April 2, 2024

Dr. Rachel Morissette
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 Dr. Rachel Morissette,

On behalf of Runke Bioengineering (Fujian) Co., Ltd. (Runke Bioengineering), we are resubmitting a GRAS notification for docosahexaenoic acid (DHA)-rich oil as a food ingredient. This is a resubmission of GRN 001156. 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,

April 2, 2024

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

# THE GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION OF DOCOSAHEXAENOIC ACID (DHA)-RICH OIL FROM SCHIZOCHYTRIUM SP. FJRK-SCH3 AS A FOOD INGREDIENT FOR USE IN INFANT FORMULA AND GENERAL FOODS

**Prepared for**

Runke Bioengineering (Fujian) Co., Ltd.
West of No. 552 Rd., Jindu Industrial Clusters Zone, Zhao'an, Zhangzhou,
Fujian Province 363500, China

**Prepared by**

Susan Cho, Ph.D., Chief Science Officer, AceOne RS, Inc., Centreville, VA
George C. Fahey, Jr., Ph.D., Professor Emeritus, University of Illinois-Urbana
Joanne Slavin, Ph.D., R.D., Professor, University of Minnesota, St. Paul, MN

# GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF DOCOSAHEXAENOIC ACID (DHA)-RICH OIL FROM SCHIZOCHYTRIUM SP. FJRK-SCH3 AS A FOOD INGREDIENT FOR USE IN INFANT FORMULA AND GENERAL FOODS

## Table of Contents

## Tables



## Figures

**List of Abbreviations**

ACAT2 = acyl-Coenzyme A:cholesterol acyltransferase 2
ADI = acceptable daily intake
ALP = alkaline phosphatase
ALT = alanine aminotransferase
AOAC = Association of Official Analytical Chemists
AOCS = American Oil Chemists' Society
aPTT = activated partial thromboplastin time
ARA = arachidonic acid
AST = aspartate aminotransferase
BAM = Bacteriological Analytical Manual
BMI = body mass index
bw = body weight
CAS = chemical abstract service
CFR = Code of Federal Regulations
cGMP = current Good Manufacturing Practices
COAs = Certificates of Analysis
DGLA = dihomo-γ-linolenic acid
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 daily intake
EFSA = European Food Safety Authority
EPA = eicosapentaenoic acid
FA = fatty acid
FAO = Food and Agriculture Organization
FCC = Food Chemicals Codex
FD&C = Federal Food, Drug, and Cosmetic
FDA = Food and Drug Administration
FR = Federal Register
FVII = Factor VII
GD = gestational days
GGT = gamma-glutamyl transferase
GMO = genetically modified organisms
GOS = galactooligosaccharide
GRAS = Generally Recognized as Safe
HACCP = Hazard Analysis Critical Control Point
HMG-CoA = 3-hydroxy-3-methylglutaryl-CoA

HOMA-IR = Homeostasis Model Assessment for Insulin Resistance
hsCRP = high-sensitivity C-reactive protein
IL = interleukin
IMCAS = Institute of Microbiology Chinese Academy of Sciences
ISO = International Standardization Organization
LCPUFA = long-chain polyunsaturated fatty acid
$LD_{50}$ = median lethal dose
LDH = lactate dehydrogenase
LDL-C = low-density lipoprotein cholesterol
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
MCPD = monochloropropanediol
MCV = mean corpuscular volume
MIGHT = Maternal obesIty/overweight control throuGh Healthy nuTrition
MN = micronucleated
MPV = mean platelet volume
NEC = necrotizing enterocolitis
NFMOA = National Fish Meal and Oil Association
NHANES = National Health and Nutrition Examination Survey
NOAEL = no-observed-adverse-effect-level
NA = not available
ND = not detected
NR = not reported
NS = not specified
OECD = Organisation for Economic Co-operation and Development
OGTT = oral glucose tolerance test
PAH = polycyclic aromatic hydrocarbon
PCB = polychlorinated biphenyl
PCTL = percentile
PCE = polychromatic erythrocyte
PT = prothrombin time
PUFA = polyunsaturated fatty acid
QC = quality control
RBC = red blood cell
rRNA = ribosomal ribonucleic acid
SDH = sorbitol dehydrogenase
TG = triglyceride
TNFα = tumor necrosis factor-alpha
U.S. = United States

U.S.C. = United States Code
USDA = United States Department of Agriculture
USP = United States Pharmacopeia
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
Tel: +86-754-86309891
E-mail: wangyinan@runke.com.cn or sales@runke.com.cn

### 1.C. Common or Trade Name

Docosahexaenoic acid-rich oil from *Schizochytrium* sp. FJRK-SCH3, DHA-rich oil from *Schizochytrium* sp. FJRK-SCH3, DHA-rich oil, docosahexaenoic acid-rich algal oil, DHA-rich 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 (GRNs) for algal oil derived from *Schizochytrium* sp. (GRNs 000137, 000732, and 001008) for which the United States Food and Drug Administration (U.S. 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 from GRN 000137 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 (U.S. FDA, 2005). Runke Bioengineering's 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 eicosapentaenoic acid (EPA), the total dietary exposure to DHA will be not more than 1.5 g/person/day and not more than 3.0 g/person/day 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 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 dairy such as bovine or goat milk-based; ages from birth to 12 months) in combination with a safe and suitable source of ARA. This level corresponds to 1.43% of total dietary fat providing 28-39 mg DHA/kg body weight (bw)/day (or 80-111 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 contains ≥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 000067, page 6; GRN 000731, page 5; GRN 000776, page 3; GRN 000777, page 3; GRN 000933, 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.

