's DHA-rich oil are not expected to pose safety concerns.

6.C. Safety Determination

Numerous human and animal studies have reported health benefits of DHA with no major adverse effects. There is broad-based and widely disseminated knowledge concerning the chemistry of the DHA-rich oil. This GRAS determination is based on the data and information generally available and consented opinion about the safety of DHA.

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

1. Analytical data from multiple lots indicate that the DHA-rich oil reliably complies with established specifications and meets all applicable purity standards. Its purity is over 35.0% DHA. No significant amounts of domoic acid, MCPDs, glycidyl esters, and other contaminants have been detected from Runke Bioengineering's DHA-rich oil.

2. As the DHA-rich oil in this GRAS notice has similar specifications and composition to those described in previous FDA GRAS notices, it is concluded that Runke Bioengineering's DHA-rich oil is substantially chemically equivalent to those described in GRNs 000137, 000553, 000731, and in particular to those described in GRN 000677. Thus, the information and data presented or reviewed in the GRN notices are pertinent when evaluating the safety of the DHA-rich oil in this GRAS notice. As noted above, the FDA did not question the safety of DHA-rich oil for the specified food uses in response to GRAS notifications on DHA-rich oil derived from *Schizochytrium* sp.

3. Runke Bioengineering's DHA-rich oil will be added to the same food categories as those currently listed in 21 CFR 184.1472(a)(3) (menhaden oil), excluding egg, meat, poultry, and fish products, at maximum use levels that are 28.57% of those specified in that regulation. Based on the final rule on menhaden oil described in 21 CFR 184.1472(a)(3), the ADI for DHA has been established as 1.5 g/person/day. In addition, algal DHA-rich oils derived from *Schizochytrium* sp. (GRNs 137 and 732) received FDA GRAS notice status to result in a maximum dietary exposure of less than 1.5 g of DHA per day. Furthermore, historical consumption of DHA supports the safety of DHA as long as the consumption level does not exceed 1.5 g/person/day. Recently published studies continue to support the safety of DHA as a food ingredient.

4. Runke Bioengineering's DHA-rich oil may be used at a maximum use level of 0.5% of total fat as DHA or 1.43% of dietary fat as Runke Bioengineering's DHA-rich oil in infant formulas for term and pre-term infants. The intended use will result in 28 to 39 mg DHA/kg bw/day or 80 to 111 mg DHA-rich oil/kg bw/day. This estimated DHA intake is consistent with current DHA

recommendations for pre-term and term infants of 18 to 60 mg/kg bw/day depending on gestational age. The intended use level is the same as other approved uses for incorporation of DHA-rich oils in infant formula for term and pre-term infants (GRNs 000553, 000677, 000731, and 000776/000777). Recently published studies continue to support the safety of DHA as a food ingredient for infants.

5. It is assumed that Runke Bioengineering's DHA-rich oil derived from *Schizochytrium* sp. will replace currently marketed DHA or other DHA sources. Thus, cumulative exposures are not expected to change.

6. In previous GRAS notices to the FDA, the safety of DHA has been established in toxicological studies in animals, and mutagenicity and genotoxicity studies, and is further supported by clinical studies in human. The NOAEL was determined to be 2,069 mg/kg bw/day in a subchronic toxicity study in rats. The EDIs under the intended use are far less than the estimated safe intake levels in infants.

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

6.D.1. Common Knowledge Element of the GRAS Determination

Several sources of DHA or DHA-rich oil derived from *Schizochytrium* sp. have been evaluated by the FDA over the past 16 years for the proposed incorporation of DHA in foods for human consumption. Relevant U.S. GRAS notifications include GRNs 000137 (FDA, 2004), 000553 (FDA, 2015), 000677 (FDA, 2017), 000731/000732 (FDA, 2018a, 2018b), 000776/000777 (FDA, 2018c, 2018d), 000836 (FDA, 2019a), and 000843/000844 (FDA, 2019b, 2019c), 000862 (FDA, 2020a), 000933 (FDA, 2020b, 000934 (FDA, 2021), and 001008 (FDA, 2022). All the GRAS notices provided information/clinical study data that supported the safety of the proposed DHA ingredients for use in human foods. In all the studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues attributable to DHA. Due to the compositional similarity and DHA content of algae-derived oils to Runke Bioengineering's DHA-rich oil, the available scientific literature on the safety of these oils supports the safety of Runke Bioengineering's DHA-rich oil derived from *Schizochytrium* sp. Given 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.

6.D.2. Technical Element of the GRAS Determination (Safety Determination)

In addition, the intended uses of DHA 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 and fatty acid profile of the proposed GRAS substance, Runke Bioengineering's DHA-rich oil derived from *Schizochytrium* sp., is substantially equivalent to those that have received FDA's 'no question' letters.

This GRAS determination for DHA is based on scientific procedures. Numerous human and animal studies examined safety-related parameters of DHA-rich oil. For general population, there are no reports of safety concerns in any of the studies as long as the consumption level does not exceed 1.5 g/person/day in the general population. In infants, no adverse effects of DHA in infant formula up to 0.96% of total fatty acids were reported.

Runke Bioengineering observes the principles of HACCP-controlled manufacturing process and cGMP and rigorously tests its final production batches to verify adherence to QC specifications. The information and data provided by Runke Bioengineering in this report and supplemented by the publicly available literature/toxicity data on DHA and DHA-rich algal oil provide a sufficient basis for an assessment of the safety of DHA-rich oil from *Schizochytrium* sp. for the proposed use as an ingredient in food.

It is concluded that Runke Bioengineering's DHA-rich oil, manufactured as described in the dossier and consistent with cGMP, and meeting appropriate food grade specifications, is GRAS based on scientific procedures for use as an ingredient in term and pre-term infant formulas and selected conventional foods at levels specified in the accompanying dossier. It is

our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions.

6.E. Discussion of Information Inconsistent with GRAS Determination

We are not aware of information that would be inconsistent with the finding that the proposed use of DHA, meeting appropriate specifications and used according to cGMP, is GRAS.

PART 7. REFERENCES

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7.B. References That Are Not Generally Available

Not applicable.

Appendix A. Certificates of Analysis

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CNAS 中国认可 检测 TESTING CNAS L3788

Analytical Report

Sample Code	502-2021-00126361	Report date	30-Dec-2021
Certificate No.	AR-21-SU-116944-01-EN		

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2021-00126361/ AR-21-SU-116944-01-EN
Client Sample Code:	样品批号：11024713 生产日期：2021.10.24
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	29-Nov-2021
Analysis Starting Date:	29-Nov-2021
Analysis Ending Date:	29-Dec-2021
Arrival Temperature (°C)	21.8
Sample Weight	140g*12

		Results	Unit	LOQ	LOD
^# SU007	Mercury (AAS) Method: BS EN 13806:2002 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
Mercury (Hg)		<0.005	mg/kg	0.005	
# SU05D	Lead (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
Lead (Pb)		<0.05	mg/kg	0.05	
# SU05E	Arsenic (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
Arsenic (As)		<0.005	mg/kg	0.005	
# SU05G	Cadmium (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
Cadmium (Cd)		<0.005	mg/kg	0.005	

		Results	Unit	LOQ	LOD
^# SU1A2	Aerobic plate count Method: US FDA BAM Chapter 3, Jan 2001 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
Aerobic Plate Count		<1.0	cfu/ml		
^ SU1A4	Salmonella Method: US FDA BAM Chapter 5, 2021 Accreditation: ISO/IEC 17025:2017 CNAS L3788				
Salmonella		Not Detected	/25 ml		
^# SU1A7	Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
Moulds		<1.0	cfu/ml		
Yeast		<1.0	cfu/ml		
^# SU1CX	E.coli Method: ISO 16649-3:2015 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
E. coli		Not Detected	/25 ml		

		Results	Unit	LOQ	LOD
^ SU207	Peroxide value Method: AOCS Cd 8b-90:2017 Accreditation: ISO/IEC 17025:2017 CNAS L3788				

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DAkkS Deutsche Akkreditierungsstelle D-PL-14292-01-00

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		Results	Unit	LOQ	LOD
	Peroxide value	0.36	meq/kg	0.05	
A# SU20L	Protein Method: AOAC 984.13 1994 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Protein	<0.1	g/100 g	0.1	
	Protein Factor	6.25			

		Results	Unit	LOQ	LOD
☆ FL023	Plant sterols and plant stanols (not enriched) Method: NMKL 198:2014				
	Brassicasterol	18	mg/100 g	1	
	Cholesterol	318	mg/100 g	1	
	Campesterol	9	mg/100 g	1	
	Campestanol	2	mg/100 g	1	
	Stigmasterol	31	mg/100 g	1	
	Unidentified sterols	328	mg/100 g	1	
	Sitosterol	112	mg/100 g	1	
	Sitostanol+ delta-5-avenasterol	6	mg/100 g	1	
	Delta-5,24-stigmastadienol	20	mg/100 g	1	
	Delta-7-stigmastenol	54	mg/100 g	1	
	delta-7-Avenasterol	11	mg/100 g	1	
	Cycloartenol	7	mg/100 g	1	
	24-Methylenecycloartanol	2	mg/100 g	1	
	Citrostadienol	7	mg/100 g	1	
	Total plant sterols + plant stanols	591	mg/100 g	1	
☆ QA00I	Acid Value Method: AOCS Cd 3d-63 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Acid value (mg KOH/g)	0.23	mg KOH/g	0.05	
	Free fatty acids (as oleic acid)	0.12	%	0.01	
☆ QA01L	p-Anisidine Value Method: AOCS Cd 18-90 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	p-Anisidine Value	8.8		1	
☆ QA307	Glyceride Profile Method: AOCS Cd 11c-93				
	Diglycerides	3.9	%	1	
	Glycerol	2.8	%	1	
	Monoglycerides	2.2	%	1	
	Triglycerides	94.2	%	1	
☆ QA383	Moisture & Volatiles (Air Oven 130C) Method: AOCS Ca 2c-25				
	Moisture & Volatiles	<0.01	%	0.01	
☆ QA966	Unsaponifiable Matter Method: AOCS Ca 6a-40				
	Unsaponifiable matter	1.19	%	0.05	
☆ QD05C	Fatty Acids-Full Omega 9,6&3 & Trans %W/W Method: AOAC 996.06 mod. Accreditation: ISO/IEC 17025:2017 A2LA 2927.01				
	C 16:4 (Hexadecatetraenoic Acid)	<0.02	%	0.02	
	C10:0 (Capric acid)	<0.02	%	0.02	
	C11:0 (Undecanoic acid)	<0.02	%	0.02	
	C12:0 (Lauric Acid)	0.04	%	0.02	
	C14:0 (Myristic acid)	0.31	%	0.02	
	C14:1 (Myristoleic acid)	<0.02	%	0.02	
	C15:0 (Pentadecanoic acid)	0.05	%	0.02	
	C15:1 (Pentadecenoic acid)	<0.02	%	0.02	
	C16:0 (Palmitic Acid)	15.93	%	0.02	
	C16:1 Omega 7	0.09	%	0.04	
	C16:1 Total (Palmitoleic Acid + isomers)	0.26	%	0.04	
	C16:2 (Hexadecadienoic Acid)	<0.02	%	0.02	
	C16:3 (Hexadecatrienoic Acid)	<0.02	%	0.02	
	C17:0 (Margaric Acid)	0.06	%	0.02	
	C17:1 (Heptadecenoic Acid)	<0.02	%	0.02	

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	Results	Unit	LOQ	LOD
C18:0 (Stearic Acid)	1.35	%	0.02	
C18:1 (Vaccenic acid)	0.17	%	0.03	
C18:1 Omega 9 (Oleic Acid)	3.88	%	0.02	
C18:1, Total (Oleic Acid + isomers)	4.09	%	0.03	
C18:2 Omega 6 (Linoleic Acid)	8.24	%	0.02	
C18:2, Total (Linoleic Acid + isomers)	8.46	%	0.02	
C18:3 Omega 3 (Alpha Linolenic Acid)	0.12	%	0.02	
C18:3 Omega 6 (Gamma Linolenic Acid)	0.13	%	0.02	
C18:3, Total (Linolenic Acid + isomers)	0.25	%	0.02	
C18:4 Omega 3 (Octadecatetraenoic Acid)	0.19	%	0.02	
C18:4 Total (Octadecatetraenoic Acid)	0.19	%	0.02	
C20:0 (Arachidic Acid)	0.24	%	0.02	
C20:1 Omega 9 (Gondoic Acid)	0.02	%	0.02	
C20:1 Total (Gondoic Acid + isomers)	0.04	%	0.02	
C20:2 Omega 6	<0.02	%	0.02	
C20:2 Total (Eicosadienoic Acid)	<0.02	%	0.02	
C20:3 Omega 3	<0.02	%	0.02	
C20:3 Omega 6	0.26	%	0.02	
C20:3, Total (Eicosatrienoic Acid)	0.26	%	0.02	
C20:4 Omega 3	0.61	%	0.02	
C20:4 Omega 6 (Arachidonic Acid)	0.19	%	0.02	
C20:4, Total (Eicosatetraenoic Acid)	0.80	%	0.02	
C20:5 Omega 3 (Eicosapentaenoic Acid)	0.42	%	0.02	
C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02	%	0.02	
C22:0 (Behenic Acid)	0.22	%	0.02	
C22:1 Omega 9 (Erucic Acid)	0.28	%	0.02	
C22:1 Total (Erucic Acid + isomers)	0.28	%	0.02	
C22:2 Docosadienoic Omega 6	<0.02	%	0.02	
C22:3 Docosatrienoic, Omega 3	0.16	%	0.02	
C22:4 Docosatetraenoic Omega 6	<0.02	%	0.02	
C22:5 Docosapentaenoic Omega 3	0.08	%	0.02	
C22:5 Docosapentaenoic Omega 6	12.31	%	0.02	
C22:5 Total (Docosapentaenoic Acid)	12.40	%	0.02	
C22:6 Docosahexaenoic Omega 3	43.01	%	0.02	
C24:0 (Lignoceric Acid)	0.13	%	0.02	
C24:1 Omega 9 (Nervonic Acid)	<0.02	%	0.02	
C24:1 Total (Nervonic Acid + isomers)	0.10	%	0.02	
C4:0 (Butyric Acid)	<0.02	%	0.02	
C6:0 (Caproic acid)	<0.02	%	0.02	
C8:0 (Caprylic acid)	<0.02	%	0.02	
Fatty Acid Profile	Reported as Fatty Acids			
Total Fat as Triglycerides	92.85	%	0.1	
Total Fatty Acids	89.13	%	0.1	
Total Monounsaturated Fatty Acids	4.62	%	0.05	
Total Omega 3 Isomers	44.61	%	0.05	
Total Omega 5 Isomers	<0.05	%	0.05	
Total Omega 6 Isomers	21.17	%	0.05	
Total Omega 7 Isomers	0.26	%	0.05	
Total Omega 9 Isomers	4.22	%	0.05	
Total Polyunsaturated Fatty Acids	65.91	%	0.05	

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		Results	Unit	LOQ	LOD
	Total Saturated Fatty Acids	18.35	%	0.05	
	Total Trans Fatty Acids	0.25	%	0.02	
☆ QD094	Free Fatty Acids (FFA) Method: AOCS Ca 5a-40; AOAC 940.28 Accreditation: ISO/IEC 17025:2017 A2LA 2927.01				
	FFA (Free Fatty Acids)	0.08	%	0.01	
● R290Z	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	0.103	EU/ml		
☆ ZME3X	Enumeration (MPN) of Enterobacter sakazakii Method: FDA BAM Chapter 29 mod.				
	Enterobacter sakazakii	< 0.3	MPN/10 ml		

COMMENT
TEST CHANGE: ordered FL025 for candies has been changed to FL023.

The content of total plant sterols and plant stanols does not contain cholesterol and non-4-desmethyl sterols (i.e. cycloartenol, 24-methylenecycloartanol, and citrostadienol).

Amount of total GC elutables is 1331 mg/100 g

Peak identifications have to be treated only as tentative for this sample matrix.

SIGNATURE

Jack He
Authorized Signatory

Shine Xie
Authorized Signatory

EXPLANATORY NOTE
LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
△ CNAS # DAkkS ○CMA
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Analytical Report

Sample Code	502-2022-00002952	Report date	27-Jan-2022
Certificate No.	AR-22-SU-007858-02		

This report is translated from report AR-22-SU-007858-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00002952/ AR-22-SU-007858-02
Client Sample Code:	批号：11024713 生产日期：2021.10.24
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	10-Jan-2022
Analysis Starting Date:	10-Jan-2022
Analysis Ending Date:	26-Jan-2022
Arrival Temperature (°C)	14.0
Sample Weight	140g*2

		Results	Unit	LOQ	LOD
☆ QA04G	Monochloropropanediols (sum of free and esters) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Total 2-MCPD (free and bound)	<0.10	mg/kg	0.1	
	Total 3-MCPD (free and bound)	0.14	mg/kg	0.1	
☆ QA0N0	Glycidyl esters (GC-MSMS) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Glycidol (calculated)	<0.10	mg/kg	0.1	

SIGNATURE

Claire Wang
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	**502-2022-00037065**	Report date	30-Apr-2022
Certificate No.	**AR-22-SU-033313-02**		

This report is translated from report AR-22-SU-033313-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00037065/ AR-22-SU-033313-02
Client Sample Code:	样品批号：11024713 生产日期： 2021.10.24
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	23-Apr-2022
Analysis Starting Date:	24-Apr-2022
Analysis Ending Date:	29-Apr-2022
Arrival Temperature (°C)	21.6
Sample Weight	280g
Sample Condition	Other

		Results	Unit	LOQ	LOD
#§ SU10Z	Cronobacter spp. in 10g Method: ISO 22964:2017				
	Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	Cronobacter spp	Not Detected	/10 g		
#§ SU1A2	Aerobic plate count Method: US FDA BAM Chapter 3, Jan 2001				
	Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Aerobic Plate Count	<10	cfu/g		
§ SU1A4	Salmonella Method: US FDA BAM Chapter 5, 2021				
	Accreditation: ISO/IEC 17025:2017 CNAS L3788				
	Salmonella	Not Detected	/25 g		
#§ SU1A7	Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001				
	Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Moulds	<10	cfu/g		
	Yeast	<10	cfu/g		
#§ SU1CX	E.coli Method: ISO 16649-3:2015				
	Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	E. coli	Not Detected	/25 g		

SIGNATURE

Tracy Li
Authorized Signatory

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Analytical Report

Sample Code	502-2022-00039296	**Report date**	03-Jul-2022
Certificate No.	AR-22-SU-056885-02		

This report is translated from report AR-22-SU-056885-01

Runke Bioengineering (Fujian) Co.,Ltd.

JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province

Our reference:	502-2022-00039296/ AR-22-SU-056885-02
Client Sample Code:	样品批号：11024713 生产日期： 2021.10.24
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample reception date:	28-Apr-2022
Analysis Starting Date:	28-Apr-2022
Analysis Ending Date:	01-Jul-2022

		Results	Unit	LOQ	LOD
● **SUDJD**	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	**<0.109**	EU/g		

SIGNATURE

Lucy Liu
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Certificate No.	AR-23-SU-007403-02	Report date	30-Jan-2023
Sample reception date:	20-Jun-2022		
Analysis Starting Date:	20-Jun-2022		
Analysis Ending Date:	28-Jan-2023		

This report is translated from report AR-23-SU-007403-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province

Sample Code:	502-2022-00063740		
Client Sample Code:	批号：11024713 生产日期：2021.10.24		
Sample described as:	Docosahexaenoic acid oil /DHA algae oil		
Sample Packaging:	Sealed metal bottle		
Arrival Temperature (°C)	26.2	**Sample Weight**	100g*2
Sample Condition	Other		

		Results	Unit	LOQ	LOD
▵# SU114	Enterobacteriaceae Method: ISO 21528-2-2017				
	Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	Enterobacteriaceae	**<10**	cfu/g		

Sample Code:	502-2023-00005399		
Client Sample Code:	批号：11024713 生产日期：2021.10.24		
Sample described as:	Docosahexaenoic acid oil /DHA algae oil		
Sample Packaging:	Sealed metal can		
Arrival Temperature (°C)	18	**Sample Weight**	140g
Sample Condition	Other		

		Results	Unit	LOQ	LOD
☆ JK590	Protein content (Roti®-Nanoquant) Method: internal method (PV 01498 V2)				
	Content of protein	**<25**	µg/g	25	

SIGNATURE

Ally Dong
Authorized Signatory

Jack He
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2022-00045887	Report date	08-Jun-2022
Certificate No.	AR-22-SU-047148-02		

This report is translated from report AR-22-SU-047148-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00045887/ AR-22-SU-047148-02
Client Sample Code:	批号：11024713 生产日期：2021.10.24
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample reception date:	13-May-2022
Analysis Starting Date:	13-May-2022
Analysis Ending Date:	07-Jun-2022

		Results	Unit	LOQ	LOD
° SUDQ7	Domoic acid	Method: Internal Method (TPM001 Version 12 2021-06)			
Domoic acid		<1	mg/kg	1	

SIGNATURE

Shine Xie
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2021-00126362	Report date	30-Dec-2021
Certificate No.	AR-21-SU-116945-01-EN		

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JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2021-00126362/ AR-21-SU-116945-01-EN
Client Sample Code:	样品批号：11027715 生产日期： 2021.10.27
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	29-Nov-2021
Analysis Starting Date:	29-Nov-2021
Analysis Ending Date:	29-Dec-2021
Arrival Temperature (°C)	21.8
Sample Weight	140g*12

		Results	Unit	LOQ	LOD
^# SU007	Mercury (AAS) Method: BS EN 13806:2002 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	Mercury (Hg)	<0.005	mg/kg	0.005	
# SU05D	Lead (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
	Lead (Pb)	<0.05	mg/kg	0.05	
# SU05E	Arsenic (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
	Arsenic (As)	<0.005	mg/kg	0.005	
# SU05G	Cadmium (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
	Cadmium (Cd)	<0.005	mg/kg	0.005	

		Results	Unit	LOQ	LOD
^# SU1A2	Aerobic plate count Method: US FDA BAM Chapter 3, Jan 2001 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Aerobic Plate Count	<1.0	cfu/ml		
^ SU1A4	Salmonella Method: US FDA BAM Chapter 5, 2021 Accreditation: ISO/IEC 17025:2017 CNAS L3788				
	Salmonella	Not Detected	/25 ml		
^# SU1A7	Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Moulds	<1.0	cfu/ml		
	Yeast	<1.0	cfu/ml		
^# SU1CX	E.coli Method: ISO 16649-3:2015 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	E. coli	Not Detected	/25 ml		

		Results	Unit	LOQ	LOD
^ SU207	Peroxide value Method: AOCS Cd 8b-90:2017 Accreditation: ISO/IEC 17025:2017 CNAS L3788				

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		Results	Unit	LOQ	LOD
	Peroxide value	0.48	meq/kg	0.05	
#☆ SU20L	Protein Method: AOAC 984.13 1994 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Protein	<0.1	g/100 g	0.1	
	Protein Factor	6.25			

		Results	Unit	LOQ	LOD
☆ FL023	Plant sterols and plant stanols (not enriched) Method: NMKL 198:2014				
	Brassicasterol	16	mg/100 g	1	
	Cholesterol	319	mg/100 g	1	
	Campesterol	11	mg/100 g	1	
	Campestanol	2	mg/100 g	1	
	Stigmasterol	32	mg/100 g	1	
	Unidentified sterols	286	mg/100 g	1	
	Sitosterol	115	mg/100 g	1	
	Sitostanol+ delta-5-avenasterol	7	mg/100 g	1	
	Delta-5,24-stigmastadienol	14	mg/100 g	1	
	Delta-7-stigmastenol	43	mg/100 g	1	
	delta-7-Avenasterol	9	mg/100 g	1	
	Cycloartenol	6	mg/100 g	1	
	24-Methylenecycloartanol	4	mg/100 g	1	
	Citrostadienol	8	mg/100 g	1	
	Total plant sterols + plant stanols	537	mg/100 g	1	
☆ QA00I	Acid Value Method: AOCS Cd 3d-63 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Acid value (mg KOH/g)	0.37	mg KOH/g	0.05	
	Free fatty acids (as oleic acid)	0.19	%	0.01	
☆ QA01L	p-Anisidine Value Method: AOCS Cd 18-90 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	p-Anisidine Value	7.8		1	
☆ QA307	Glyceride Profile Method: AOCS Cd 11c-93				
	Diglycerides	4.7	%	1	
	Glycerol	2.9	%	1	
	Monoglycerides	3.2	%	1	
	Triglycerides	92.1	%	1	
☆ QA383	Moisture & Volatiles (Air Oven 130C) Method: AOCS Ca 2c-25				
	Moisture & Volatiles	<0.01	%	0.01	
☆ QA966	Unsaponifiable Matter Method: AOCS Ca 6a-40				
	Unsaponifiable matter	1.28	%	0.05	
☆ QD05C	Fatty Acids-Full Omega 9,6&3 & Trans %W/W Method: AOAC 996.06 mod. Accreditation: ISO/IEC 17025:2017 A2LA 2927.01				
	C 16:4 (Hexadecatetraenoic Acid)	<0.02	%	0.02	
	C10:0 (Capric acid)	<0.02	%	0.02	
	C11:0 (Undecanoic acid)	<0.02	%	0.02	
	C12:0 (Lauric Acid)	0.03	%	0.02	
	C14:0 (Myristic acid)	0.29	%	0.02	
	C14:1 (Myristoleic acid)	<0.02	%	0.02	
	C15:0 (Pentadecanoic acid)	0.04	%	0.02	
	C15:1 (Pentadecenoic acid)	<0.02	%	0.02	
	C16:0 (Palmitic Acid)	15.53	%	0.02	
	C16:1 Omega 7	0.08	%	0.04	
	C16:1 Total (Palmitoleic Acid + isomers)	0.23	%	0.04	
	C16:2 (Hexadecadienoic Acid)	<0.02	%	0.02	
	C16:3 (Hexadecatrienoic Acid)	<0.02	%	0.02	
	C17:0 (Margaric Acid)	0.05	%	0.02	
	C17:1 (Heptadecenoic Acid)	<0.02	%	0.02	