**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 (Table 1).

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 the U.S. FDA upon request by contacting Susan Cho at AceOne RS, Inc. or Sunny Tsai at Runke Bioengineering at the address above. The data and information will be made available to the U.S. 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: April 1, 2024
Title: Export Manager

Address correspondence to
Susan S. Cho, Ph.D.
Lead Expert Panel Member
AceOne RS, Inc.
14631 Route 29, Suite 313
Centreville, VA 20121
Tel: +1-301-875-6454
scho@aceoners.com or Susanscho1@yahoo.com

**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 from *Schizochytrium* sp. FJRK-SCH3, DHA-rich oil from *Schizochytrium* sp. FJRK-SCH3, Docosahexaenoic acid-rich oil, DHA-rich oil, docosahexaenoic acid-rich algal oil, DHA-rich algal oil, DHA algal oil, DHA oil, docosahexaenoic acid-rich single-cell oil, or DHA single cell oil.

#### 2.A.1.2. Chemical Names

Its systematic name is *all-cis*-docosa-4,7,10,13,16,19-hexa-enoic acid (22:6) (Figure 1) esterified to glycerol and its shorthand name is 22:6(n-3).

#### 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) have several CAS numbers including 32765-69-8.

#### 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 g/mol

#### 2.A.1.6. Structural Formula

Figure 1 shows the structure of DHA. Docosahexaenoic acid is a long chain, polyunsaturated fatty acid (PUFA), 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).

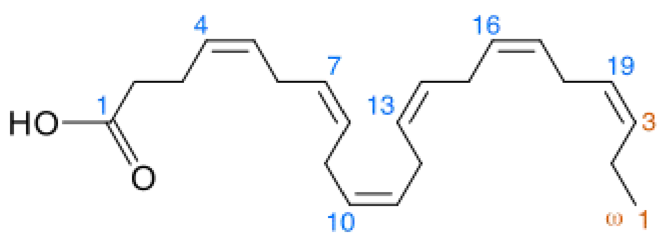



Figure 1. Structure of Docosahexaenoic Acid (DHA)

**2.A.1.7. Physical Properties**

Density of DHA, 0.943 g/cm$^3$

**2.A.1.8. Background**

Docosahexaenoic acid is a long-chain PUFA 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 42.5%. The Certificates of Analysis (COAs) for DHA-rich oil are presented in Appendix A.

<u>Shellfish Poison</u>

No amnesic shellfish poison (domoic acid) was found in Runke Bioengineering's DHA-rich oil (see Table 2 and 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 the manufacturing process, it is not expected to have significant amounts of MCPDs and glycidyl esters in the DHA-rich oils. Analysis of 3 batches showed that the concentrations of MCPDs (2- and 3-MCPD) and glycidyl esters were near or below detection levels in the Runke Bioengineering's DHA-rich oil (Table 2). 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) (Table 2; Appendix A).

Overall, Runke Bioengineering's DHA-rich oil is not expected to have a safety risk associated with potential contaminants such as shellfish poison, MCPDs, 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**

<u>DHA-Rich Oil Manufacturing Process</u>

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 and 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 DHA is produced by a heterotrophic fermentation process with a marine micro-algae of the genus *Schizochytrium* sp. (strain 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

Once the fermentation is complete, the fermentation broth is transferred to the shear agitator, and then the fermentation broth is heated to 50-100℃ with stirring at 50-300 rpm for 4-20 hours to break algal cells, followed by centrifugation to separate crude DHA-rich oil from algal cells. The resulting supernatant, also known as the DHA-rich crude oil, is collected in stainless steel tanks and overlaid with nitrogen.

c. Refining

For caustic refining, sodium hydroxide is added to crude DHA oil and stirred at 60-80℃ for a period of time. The resulting gums (hydrated phosphatides) and soap stock (neutralized fatty acids) are removed by settling or centrifugation. Crude oil is heated to 70-90℃ and is washed with pure hot water (70-100℃) to remove the water-soluble impurities that could not be removed in the settling (or precipitation) or centrifugation process. In the process of oil washing, sodium sulfate can be added according to 0.1-1% of the amount of alkali to prevent the emulsification of oil, and the excess water is removed by vacuum after the oil washing is completed.