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	Results	Unit	LOQ	LOD
C18:0 (Stearic Acid)	1.32	%	0.02	
C18:1 (Vaccenic acid)	0.15	%	0.03	
C18:1 Omega 9 (Oleic Acid)	4.05	%	0.02	
C18:1, Total (Oleic Acid + isomers)	4.24	%	0.03	
C18:2 Omega 6 (Linoleic Acid)	9.13	%	0.02	
C18:2, Total (Linoleic Acid + isomers)	9.32	%	0.02	
C18:3 Omega 3 (Alpha Linolenic Acid)	0.13	%	0.02	
C18:3 Omega 6 (Gamma Linolenic Acid)	0.11	%	0.02	
C18:3, Total (Linolenic Acid + isomers)	0.25	%	0.02	
C18:4 Omega 3 (Octadecatetraenoic Acid)	0.19	%	0.02	
C18:4 Total (Octadecatetraenoic Acid)	0.19	%	0.02	
C20:0 (Arachidic Acid)	0.21	%	0.02	
C20:1 Omega 9 (Gondoic Acid)	0.03	%	0.02	
C20:1 Total (Gondoic Acid + isomers)	0.05	%	0.02	
C20:2 Omega 6	<0.02	%	0.02	
C20:2 Total (Eicosadienoic Acid)	<0.02	%	0.02	
C20:3 Omega 3	<0.02	%	0.02	
C20:3 Omega 6	0.20	%	0.02	
C20:3, Total (Eicosatrienoic Acid)	0.20	%	0.02	
C20:4 Omega 3	0.52	%	0.02	
C20:4 Omega 6 (Arachidonic Acid)	0.22	%	0.02	
C20:4, Total (Eicosatetraenoic Acid)	0.73	%	0.02	
C20:5 Omega 3 (Eicosapentaenoic Acid)	0.46	%	0.02	
C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02	%	0.02	
C22:0 (Behenic Acid)	0.20	%	0.02	
C22:1 Omega 9 (Erucic Acid)	0.21	%	0.02	
C22:1 Total (Erucic Acid + isomers)	0.21	%	0.02	
C22:2 Docosadienoic Omega 6	<0.02	%	0.02	
C22:3 Docosatrienoic, Omega 3	0.12	%	0.02	
C22:4 Docosatetraenoic Omega 6	<0.02	%	0.02	
C22:5 Docosapentaenoic Omega 3	0.07	%	0.02	
C22:5 Docosapentaenoic Omega 6	10.60	%	0.02	
C22:5 Total (Docosapentaenoic Acid)	10.68	%	0.02	
C22:6 Docosahexaenoic Omega 3	41.71	%	0.02	
C24:0 (Lignoceric Acid)	0.11	%	0.02	
C24:1 Omega 9 (Nervonic Acid)	<0.02	%	0.02	
C24:1 Total (Nervonic Acid + isomers)	0.04	%	0.02	
C4:0 (Butyric Acid)	<0.02	%	0.02	
C6:0 (Caproic acid)	<0.02	%	0.02	
C8:0 (Caprylic acid)	<0.02	%	0.02	
Fatty Acid Profile	Reported as Fatty Acids			
Total Fat as Triglycerides	89.86	%	0.1	
Total Fatty Acids	86.26	%	0.1	
Total Monounsaturated Fatty Acids	4.63	%	0.05	
Total Omega 3 Isomers	43.20	%	0.05	
Total Omega 5 Isomers	<0.05	%	0.05	
Total Omega 6 Isomers	20.28	%	0.05	
Total Omega 7 Isomers	0.23	%	0.05	
Total Omega 9 Isomers	4.33	%	0.05	
Total Polyunsaturated Fatty Acids	63.60	%	0.05	

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		Results	Unit	LOQ	LOD
	Total Saturated Fatty Acids	17.81	%	0.05	
	Total Trans Fatty Acids	0.22	%	0.02	
☆ QD094	Free Fatty Acids (FFA) Method: AOCS Ca 5a-40; AOAC 940.28 Accreditation: ISO/IEC 17025:2017 A2LA 2927.01				
	FFA (Free Fatty Acids)	0.10	%	0.01	
• R290Z	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	0.141	EU/ml		
☆ ZME3X	Enumeration (MPN) of Enterobacter sakazakii Method: FDA BAM Chapter 29 mod.				
	Enterobacter sakazakii	< 0.3	MPN/10 ml		

COMMENT
TEST CHANGE: ordered FL025 for candies has been changed to FL023.

The content of total plant sterols and plant stanols does not contain cholesterol and non-4-desmethyl sterols (i.e. cycloartenol, 24-methylenecycloartanol, and citrostadienol).

Amount of total GC elutables is 1346 mg/100 g

Peak identfitications have to be treated only as tentative for this sample matrix.

SIGNATURE

Jack He
Authorized Signatory

Shine Xie
Authorized Signatory

EXPLANATORY NOTE
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Analytical Report

Sample Code	502-2022-00002953	Report date	27-Jan-2022
Certificate No.	AR-22-SU-007859-02		

This report is translated from report AR-22-SU-007859-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00002953/ AR-22-SU-007859-02
Client Sample Code:	批号：11027715 生产日期：2021.10.27
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	10-Jan-2022
Analysis Starting Date:	10-Jan-2022
Analysis Ending Date:	26-Jan-2022
Arrival Temperature (°C)	14.0
Sample Weight	140g*2

		Results	Unit	LOQ	LOD
☆ QA04G	Monochloropropanediols (sum of free and esters) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Total 2-MCPD (free and bound)	<0.10	mg/kg	0.1	
	Total 3-MCPD (free and bound)	0.14	mg/kg	0.1	
☆ QA0N0	Glycidyl esters (GC-MSMS) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Glycidol (calculated)	<0.10	mg/kg	0.1	

SIGNATURE

Claire Wang
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2022-00037066	Report date	30-Apr-2022
Certificate No.	AR-22-SU-033314-02		

This report is translated from report AR-22-SU-033314-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00037066/ AR-22-SU-033314-02
Client Sample Code:	样品批号：11027716 生产日期： 2021.10.27
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	23-Apr-2022
Analysis Starting Date:	24-Apr-2022
Analysis Ending Date:	29-Apr-2022
Arrival Temperature (°C)	21.6
Sample Weight	280g
Sample Condition	Other

		Results	Unit	LOQ	LOD
+# SU10Z	Cronobacter spp. in 10g Method: ISO 22964:2017				
	Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	Cronobacter spp	Not Detected	/10 g		
+# SU1A2	Aerobic plate count Method: US FDA BAM Chapter 3, Jan 2001				
	Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Aerobic Plate Count	<10	cfu/g		
+ SU1A4	Salmonella Method: US FDA BAM Chapter 5, 2021				
	Accreditation: ISO/IEC 17025:2017 CNAS L3788				
	Salmonella	Not Detected	/25 g		
+# SU1A7	Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001				
	Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Moulds	<10	cfu/g		
	Yeast	<10	cfu/g		
+# SU1CX	E.coli Method: ISO 16649-3:2015				
	Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	E. coli	Not Detected	/25 g		

SIGNATURE

Tracy Li
Authorized Signatory

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Analytical Report

Sample Code	502-2022-00039297	**Report date**	03-Jul-2022
Certificate No.	AR-22-SU-056886-02		

This report is translated from report AR-22-SU-056886-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province

Our reference:	502-2022-00039297/ AR-22-SU-056886-02
Client Sample Code:	样品批号：11027715 生产日期： 2021.10.27
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample reception date:	28-Apr-2022
Analysis Starting Date:	28-Apr-2022
Analysis Ending Date:	01-Jul-2022

		Results	Unit	LOQ	LOD
• **SUDJD**	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	**<0.109**	EU/g		

SIGNATURE

Lucy Liu
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Certificate No.	AR-23-SU-007404-02	Report date	30-Jan-2023
Sample reception date:	20-Jun-2022		
Analysis Starting Date:	20-Jun-2022		
Analysis Ending Date:	28-Jan-2023		

This report is translated from report AR-23-SU-007404-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province

Sample Code:	502-2022-00063741		
Client Sample Code:	批号：11027715 生产日期：2021.10.27		
Sample described as:	Docosahexaenoic acid oil /DHA algae oil		
Sample Packaging:	Sealed metal bottle		
Arrival Temperature (°C)	26.2	**Sample Weight**	100g*2
Sample Condition	Other		

		Results	Unit	LOQ	LOD
▵# SU114	Enterobacteriaceae Method: ISO 21528-2-2017				
	Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	Enterobacteriaceae	**<10**	cfu/g		

Sample Code:	502-2023-00005400				
Client Sample Code:	批号：11027715 生产日期：2021.10.27				
Sample described as:	Docosahexaenoic acid oil /DHA algae oil				
Sample Packaging:	Sealed metal can				
Arrival Temperature (°C)	18	**Sample Weight**	140g		
Sample Condition	Other				

		Results	Unit	LOQ	LOD
☆ JK590	Protein content (Roti®-Nanoquant) Method: internal method (PV 01498 V2)				
	Content of protein	**<25**	µg/g	25	

SIGNATURE

Ally Dong
Authorized Signatory

Jack He
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2022-00045888	Report date	08-Jun-2022
Certificate No.	AR-22-SU-047149-02		

This report is translated from report AR-22-SU-047149-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00045888/ AR-22-SU-047149-02
Client Sample Code:	批号：11027715 生产日期：2021.10.27
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample reception date:	13-May-2022
Analysis Starting Date:	13-May-2022
Analysis Ending Date:	07-Jun-2022

		Results	Unit	LOQ	LOD
• SUDQ7	Domoic acid Method: Internal Method (TPM001 Version 12 2021-06)				
Domoic acid		<1	mg/kg	1	

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Shine Xie
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2021-00126363	Report date	30-Dec-2021
Certificate No.	AR-21-SU-116946-01-EN		