The oil phase is collected and is bleached with activated clay. The clay is removed using a stainless-steel plate filter. The bleached oil is chilled and is held to crystallize any remaining waxes, as necessary to achieve the desired level of clarity. Solids from this step may be removed by centrifugation and/or filtration. Winterized oil is deodorized with steam at high temperature under vacuum. The product is then cooled, and tocopherols and sunflower oil are added to prevent oxidation and standardize DHA content. Filtration is the last refining step after the addition of safe and suitable antioxidants (vitamin E and ascorbyl palmitate) to ensure stability.

d. Packaging

The product is packaged in airtight containers. Figure 2 presents the 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 are used in accordance with applicable regulations, are GRAS for their intended use, or are the subject of an effective food contact notification. They are commonly used in food ingredient manufacturing processes and all production processes used are processes traditionally used in food manufacturing.

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 are also 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

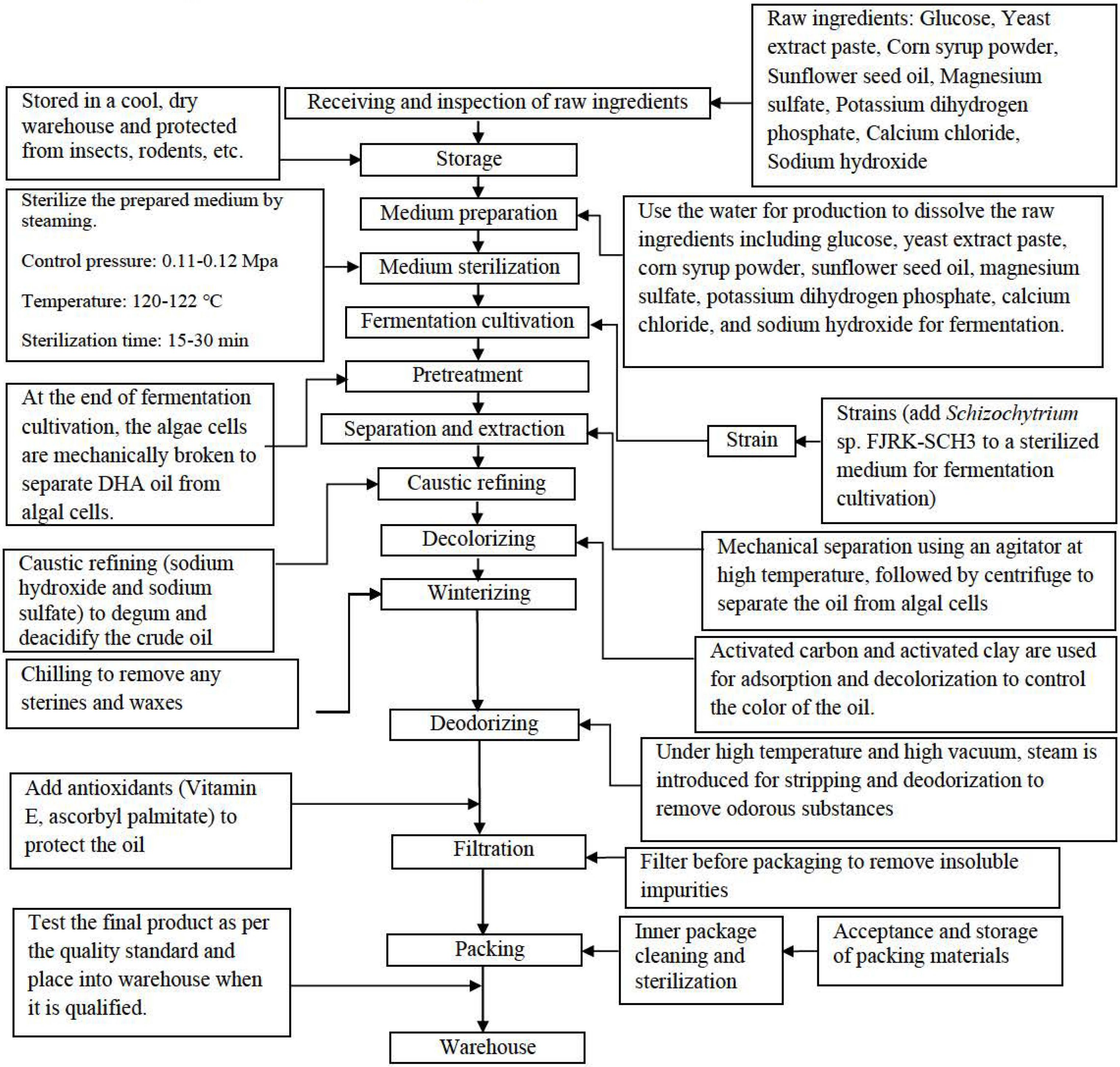



Figure 2. Manufacturing Flow Diagram of DHA-Rich Oil

The production method (algal fermentation) is similar to those described by other companies whose production methods for DHA-rich oil received 'no question' letters from the U.S. FDA (GRN 000137 – U.S. FDA, 2004; GRN 000553 – U.S. FDA, 2015; GRN 000677 – U.S. FDA, 2017; GRN 000731/000732 – U.S. FDA, 2018a, 2018b; GRN 000776/000777 – U.S. FDA, 2018c, 2018d; GRN 000836 – U.S. FDA 2019a; GRN 000843 – U.S. FDA 2019b; GRN 000844 – U.S. FDA 2019c; GRN 000862 – U.S. FDA 2020a; GRN 000933 – U.S. FDA 2020b; GRN 000934 – U.S. FDA 2021; GRN 001008 – U.S. 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 a non-toxigenic and non-pathogenic strain of the marine alga *Schizochytrium* sp.

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. 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).

*Schizochytrium* sp. is a thraustochytrid and a member of the Chromista kingdom (Stramenopila). *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 *Schizochytrium* sp. 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 humans 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. Chemical analysis of the finished DHA-rich oil ingredient confirmed the absence of common shellfish toxin, domoic acid (Table 2; Appendix A). The taxonomic classification of *Schizochytrium* sp. FJRK-SCH3 is presented in Table 3.

Table 3. 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 (FAs), trans-FAs, unsaponifiable content, peroxide value, p-anisidine value, moisture, and heavy metals (arsenic, cadmium, mercury, and lead), docosapentaenoic acid (DPA, n-6), ARA, eicosapentaenoic acid (EPA), and microbiology parameters (such as *Escherichia coli, Cronobacter* species, and *Salmonella*) (Table 4). Physical and chemical tests applied to the quality control (QC) characterization of the oil are mostly adapted from American Oil Chemists' Society (AOCS), Association of Official Analytical Chemists (AOAC), or the Official Methods and Recommended Practices of International Standardization Organization (ISO). All analytical methods were validated for their intended use.