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JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2021-00126363/ AR-21-SU-116946-01-EN
Client Sample Code:	样品批号：11030717 生产日期： 2021.10.30
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	29-Nov-2021
Analysis Starting Date:	29-Nov-2021
Analysis Ending Date:	29-Dec-2021
Arrival Temperature (°C)	21.8
Sample Weight	140g*12

		Results	Unit	LOQ	LOD
^# SU007	Mercury (AAS) Method: BS EN 13806:2002 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
Mercury (Hg)		<0.005	mg/kg	0.005	
# SU05D	Lead (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
Lead (Pb)		<0.05	mg/kg	0.05	
# SU05E	Arsenic (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
Arsenic (As)		<0.005	mg/kg	0.005	
# SU05G	Cadmium (ICP-MS) Method: BS EN ISO 17294-2 2016 mod. Accreditation: ISO/IEC 17025:2017 DAkkS D-PL-14292-01-00				
Cadmium (Cd)		<0.005	mg/kg	0.005	

		Results	Unit	LOQ	LOD
^# SU1A2	Aerobic plate count Method: US FDA BAM Chapter 3, Jan 2001 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
Aerobic Plate Count		<1.0	cfu/ml		
^ SU1A4	Salmonella Method: US FDA BAM Chapter 5, 2021 Accreditation: ISO/IEC 17025:2017 CNAS L3788				
Salmonella		Not Detected	/25 ml		
^# SU1A7	Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
Moulds		<1.0	cfu/ml		
Yeast		<1.0	cfu/ml		
^# SU1CX	E.coli Method: ISO 16649-3:2015 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
E. coli		Not Detected	/25 ml		

		Results	Unit	LOQ	LOD
^ SU207	Peroxide value Method: AOCS Cd 8b-90:2017 Accreditation: ISO/IEC 17025:2017 CNAS L3788				

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		Results	Unit	LOQ	LOD
	Peroxide value	0.24	meq/kg	0.05	
^# SU20L	Protein Method: AOAC 984.13 1994 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Protein	<0.1	g/100 g	0.1	
	Protein Factor	6.25			
		Results	Unit	LOQ	LOD
☆ FL023	Plant sterols and plant stanols (not enriched) Method: NMKL 198:2014				
	Brassicasterol	18	mg/100 g	1	
	Cholesterol	324	mg/100 g	1	
	Campesterol	9	mg/100 g	1	
	Campestanol	2	mg/100 g	1	
	Stigmasterol	31	mg/100 g	1	
	Unidentified sterols	326	mg/100 g	1	
	Sitosterol	109	mg/100 g	1	
	Sitostanol+ delta-5-avenasterol	5	mg/100 g	1	
	Delta-5,24-stigmastadienol	20	mg/100 g	1	
	Delta-7-stigmastenol	54	mg/100 g	1	
	delta-7-Avenasterol	11	mg/100 g	1	
	Cycloartenol	8	mg/100 g	1	
	24-Methylenecycloartanol	3	mg/100 g	1	
	Citrostadienol	6	mg/100 g	1	
	Total plant sterols + plant stanols	584	mg/100 g	1	
☆ QA00I	Acid Value Method: AOCS Cd 3d-63 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Acid value (mg KOH/g)	0.21	mg KOH/g	0.05	
	Free fatty acids (as oleic acid)	0.11	%	0.01	
☆ QA01L	p-Anisidine Value Method: AOCS Cd 18-90 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	p-Anisidine Value	9.6		1	
☆ QA307	Glyceride Profile Method: AOCS Cd 11c-93				
	Diglycerides	3.7	%	1	
	Glycerol	2.7	%	1	
	Monoglycerides	1.8	%	1	
	Triglycerides	94.5	%	1	
☆ QA383	Moisture & Volatiles (Air Oven 130C) Method: AOCS Ca 2c-25				
	Moisture & Volatiles	<0.01	%	0.01	
☆ QA966	Unsaponifiable Matter Method: AOCS Ca 6a-40				
	Unsaponifiable matter	1.33	%	0.05	
☆ QD05C	Fatty Acids-Full Omega 9,6&3 & Trans %W/W Method: AOAC 996.06 mod. Accreditation: ISO/IEC 17025:2017 A2LA 2927.01				
	C 16:4 (Hexadecatetraenoic Acid)	<0.02	%	0.02	
	C10:0 (Capric acid)	<0.02	%	0.02	
	C11:0 (Undecanoic acid)	<0.02	%	0.02	
	C12:0 (Lauric Acid)	0.04	%	0.02	
	C14:0 (Myristic acid)	0.36	%	0.02	
	C14:1 (Myristoleic acid)	<0.02	%	0.02	
	C15:0 (Pentadecanoic acid)	0.06	%	0.02	
	C15:1 (Pentadecenoic acid)	<0.02	%	0.02	
	C16:0 (Palmitic Acid)	16.36	%	0.02	
	C16:1 Omega 7	0.09	%	0.04	
	C16:1 Total (Palmitoleic Acid + isomers)	0.26	%	0.04	
	C16:2 (Hexadecadienoic Acid)	<0.02	%	0.02	
	C16:3 (Hexadecatrienoic Acid)	<0.02	%	0.02	
	C17:0 (Margaric Acid)	0.06	%	0.02	
	C17:1 (Heptadecenoic Acid)	<0.02	%	0.02	

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	Results	Unit	LOQ	LOD
C18:0 (Stearic Acid)	1.33	%	0.02	
C18:1 (Vaccenic acid)	0.16	%	0.03	
C18:1 Omega 9 (Oleic Acid)	3.54	%	0.02	
C18:1, Total (Oleic Acid + isomers)	3.75	%	0.03	
C18:2 Omega 6 (Linoleic Acid)	7.50	%	0.02	
C18:2, Total (Linoleic Acid + isomers)	7.81	%	0.02	
C18:3 Omega 3 (Alpha Linolenic Acid)	0.12	%	0.02	
C18:3 Omega 6 (Gamma Linolenic Acid)	0.14	%	0.02	
C18:3, Total (Linolenic Acid + isomers)	0.26	%	0.02	
C18:4 Omega 3 (Octadecatetraenoic Acid)	0.21	%	0.02	
C18:4 Total (Octadecatetraenoic Acid)	0.21	%	0.02	
C20:0 (Arachidic Acid)	0.24	%	0.02	
C20:1 Omega 9 (Gondoic Acid)	0.03	%	0.02	
C20:1 Total (Gondoic Acid + isomers)	0.06	%	0.02	
C20:2 Omega 6	0.03	%	0.02	
C20:2 Total (Eicosadienoic Acid)	0.03	%	0.02	
C20:3 Omega 3	<0.02	%	0.02	
C20:3 Omega 6	0.28	%	0.02	
C20:3, Total (Eicosatrienoic Acid)	0.28	%	0.02	
C20:4 Omega 3	0.62	%	0.02	
C20:4 Omega 6 (Arachidonic Acid)	0.23	%	0.02	
C20:4, Total (Eicosatetraenoic Acid)	0.85	%	0.02	
C20:5 Omega 3 (Eicosapentaenoic Acid)	0.37	%	0.02	
C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02	%	0.02	
C22:0 (Behenic Acid)	0.24	%	0.02	
C22:1 Omega 9 (Erucic Acid)	0.35	%	0.02	
C22:1 Total (Erucic Acid + isomers)	0.35	%	0.02	
C22:2 Docosadienoic Omega 6	<0.02	%	0.02	
C22:3 Docosatrienoic, Omega 3	0.17	%	0.02	
C22:4 Docosatetraenoic Omega 6	0.02	%	0.02	
C22:5 Docosapentaenoic Omega 3	0.08	%	0.02	
C22:5 Docosapentaenoic Omega 6	12.60	%	0.02	
C22:5 Total (Docosapentaenoic Acid)	12.68	%	0.02	
C22:6 Docosahexaenoic Omega 3	42.76	%	0.02	
C24:0 (Lignoceric Acid)	0.13	%	0.02	
C24:1 Omega 9 (Nervonic Acid)	<0.02	%	0.02	
C24:1 Total (Nervonic Acid + isomers)	0.07	%	0.02	
C4:0 (Butyric Acid)	<0.02	%	0.02	
C6:0 (Caproic acid)	<0.02	%	0.02	
C8:0 (Caprylic acid)	<0.02	%	0.02	
Fatty Acid Profile	Reported as Fatty Acids			
Total Fat as Triglycerides	92.47	%	0.1	
Total Fatty Acids	88.77	%	0.1	
Total Monounsaturated Fatty Acids	4.31	%	0.05	
Total Omega 3 Isomers	44.34	%	0.05	
Total Omega 5 Isomers	<0.05	%	0.05	
Total Omega 6 Isomers	20.80	%	0.05	
Total Omega 7 Isomers	0.25	%	0.05	
Total Omega 9 Isomers	3.95	%	0.05	
Total Polyunsaturated Fatty Acids	65.35	%	0.05	

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		Results	Unit	LOQ	LOD
	Total Saturated Fatty Acids	18.84	%	0.05	
	Total Trans Fatty Acids	0.26	%	0.02	
☆ QD094	Free Fatty Acids (FFA) Method: AOCS Ca 5a-40; AOAC 940.28 Accreditation: ISO/IEC 17025:2017 A2LA 2927.01				
	FFA (Free Fatty Acids)	0.08	%	0.01	
• R290Z	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	0.133	EU/ml		
☆ ZME3X	Enumeration (MPN) of Enterobacter sakazakii Method: FDA BAM Chapter 29 mod.				
	Enterobacter sakazakii	< 0.3	MPN/10 ml		

COMMENT

TEST CHANGE: ordered FL025 for candies has been changed to FL023.