Table 4 presents the specifications of Runke Bioengineering's DHA-rich oil in comparison with those described in GRNs 000137 (page 21, stamped page 26), 000553 (pages 17-18, stamped pages 23-24), 000677 (page 15), 000731 (page 18), and 000933 (pages 17-18). The specifications of Runke Bioengineering's DHA-rich oil were also compared with the 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-rich oil to preweaning farm piglets (Fedorova-Dahms et al., 2014) and human infants (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, 2023).

Table 5 summarizes the analytical values for Runke Bioengineering's DHA-rich oil. Three non-consecutive lots of DHA-rich oil were subjected to analytical testing for various parameters. These data demonstrate a reproducible and representative process capable of meeting the proposed product specifications. Analytical results ensured that Runke

Bioengineering's DHA-rich oil meets the specifications. The specifications for DHA, free fatty acid (as % oleic acid), unsaponifiable matter, peroxide value, anisidine value, total oxidation value, DPA, ARA, and EPA meet or exceed the FCC standards (Table 5).

Algal oil (≥35% DHA) consists of a mixture of TGs where the predominant fatty acid is DHA (on average ~42.5% of total FAs). 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.

Fatty Acid Composition

The identified components present in DHA-rich oil have a demonstrated history of safe consumption. Tables 6 and 7 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 (15.5-16.4%) and DPA n-6 (10.6-12.3%) are the predominant FAs next to DHA (Tables 6 and 7). Minor FAs include oleic acid (3.5-4.0%), stearic acid (1.32-1.35%), ARA(0.19-0.23%), and EPA (0.37-0.46%). All FAs present in DHA-rich oil are present in the diet from vegetable and animal sources and, thus, do not pose a safety concern.

The analytical values for DPA are comparable between the subject of this GRAS notice (10-15%) and FCC specifications for algal DHA oil derived from *Schizochytrium* sp. (10.5-16.5%). Compared to the specifications listed in the FCC monograph for algal oil from *Schizochytrium* sp., the levels of dihomo-γ-linolenic acid (DGLA), ARA, and EPA in Runke Bioengineering's DHA-rich oil are below the FCC specifications for corresponding FAs (Runke Bioengineering vs. $FCC_{\textit{Schizochytrium}\ \text{sp.}}$: DGLA, 0.25 vs. 1.7-2.8%; ARA, 0.21 vs. 0.6-1.3 %; EPA, 0.42 vs. 1.3-3.9). Thus, the resulting exposure to DGLA, ARA, EPA, and DPA in the finished product will be equal to or less than oils that comply with the FCC specifications, whereas the amount of DHA exceeds the DHA specification in the FCC monograph. It is noteworthy that the subject of this notice is intended to be used as a source of DHA, consumers will be exposed to equal to or less amounts of DLGA, ARA, EPA, and DPA when the subject of this GRAS Notice is used to deliver the same target DHA level as algal oils that comply with the specifications listed in the FCC monograph. Because the oil contains similar amounts of these long chain PUFAs (the sum of DHA, DLGA, ARA, EPA, and DPA) compared to FCC specifications (Runke Bioengineering vs. $FCC_{\textit{Schizochytrium}\ \text{sp}}$: 53.1-56.2 vs. ~54.5%), the differences in FA profile are not expected to impact the stability or the amount of antioxidants added to the DHA-rich oil to maintain stability. Overall, differences in FA profile in the DHA, DGLA, ARA, EPA, and DPA content compared to food-grade algal oil meeting the FCC specifications do not affect Runke Bioengineering's conclusion that the subject of this notice is safe for intended use.

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 Runke Bioengineering's DHA-rich oil (smaller amounts compared to FCC-grade algal oil) would not impact the safety of the oil.

Table 4. Specifications of DHA-Rich Oil

| Parameter | Current notice | GRN 137[a] | GRN 553[b] | GRN 677[b] | GRN 731[b] | GRN 933 | GRN 1008 | FCC[c] | FCC[d] | Methods of Analysis for the Current Notice |
|---|---|---|---|---|---|---|---|---|---|---|
| DHA, % | ≥35 | 32 – 45[f] | ≥35[f] | ≥35[f] | >45[e] | ≥36[e] | ≥45 | ≥30 [f] | 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 | ≤0.5 | NS | | AOCS Cd 3d-63 |
| Free fatty acid, as % oleic acid | ≤0.4 | | ≤0.4 | | < 0.1 | ≤0.4 | ≤0.4 | ≤0.4 | ≤0.4 | AOCS Cd 3d-63 |
| Trans FAs, relative area % | ≤1.0 | ≤2.0 | ≤3.5 | ≤2.0 | <1.0 | ≤1.0 | ≤2.0 | NS | | AOCS 996.06 mod. |
| Unsaponifiable matter, % | ≤3.5 | ≤4.5 | ≤3.5 | ≤3.5 | <1.0 | ≤3.0 | ≤3.5 | ≤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 | ≤5.0 | AOCS Cd 8b-90:2017 |
| p-Anisidine value | ≤20.0 | NS | NS | NS | NS | NS | NS | ≤20.0 | NS | AOCS Cd 18-90 |
| Total oxidation value | ≤20 | NS | NS | NS | NS | NS | NS | ≤26 | NS | |
| Moisture (direct drying method), wt% | ≤0.1 | ≤0.1 | ≤0.02 | ≤0.05 | <0.1 | ≤0.1 | ≤0.05 | NS | NS | AOCS Ca 2c-25 |
| Lead, ppm | ≤0.1 | ≤0.2 | ≤0.1 | <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 | NS | ≤0.1 | |
| Cadmium, ppm | ≤0.1 | | ≤0.1 | | <0.1 | ≤0.1 | ≤0.2 | NS | NS | |
| Mercury, ppm | ≤0.04 | <0.2 | ≤0.04 | <0.1 | <0.01 | ≤0.04 | ≤0.04 | ≤0.1 | ≤0.1 | BS EN 13806:2002 |
| DPA, n-6, % | ≤16.5 | 10 - 20 | NS | NS | NS | NS | NS | ≤16.5 | 0-0.1 | AOAC 996.06 mod. |
| ARA, % | ≤1.3 | NS | NS | NS | NS | NS | NS | ≤1.3 | | AOAC 996.06 mod. |
| EPA, % | ≤2.0 | NS | ≤10 | NS | NS | NS | NS | ≤3.9 | 0-0.1 | AOAC 996.06 mod. |
| *Escherichia coli*/25 g | Absent in 25 g | NS | NS | NS | NS | NS | NS | NS | NS | ISO 16649-3:2015 |
| Cronobacter sp./10 g | ND in 10g | NS | NS | NS | NS | NS | NS | NS | NS | ISO 22964:2017 |
| Salmonella/25 g | Absent in 25 g | NS | NS | NS | NS | NS | Absent in 375 g | NS | NS | U.S. FDA BAM Ch 5, April 2001 |

[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%.
Abbreviations: 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; ND = not detected; NS = not specified.
Sources: GRN 000137, p 24 (stamped p 29); GRN 000553, p 17 (stamped p 23); GRN 000677, p 15; GRN 000731, p 18; GRN 000933, p 17; DHA specification, GRN 001008, DHA from Agency response letter and other parameters from p 4 of the amendment dated 11/3/21.

Table 5. 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 FAs as oleic acid, % | 0.12 | 0.19 | 0.11 | 0.01 | 0.14 |
| Free FAs, % | 0.08 | 0.10 | 0.08 | 0.01 | 0.09 |
| Total trans FAs, % | 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 |
| Total oxidation value | 9.52 | 8.76 | 10.08 | | 9.4 |
| 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 |
| *Escherichia coli*/25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | | Absent in 25 g |

| | | | | | |
|---|---|---|---|---|---|
| *Cronobacter* sp./10 g | ND in 10 g | ND in 10 g | ND in 10 g | | ND in 10 g |
| Salmonella/25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | | Absent in 25 g |

*Samples were taken from 3 non-consecutive batches. Total oxidation values were calculated using the following formula: anisidine value +2 peroxide value = total oxidation value.