The content of total plant sterols and plant stanols does not contain cholesterol and non-4-desmethyl sterols (i.e. cycloartenol, 24-methylenecycloartanol, and citrostadienol).

Amount of total GC elutables is 1365 mg/100 g

Peak identifications have to be treated only as tentative for this sample matrix.

SIGNATURE

Jack He
Authorized Signatory

Shine Xie
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2022-00002954	Report date	27-Jan-2022
Certificate No.	AR-22-SU-007860-02		

This report is translated from report AR-22-SU-007860-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00002954/ AR-22-SU-007860-02
Client Sample Code:	批号：11030717 生产日期：2021.10.30
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	10-Jan-2022
Analysis Starting Date:	10-Jan-2022
Analysis Ending Date:	26-Jan-2022
Arrival Temperature (℃)	14.0
Sample Weight	140g*2

		Results	Unit	LOQ	LOD
☆ QA04G	Monochloropropanediols (sum of free and esters) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Total 2-MCPD (free and bound)	<0.10	mg/kg	0.1	
	Total 3-MCPD (free and bound)	0.14	mg/kg	0.1	
☆ QA0N0	Glycidyl esters (GC-MSMS) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Glycidol (calculated)	<0.10	mg/kg	0.1	

SIGNATURE

Claire Wang
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2022-00037067	Report date	30-Apr-2022
Certificate No.	AR-22-SU-033315-02		

This report is translated from report AR-22-SU-033315-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00037067/ AR-22-SU-033315-02
Client Sample Code:	样品批号：11030717 生产日期： 2021.10.30
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	23-Apr-2022
Analysis Starting Date:	24-Apr-2022
Analysis Ending Date:	29-Apr-2022

Arrival Temperature (°C)	21.6	Sample Weight	280g
Sample Condition	Other		

		Results	Unit	LOQ	LOD
+# SU10Z	Cronobacter spp. in 10g Method: ISO 22964:2017 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	Cronobacter spp	Not Detected	/10 g		
+# SU1A2	Aerobic plate count Method: US FDA BAM Chapter 3, Jan 2001 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Aerobic Plate Count	<10	cfu/g		
+ SU1A4	Salmonella Method: US FDA BAM Chapter 5, 2021 Accreditation: ISO/IEC 17025:2017 CNAS L3788				
	Salmonella	Not Detected	/25 g		
+# SU1A7	Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001 Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Moulds	<10	cfu/g		
	Yeast	<10	cfu/g		
+# SU1CX	E.coli Method: ISO 16649-3:2015 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	E. coli	Not Detected	/25 g		

SIGNATURE

Tracy Li
Authorized Signatory

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Analytical Report

Sample Code	502-2022-00039298	**Report date**	03-Jul-2022
Certificate No.	AR-22-SU-056887-02		

This report is translated from report AR-22-SU-056887-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province

Our reference:	502-2022-00039298/ AR-22-SU-056887-02
Client Sample Code:	样品批号：11030717 生产日期： 2021.10.30
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample reception date:	28-Apr-2022
Analysis Starting Date:	28-Apr-2022
Analysis Ending Date:	01-Jul-2022

		Results	Unit	LOQ	LOD
• **SUDJD**	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	**<0.109**	EU/g		

SIGNATURE

Lucy Liu
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Certificate No.	AR-23-SU-007405-02	Report date	30-Jan-2023
Sample reception date:	20-Jun-2022		
Analysis Starting Date:	20-Jun-2022		
Analysis Ending Date:	28-Jan-2023		

This report is translated from report AR-23-SU-007405-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province

Sample Code:	502-2022-00063742
Client Sample Code:	批号：11030717 生产日期：2021.10.30
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal bottle
Arrival Temperature (°C)	26.2
Sample Weight	100g*2
Sample Condition	Other

		Results	Unit	LOQ	LOD
▵# SU114	Enterobacteriaceae Method: ISO 21528-2-2017 Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	Enterobacteriaceae	<10	cfu/g		

Sample Code:	502-2023-00005401
Client Sample Code:	批号：11030717 生产日期：2021.10.30
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample Packaging:	Sealed metal can
Arrival Temperature (°C)	18
Sample Weight	140g
Sample Condition	Other

		Results	Unit	LOQ	LOD
☆ JK590	Protein content (Roti®-Nanoquant) Method: internal method (PV 01498 V2)				
	Content of protein	<25	µg/g	25	

SIGNATURE

Ally Dong
Authorized Signatory

Jack He
Authorized Signatory

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Analytical Report

Sample Code	502-2022-00045889	Report date	08-Jun-2022
Certificate No.	AR-22-SU-047150-02		

This report is translated from report AR-22-SU-047160-01

Runke Bioengineering (Fujian) Co.,Ltd.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

Our reference:	502-2022-00045889/ AR-22-SU-047150-02
Client Sample Code:	批号：11030717 生产日期：2021.10.30
Sample described as:	Docosahexaenoic acid oil /DHA algae oil
Sample reception date:	13-May-2022
Analysis Starting Date:	13-May-2022
Analysis Ending Date:	07-Jun-2022

		Results	Unit	LOQ	LOD
• SUDQ7	Domoic acid	Method: Internal Method (TPM001 Version 12 2021-06)			
Domoic acid		<1	mg/kg	1	

SIGNATURE

Shine Xie
Authorized Signatory

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Appendix B. Identification of Runke Bioengineering's Strain

Institute of Microbiology Chinese Academy of Sciences (IMCAS) Report

TEST REPORT

IMCAS Report No. 2023JB157

Applicant: Fujian Runke Bioengineering Corp., Ltd.

Sample described: Microbial culture (strain FJRK-SCH3)

Sample quantity: One strain | Date of sampling: 2023.04

Tested by: Bing-Da SUN | Signature:

Approved by: Yu-Guang ZHOU | Signature:

(The next results only refer to the received samples. The name, Institute of Microbiology Chinese Academy of Sciences, shall not be used for commercial purpose without the prior written consent of the service provider.)

Conclusion of Identification:

According to the results of the morphological, physiological properties, sequence of 18S rRNA gene, the strain FJRK-SCH3 belongs to:

Schizochytrium sp.

Institute of Microbiology
Chinese Academy of Sciences
June 19, 2023

TEST REPORT

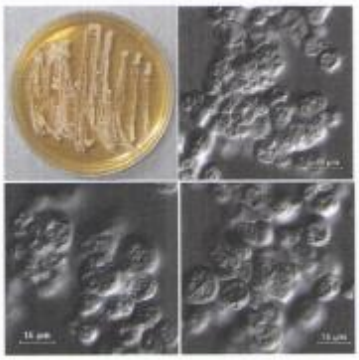
IMCAS Report No. 2023JB57

Applicant: Fujian Runke Bioengineering Corp., Ltd.

(continue)

1. Morphological properties

Fast growing on seawater agar medium, 2~4 mm diam after five days of incubation at 25 °C, colonies large by continuous binary cell divisions, white, becoming light brown when old. Thallus thin-walled, globose, transparent, pale orange, 6.5~18.0 μm. Ectoplasmic nets and Zoospores not observed.