Abbreviations: DHA = docosahexaenoic acid; FA = fatty acids; LOQ = limit of quantitation; ND=not detected.

Table 6. Fatty Acid Profile and Glyceride Composition of Runke Bioengineering's DHA-Rich Oil

| Parameters, wt% | Lot number | | | Mean | Range |
|---|---|---|---|---|---|
| | 11024713 | 11027715 | 11030717 | | |
| C16:4 (Hexadecatetraenoic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C10:0 (Capric acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C11:0 (Undecanoic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C12:0 (Lauric acid) | 0.04 | 0.03 | 0.04 | 0.04 | 0.03-0.04 |
| C14:0 (Myristic acid) | 0.31 | 0.29 | 0.36 | 0.32 | 0.31-0.36 |
| C14:1 (Myristoleic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C15:0 (Pentadecanoic acid) | 0.05 | 0.04 | 0.06 | 0.05 | 0.04-0.06 |
| C15:1 (Pentadecenoic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C16:0 (Palmitic acid) | 15.93 | 15.53 | 16.36 | 15.94 | 15.5-16.4 |
| C16:1 Omega 7 | 0.09 | 0.08 | 0.09 | 0.09 | 0.08-0.09 |
| C16:1 Total (Palmitoleic acid + isomers) | 0.26 | 0.23 | 0.26 | 0.25 | 0.23-0.26 |
| C16:2 (Hexadecadienoic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C16:3 (Hexadecatrienoic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C17:0 (Margaric acid) | 0.06 | 0.05 | 0.06 | 0.06 | 0.05-0.06 |
| C17:1 (Heptadecenoic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C18:0 (Stearic acid) | 1.35 | 1.32 | 1.33 | 1.33 | 1.32-1.35 |
| C18:1 (Vaccenic acid) | 0.17 | 0.15 | 0.16 | 0.16 | 0.15-0.17 |
| C18:1 Omega 9 (Oleic acid) | 3.88 | 4.05 | 3.54 | 3.82 | 3.54-4.05 |
| C18:1 Total (Oleic acid + isomers) | 4.09 | 4.24 | 3.75 | 4.03 | 3.75-4.24 |
| C18:2 Omega 6 (Linoleic acid) | 8.24 | 9.13 | 7.50 | 8.29 | 7.50-9.13 |
| C18:2 Total (Linoleic acid + isomers) | 8.46 | 9.32 | 7.81 | 8.53 | 7.81-9.32 |

| | | | | | |
|---|---|---|---|---|---|
| C18:3 Omega 3 (Alpha linolenic acid) | 0.12 | 0.13 | 0.12 | 0.12 | 0.12-0.13 |
| C18:3 Omega 6 (Gamma linolenic acid) | 0.13 | 0.11 | 0.14 | 0.13 | 0.11-0.14 |
| C18:3 Total (Linolenic acid + isomers) | 0.25 | 0.25 | 0.26 | 0.25 | 0.25-0.26 |
| C18:4 Omega 3 (Octadecatetraenoic acid) | 0.19 | 0.19 | 0.21 | 0.20 | 0.19-0.21 |
| C18:4 Total (Octadecatetraenoic acid) | 0.19 | 0.19 | 0.21 | 0.20 | 0.19-0.21 |
| C20:0 (Arachidic acid) | 0.24 | 0.21 | 0.24 | 0.23 | 0.21-0.24 |
| C20:1 Omega 9 (Gondoic acid or 11-eicosenoic acid) | 0.02 | 0.03 | 0.03 | 0.03 | 0.02-0.03 |
| C20:1 Total (Gondoic acid + isomers) | 0.04 | 0.05 | 0.06 | 0.05 | 0.05-0.06 |
| C20:2 Omega 6 | <0.02 | <0.02 | 0.03 | <0.02 | <0.02-0.03 |
| C20:2 Total (Eicosadienoic acid) | <0.02 | <0.02 | 0.03 | <0.02 | <0.02-0.03 |
| C20:3 Omega 3 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| **C20:3 Omega 6 (DGLA)** | **0.26** | **0.20** | **0.28** | **0.25** | **0.20-0.28** |
| C20:3 Total (Eicosatrienoic acid) | 0.26 | 0.20 | 0.28 | 0.25 | 0.20-0.28 |
| C20:4 Omega 3 | 0.61 | 0.52 | 0.62 | 0.58 | 0.52-0.61 |
| **C20:4 ARA, Omega 6** | **0.19** | **0.22** | **0.23** | **0.21** | **0.19-0.23** |
| C20:4 Total (Eicosatetraenoic acid) | 0.80 | 0.73 | 0.85 | 0.79 | 0.73-0.85 |
| **C20:5 EPA, Omega 3** | **0.42** | **0.46** | **0.37** | **0.42** | **0.37-0.46** |
| C21:5 Omega 3 (Heneicosapentaenoic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C22:0 (Behenic acid) | 0.22 | 0.20 | 0.24 | 0.22 | 0.20-0.24 |
| C22:1 Omega 9 (Erucic acid) | 0.28 | 0.21 | 0.35 | 0.28 | 0.21-0.35 |
| C22:1 Total (Erucic acid + isomers) | 0.28 | 0.21 | 0.35 | 0.28 | 0.21-0.35 |
| C22:2 Docosadienoic Omega 6 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C22:3 Docosatrienoic, Omega 3 | 0.16 | 0.12 | 0.17 | 0.15 | 0.12-0.17 |
| C22:4 Docosatetraenoic Omega 6 | <0.02 | <0.02 | 0.02 | 0.02 | <0.02-0.02 |
| C22:5 Docosapentaenoic Omega 3 | 0.08 | 0.07 | 0.08 | 0.08 | 0.07-0.08 |
| **C22:5 DPA, Omega 6** | **12.31** | **10.60** | **12.60** | **11.84** | **10.6-12.3** |
| C22:5 Total Docosapentaenoic acid (DPA) | 12.40 | 10.68 | 12.68 | 11.92 | 10.7-12.7 |
| **C22:6 DHA, Omega 3** | **43.01** | **41.71** | **42.76** | **42.49** | **41.7-43.0** |
| C24:0 (Lignoceric acid) | 0.13 | 0.11 | 0.13 | 0.12 | 0.11-0.13 |

| | | | | | |
|---|---|---|---|---|---|
| C24:1 Omega 9 (Nervonic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C24:1 Total (Nervonic acid + isomers) | 0.10 | 0.04 | 0.07 | 0.07 | 0.04-0.07 |
| C4:0 (Butyric acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C6:0 (Caproic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| C8:0 (Caprylic acid) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| Total Fat as Triglycerides | 92.85 | 89.86 | 92.47 | 91.73 | 89.9-92.9 |
| Total FAs | 89.13 | 86.26 | 88.77 | 88.05 | 86.3-89.1 |
| Glycerides Composition | | | | | |
| Diglycerides | 3.9 | 4.7 | 3.7 | 4.1 | 3.7-4.7 |
| Glycerol | 2.8 | 2.9 | 2.7 | 2.8 | 2.7-2.9 |
| Monoglycerides | 2.2 | 3.2 | 1.8 | 2.4 | 1.8-3.2 |
| Triglycerides | 94.2 | 92.1 | 94.5 | 93.6 | 92.1-94.5 |

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

Abbreviations: ARA = arachidonic acid; DGLA = dihomo-γ-linolenic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; FAs = fatty acids; LOQ = limit of quantitation.

Table 7. 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 | GRN 1008 | FCC[c,*] | FCC[d,*] |
|---|---|---|---|---|---|---|---|---|---|
| DHA (Docosahexaenoic acid) specifications | **≥35** | 32 - 45 | ≥35 | ≥35 | >45 | ≥36 | ≥45 | **≥30** | **35-47** |
| Actual content, % | **41.7-43.0** | 35.0 | 43.3 | 40.22 | 50.7 | 38.87 | 57.32 | | |
| Other Fatty Acids, g/100g | | | | | | | | | |
| C 20:3n6 (homo-gamma-Linolenic acid) | **0.25** | | <0.1 | <0.11 | 0.21 | 0.19 | 0.25 | **≤2.8** | **0-0.1** |
| C 20:4n6 (Arachidonic acid; ARA) | **0.19-0.23** | 0.94 | 0.69 | 0.70 | 0.15 | 1.01 | 0.14 | **≤1.3** | |
| C 20:5n3 (Eicosapentaenoic acid; EPA) | **0.37-0.46** | 2.63 | 6.23 | 1.18 | 0.70 | 0.31 | 0.47 | **≤3.9** | **0-0.1** |
| C 22-5n6 (Docosapentaenoic acid; DPA) | **10.6-12.3** | 13.5 | 2.53 | 7.81 | 10.33 | 8.76 | 13.95 | **≤16.5** | **0-0.1** |
| Sum of DHA, DGLA, ARA, EPA and DPA | **53.1-56.2** | | | | | | | **~54.5** | **35-47.3** |
| 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 | 0.06 | | |
| C 14:0 (Myristic acid) | 0.32 | 10.11 | 1.18 | 11.87 | 0.82 | 1.29 | 0.55 | | |
| 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 | 0.08 | | |
| C 15:1 (Pentadecenoic acid) | <0.02 | | | | 0.07 | <0.02 | ND | | |
| C 16:0 (Palmitic acid) | 15.94 | 23.68 | 13.78 | 25.43 | 20.96 | 26.20 | 18.26 | | |
| C 16:1 (Palmitoleic acid) | 0.09 | 1.76 | <0.10 | 3.42 | 0.51 | 0.19 | 0.23 | | |
| C 17:0 (Margaric acid or Heptadecanoic acid) | 0.06 | | <0.10 | <0.12 | 0.08 | 0.84 | 0.08 | | |
| C 18:0 (Stearic acid) | 1.33 | 0.45 | 1.65 | 0.82 | 1.30 | 1.12 | 1.06 | | |
| C 18:1 (Oleic acid) | 3.82 | NA | | 4.77 | 0.27 | 1.83 | 1.27 | | |
| 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 | 1.77 | NA | 0-1.0 |
| C 18:3n3 (alpha-Linolenic acid) | 0.12 | | <0.10 | NA | 0.14 | 0.48 | 0.18 | | |
| C 18:3n6 (gamma-Linolenic acid) | 0.13 | | NA | 0.23 | 0.09 | 0.12 | 0.10 | | |
| C 20:0 (Arachidic acid) | 0.23 | | 0.32 | <0.10 | 0.29 | 0.20 | 0.23 | | |
| C 20:1 (Eicosenoic acid) | | | | <0.06 | < 0.02 | <0.03 | | | |
| C 20:2n6 (Eicosadienoic acid) | <0.02 | | 0.13 | | < 0.02 | <0.03 | ND | | |