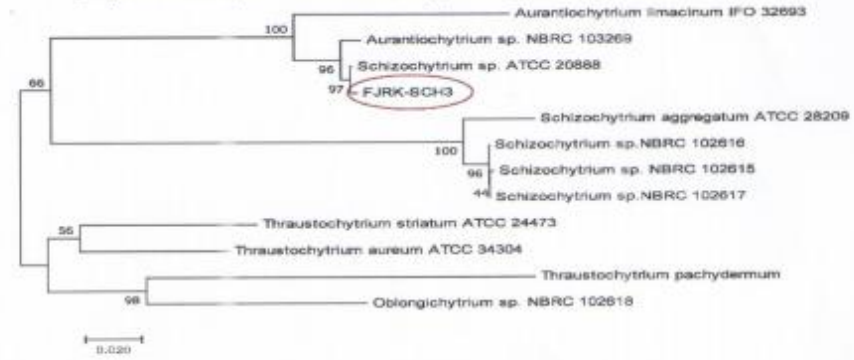


2. Partial sequence of 18S rRNA gene

Part 1: 5'- GCATGTGTAAGTATAAGCGAATTATACTGTGAAACTGCGAACGGCTCATTATATCAGTTATAATCCCTTCGG
TAGTTCCTTTATACGGATACCTGCAGTAATTCTGGAATTAATACGTGCTGTACGGGCCCGACTTTCGGGGAGGGCCGCACTTA
TTAGGTCTAAGCCAACTCTCTTGGTGAGTCATGATAATTGAGCAGATCGCTTTTCGGAGCGATGAATCGTTTGAGTTTCTGCC
CCATCAGTTGTCGACGGTAGGGTATTGGCCTACGGTGACTATAACGGGTGACGGGGAGTTAGGGCTCGACTCCGGAGAGGGAG
CCTGAGAGACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGTAAATTACCCAATGTGGACTCCACGAGGTAGTGACGAGAAA
TATCAATGCGGGGCGCTTCGCGTCTTGCTATTGGAATGAGAGCAATGTAAAACCCTCATCGAGGATCAACTGGAGGGCAAGTC
TGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAGAAGCGTATGCTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTG
GCGTGGGAGCCCAGGCCTGGGTGCGAATGTGCCTTGTTATTGCCTTGCGGCTCCTTTGCCATCCTCGTCTATCTTTGTGATAG
GCGTCCTTCACTGTAATCAAAGCAGAGTGTTCCAAGCAGGCCGTAGGGCCGGTATGTTTATTATGGGATGATCAGATAGGACT
CGGGTGCTATTTTGTTGGTTTGCACATCTGAGTAATGATTAATAGGAACAGTCGGGGGTATCCGTATTTAGGAGCTAGAGGTG
AAATTCTTGGATTTCCGAAAGACGAACTACAGCGAAGGCATTTACCAAGCATGTTTTCATTAATCAAGAACGAAAGTCTGGGG
ATCGAAGATGATTAGATACCATCGTAGTCTAGACCGTAAACGATG-3'

Part 2: 5'- TTGCTTTGTCGGAAGGCATGGCTAATCCTTTGAACGCCCATCGTGCTGGGGCTAGATTTTTGCAATTATTA
ATCTCCAACGAGGAATTCCTAGTAAACGCAAGTCATCAGCTTGCATTGAATACGTCCCTGCCCTTTGTACACACCGCCCGTCG
CACCTACCGATTGAACGGTCCGATGAAACCATGGGACTACCTTTTGAGCGTTT -3'

3. Phylogenetic analysis base on rRNA gene sequencing data



Appendix C. Expert Panel Consensus Statement

Introduction

Runke Bioengineering (Fujian) Co., Ltd. ("Runke Bioengineering") convened a panel of independent scientists (the "Expert Panel"), qualified by their scientific training and relevant national and international experience to evaluate the safety of a food ingredient, to conduct a critical and comprehensive evaluation of the available pertinent data and information on docosahexaenoic acid (DHA) and to determine whether the proposed uses in food would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Expert Panel consisted of the following qualified experts: George C. Fahey, Ph.D. (Professor Emeritus, University of Illinois at Urbana-Champaign), Joanne Slavin, Ph.D., R.D. (Professor, University of Minnesota), and Susan S. Cho, Ph.D. (AceOne RS, Inc.).

The Expert Panel, independently and collectively, critically evaluated scientific information and data compiled from the literature. The Expert Panel evaluated other information deemed appropriate or necessary. To the best of our knowledge, this determination 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 The Generally Recognized as Safe (GRAS) status for the uses of this ingredient in food.

Common Knowledge Element of the GRAS Determination

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available through published, peer reviewed scientific papers related to the safety assessment. These scientific articles include published preclinical studies and human clinical studies as well as scientific review articles. The second common knowledge element required for a GRAS determination is consensus among qualified scientists that the safety of the proposed uses of the substance has been demonstrated. Numerous GRAS notifications were submitted to the U.S. FDA regarding the use of DHA as an ingredient in infant formulas and selected conventional foods. These include FDA's no question letters for infant formula applications (GRN 000553 - FDA, 2015; GRN 000677 - FDA, 2017; GRN 000731 - FDA, 2018a, GRNs 000776/000777 - FDA, 2018c, 2018d; GRN 000862 – FDA, 2020a; GRN 000933 – FDA, 2020b; GRN 000934 – FDA, 2021; GRN 001008-FDA, 2022) and selected conventional food applications (GRN 000137 - FDA, 2004; GRN 000732 - FDA, 2018b; GRN 000836 - FDA 2019a; GRN 000843/000844 – FDA, 2019b, 2019c; GRN 000862 – FDA, 2020a; GRN 000933 – FDA, 2020b; GRN 000934 – FDA, 2021; GRN 001008-FDA, 2022). These notifications all received 'no question' letters from the U.S. FDA. Exempt infant formula refers to formulas for pre-term infants only and does not include use in other exempt formulas (e.g., hypoallergenic formulas, formulas for inborn errors of metabolism). In addition, FDA issued

a final rule on menhaden oil ensuring daily intakes of EPA and DHA do not exceed 3 g/person/day (FDA, 2005).

The Expert Panel agrees that there are adequate data in the scientific literature to conclude that DHA is a common component of infant formulas, that various DHA-rich oils have been reviewed and approved as food ingredients for human use by the U.S. FDA and other expert panels, and that the weight of the available evidence demonstrates that the proposed uses are safe.

Technical Element of the GRAS Determination

DHA is a long-chain polyunsaturated fatty acid (LCPUFA) that is a primary structural component of the human brain, retina, and other tissues. DHA's structure is a 22-carbon chain carboxylic acid with 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. It can be obtained directly from maternal milk, algal oil, or fish oil.

Runke Bioengineering intends to market the DHA-rich oil as an ingredient in exempt (pre-term and/or low birth weight infants; amino acid- and/or extensively hydrolyzed protein-based) and non-exempt infant formulas (term infants; soy-, whey-, and/or milk-based; ages from birth to 12 months) in combination with a safe and suitable source of arachidonic acid (ARA). The maximum use level will be 0.5% of total fat as DHA. This level corresponds to a maximum use level of 1.43% of dietary fat as DHA-rich oil because it has ≥35% DHA. The ratio of DHA to ARA would range from 1:1 to 1:2. Runke Bioengineering's DHA-rich oil will be added to ready-to-drink or powder form of infant formulas from which reconstituted infant formulas can be prepared. The intended use level is similar to all other approved uses for incorporation of DHA or DHA-rich oil in infant formula (GRNs 000553, 000677, 000731, 000776, 000777, 000862, 000933, 000934, and 001008). In addition, Runke Bioengineering intends for the DHA-rich oil (containing ≥35% DHA) to be used in the same food categories as those listed in GRNs 000137 and 000732 and in 21 CFR 184.1472(a)(3) (menhaden oil), except in egg, meat, poultry, and fish products, at maximum use levels that are 28.57% of those specified in 21 CFR 184.1472(a)(3), which was finalized in 2005 (FDA, 2005).

Runke Bioengineering's DHA-rich oil is produced by a fermentative process using the non-toxigenic, non-pathogenic *Schizochytrium* sp. strain. All raw materials and processing aids used in the fermentation and manufacturing processes are food grade. Runke Bioengineering observes the principles of Hazard Analysis Critical Control Point (HACCP)-controlled manufacturing process and current Good Manufacturing Practices

(cGMP) and rigorously tests its final production batches to verify adherence to quality control specifications. Based on certificates of analysis (COAs), the Expert Panel concluded that Runke Bioengineering's DHA-rich oil meets specifications for chemical identity, fatty acid profile, and contaminants (heavy metals) and is free of contaminants such as domoic acid and monochloropropanediols (MCPDs) and glycidyl esters.

The bioequivalence of two types of algal DHA-rich oils (derived from either *Crypthecodinium cohnii* [DHASCO®] or *Schizochytrium* sp. [DHASCO-B®]) was demonstrated in preweaning farm piglets and in humans when administered in a blend with ARA oil (Fedorova-Dahms et al., 2014; Yeiser et al., 2016).

Animal Toxicity Studies

The DHA content of Runke Bioengineering's DHA-rich oil is at least 35% by weight, comparable to concentrations described in the previous GRAS notices (GRNs 000137, 000553, 000677, 000731, 000732, 000776, 000862, 000843, 000933, 000934, and 001008) which are acknowledged as GRAS by the FDA. The no-observed-adverse-effect-level (NOAEL) of Runke Bioengineering's DHA-rich oil was determined to be 5,000 mg/kg bw/day, the highest level tested in a battery of toxicity studies including a 90 day toxicity study with an in utero exposure (Lewis et al., 2016) and developmental and reproductive toxicity studies (Falk et al., 2017).

Other sources of DHA-rich oil and DHA-rich microalgae (DRM) have been evaluated by *in vitro* and *in vivo* genotoxicity studies, subchronic toxicity studies in rats with and without *in utero* phase, maternal and developmental toxicity in rats and rabbits, and reproductive and developmental toxicity in rats. DHA was reported as non-mutagenic and non-clastogenic in all studies conducted. In subchronic toxicity studies with an *in utero* phase, the NOAELs for F_1 ranged from 2,069 (females - Schmitt et al., 2012b) to 4,399 mg/kg bw/day (females - Fedorova-Dahms et al., 2011b) in rats. From reproductive and developmental toxicity studies of DHA-rich oils, the NOAELs for F_0 were found to range from 2,000 (Schmitt et al., 2012b) to 8,322 mg/kg bw/day (F_0 females during lactation) in rats (Fedorova-Dahms et al., 2011b).

However, in a reproductive and developmental toxicity study in rabbits by Hammond et al. (2001), both the high-dose (1,800 mg/kg/day) DRM and fish oil control groups experienced marked and sustained reduction in food consumption during the prenatal period and a slight increase in abortions. The NOAELs were determined to be 600 mg/kg bw/day for maternal toxicity and 1,800 mg/kg bw/day, the highest level tested, for developmental toxicity in rabbits (corresponding to 130 mg DHA-rich oil/kg bw/day for maternal toxicity and 392 mg DHA-rich oil/kg bw/day for developmental toxicity). However, the authors noted that

abortions occurred spontaneously more frequently in rabbits than in other commonly used laboratory species and that the incidences of abortions in both the high-dose DRM and fish oil control groups fell within the historical limits for the laboratory.

On the basis of these findings, the Expert Panel concluded that NOAEL of Runke Bioengineering's DHA-rich oil was 5,000 mg/kg bw/day in rats. However, in subchronic toxicity studies with an *in utero* phase, the NOAELs for F_1 ranged from 2,069 (females - Schmitt et al., 2012b) to 4,399 mg/kg bw/day (females - Fedorova-Dahms et al., 2011b) in rats.

Human Clinical Studies

Human clinical studies reported daily doses of DHA instead of DHA-rich oil. This review includes studies published between January 2021 and May 2023.

Studies of DHA in Adults

Since January 2021, no new studies of DHA from *Schizochytrium* sp. or algal sources have been published in adults. Previous GRAS notices reported that daily doses of up to 2 g DHA from algal sources were not associated with treatment-related adverse effects (Molfino et al., 2017, 2019; MacDonald and Sieving, 2018; Sanders et al., 2006; Smith et al., 2018) (GRN 000933 pages 41 and 44; GRN 001008, pages 61-62).

Studies of DHA in Pregnant Women and Offspring

Since January 2021, a few new studies of DHA derived from *Schizochytrium* sp. in pregnant women were published (Fougère et al., 2021; Garmendia et al., 2021). No adverse effects of DHA supplementation were reported on measured outcomes.

Overall, the review of recent human clinical trials is consistent with the conclusions of the previous GRAS notices (GRNs 000137, 000732, 000933, 000934, and 001008) that intake of DHA is safe as long as the daily intake does not exceed 1.5 g/person/day.

Term Infants

No studies published since January 2021 have been identified from the literature related to algal DHA intake in term infants. Previous GRAS notices stated that algal DHA, up to 0.96% of total fatty acids (or up to 51-61 mg DHA/kg bw/day), in combination with ARA was well tolerated, and no adverse effects were noted on the measured outcomes including gastrointestinal tolerance, adverse events, growth, RBC concentrations of fatty acids, visual acuity, cognitive function, and/or school readiness in both pre-term and term infants. In addition, studies of term infants have not reported adverse events or adverse effects on

allergies, tolerance, or adverse events associated with DHA-supplemented infant formulae when DHA was supplemented up to 0.96% of total fatty acids (Birch et al., 2010a, 2010b; Burks et al., 2008; D'Vaz et al., 2012; Fleddermann et al., 2014; Hoffman et al., 2008). Thus, it is concluded that the literature supports the intended use of DHA at 0.5% of total fatty acids in term infants.

Pre-term Infants

A few pre-term infant studies specifically discussed the effects of DHA supplementation on gastrointestinal adverse events or food allergy. These studies did not report adverse effects or events associated with DHA supplementation in pre-term infants (Clandinin et al., 2005; Manley et al., 2011). The studies by Gunaratne et al. (2019) and Manley et al. (2011) employed DHA from fish oil sources to evaluate allergy parameters in pre-term infants. As it is not expected that safety profiles of DHA derived from fish oil and algal oil would be different, the findings from studies employing DHA from fish oil sources are pertinent when evaluating the safety of DHA from algal oil. Thus, the findings from these 2 studies of DHA from fish oils were included as corroborative data to support the safety of algal DHA oil derived from *Schizochytrium* sp.

Recently published clinical trial by Bernabe-García et al. (2021), Frost et al. (2021), and Hewawasam et al. (2021) reported that daily doses up to 75 mg of DHA/kg bw (corresponding to 1.3% of total fatty acids as DHA) did not result in any adverse effects on whole blood long chain polyunsaturated fatty acid levels, cognition, or the incidence of necrotizing enterocolitis (NEC) and fatty acid profile of erythrocyte membranes from pre-term infants. In addition, GRNs 000553, 000677, 000731, 000776, 000777, 000862, 000933, 000934, and 001008 presented comprehensive summaries of clinical study literature regarding supplementation of DHA from algal oil sources to infant formula (FDA, 2015, 2017, 2018a, 2018c, 2018d, 2020a, 2020b, 2021, 2022). These GRAS notices concluded that supplementation of DHA (from *Schizochytrium* sp.), in combination with a safe source of ARA, to infant formula was safe in term and pre-term infants.

In summary, based on the substantial equivalence of Runke Bioengineering's DHA-rich oil to other algal DHA-rich oils whose safety has already been established, the intended use levels commensurate with safe dose levels tested in human clinical studies, animal toxicology studies, and mutagenicity and genotoxicity studies on various DHA-rich oil ingredients, and the history of safe use in humans, the Expert Panel concluded that Runke Bioengineering's intended use of its DHA-rich oil in term and pre-term infant formula and selected conventional foods is safe.

Conclusion

We, the undersigned members of the Expert Panel, have individually, collectively, and critically evaluated the materials summarized above on the safety of Runke Bioengineering's DHA-rich oil and other information deemed appropriate and unanimously conclude that Runke Bioengineering's DHA-rich oil, manufactured as described in the dossier and consistent with cGMP, and meeting appropriate food grade specifications, is GRAS based on scientific procedures for use as an ingredient in term and pre-term infant formula and selected conventional foods at levels specified in the accompanying dossier. It is our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions.

Expert Panel Members:

[redacted]

Susan Cho, Ph.D.
AceOne RS, Inc, Fairfax, VA

8/12/2023
Date

[redacted]

George C. Fahey, Jr, Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL

8/11/23
Date

[redacted]

Joanne Slavin, Ph.D.
Professor, University of Minnesota, St. Paul, MN

8-12-23
Date

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FDA Form 3667

Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001156	Sep 7, 2023
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

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

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

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

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Sunny Tsai	Export Manager

Organization *(if applicable)*: Runke Bioengineering (Fujian) Co., Ltd.

Mailing Address *(number and street)*: West of No. 552 Rd., Jindu Industrial Clusters Zone, Zhao'an

City	State or Province	Zip Code/Postal Code	Country
Zhangzhou	Fujian Province	363500	China

Telephone Number	Fax Number	E-Mail Address
86-754-86309891		wangyinan@runke.com.cn

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

Name of Contact Person	Position or Title
Susan Cho	Chief Science Officer

Organization *(if applicable)*: AceOne RS, Inc.

Mailing Address *(number and street)*: 5903 Hampton Forest Way

City	State or Province	Zip Code/Postal Code	Country
Fairfax	Virginia	22030	United States of America

Telephone Number	Fax Number	E-Mail Address
(301) 875-6454	(703) 998-0103	scho@aceoners.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Docosahexaenoic acid (DHA)-rich oil

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

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

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

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

☒ a) GRAS Notice No. GRN 000553

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

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

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

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

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

SECTION D – INTENDED USE

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

General Foods: Runke Bioengineering intends for the DHA-rich oil (containing 35% DHA) to be used in the same food categories as those listed in GRN 000137 (future intended use levels listed on pages 22-23; stamped page 27-28), GRN 000732 (pages 4-5), GRN 000933 (page 7), GRN 000934 (page 25) and GRN 001008 (page 24), and in 21 CFR 184.1472(a)(3) (menhaden oil), except in egg, meat, poultry, and fish products, at maximum use levels that are 28.57% of those specified in 21 CFR 184.1472(a)(3), which was finalized in 2005 (FDA, 2005). Runke Bioengineering intends for the DHA-rich oil will be used as the sole added source of DHA in any given food category, or if blended with a source of EPA, the total dietary exposure to DHA will be not more than 1.5 g/person/day (g/p/d) and

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

(Check one)

☐ Yes ☒ No

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

(Check one)

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

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

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

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

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

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

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

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

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

Other Information

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

☐ Yes ☒ No

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

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Runke Bioengineering (Fujian) Co., Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Docosahexaenoic acid (DHA)-rich oil
(name of notified substance)

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

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

West of No. 552 Rd., Jindu Industrial Clusters Zone, Zhao'an, Zhangzhou, Fujian Province 363500, China
(address of notifier or other location)

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

3. Signature of Responsible Official, Agent, or Attorney	**Printed Name and Title**	**Date (mm/dd/yyyy)**
	Susan Cho, Chief Science Officer, AceOne RS, Inc.	09/07/2023

SECTION G – LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	DHAcoverletter9-7-2023.pdf	Administrative
	DHA-GRAS-RunkeFinalSubmittedtoFDA9-7-2023.pdf	Administrative