| C 20:3n3 (Eicosatrienoic acid) | <0.02 | | <0.1 | | 1.34 | <0.03 | ND | | |
| C 21:0 (Heneicosanoic acid) | | | | | 0.04 | | | | |
| C 22:0 (Behenic acid) | 0.22 | | | <0.10 | 0.15 | 0.12 | 0.18 | | |
| C 22:1n9 (Erucic acid) | 0.28 | | | | < 0.02 | | | | |
| C 22:2n6 (Docosadienoic acid) | <0.02 | | 0.53 | | < 0.02 | <0.02 | ND | | |
| C 22-5n3 (Docosapentaenoic acid) | 0.08 | | 0.76 | | 0.11 | 0.08 | 0.14 | | |
| C 23:0 (Tricosanoic acid) | | | | | < 0.02 | | | | |
| C 24:0 (Lignoceric acid) | 0.12 | | | <0.10 | 0.15 | <0.054 | ND | | |
| C 24:1 (Nervonic acid) | <0.02 | | | | 0.41 | <0.02 | ND | | |

[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%.
Abbreviations: FCC = Food Chemicals Codex; NA = not available; ND = not detected.
Sources: GRN 000137, p 24 (stamped p 29); GRN 000553, p 18-20 (stamped p 24-26); GRN 000677, p 20; GRN 000731, p 20-21; GRN 000933, p 20-23; GRN 001008, p 16-18.

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 8). Total aerobic plate counts, yeast, molds, and Enterobacteriaceae counts are below the detection limit (<10 cfu/g). COAs are presented in Appendix A.

Table 8. 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 | U.S. FDA BAM Ch 3, Jan 2001 |
| Yeast, cfu/g | <10 | <10 | <10 | U.S. 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 in 10 g | ND in 10 g | ND in 10 g | ISO 22964:2017 |
| Salmonella/25 g | Absent in 25 g | Absent in 25 g | Absent in 25 g | U.S. FDA BAM Ch 5, April 2001 |
| Enterobacteriaceae, cfu/g | <10 | <10 | <10 | ISO 21528-2-2017 |

Abbreviations: BAM = Bacteriological Analytical Manual; cfu = colony forming units; Ch = chapter; ISO = International Standardization Organization; ND = not detected.

Sterols

Total plant sterol and stanol (wt%/v) content in Runke Bioengineering's DHA-rich oil was 0.571 wt% (Tables 9 and 10). Cholesterol was the most abundant sterol (0.32 wt%), followed by sitosterol (0.112), delta-7-stigmastenol (0.054), stigmasterol (0.031), brassicasterol (0.018), and delta-7-avenasterol (0.01). Table 10 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). The total sterol level is comparable to the average total sterol values calculated from the values reported in GRN 000553 (0.54 wt%) and GRN 000677 (0.15 wt%), but much lower than the value reported in GRN 000137 (3.1 wt%) (Table 10). It is noteworthy that GRN 000137 reported much higher total sterol concentrations compared to other GRAS notices. The mean total sterol intake was estimated to be between 41−66 mg/day in infants aged 0.5 to 5 months old consuming infant formulas (Claumarchirant et al., 2015).

As stated in GRN 000137 (stamped page 14), the lipid fraction of *Schizochytrium* sp. algae is comprised mainly of FAs 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. However, all the sterols that are present in the subject of this GRAS determination were not directly quantified to compare 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, sitostanol, 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 identify, 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. Although Runke Bioengineering was not able to quantify all of the sterols that are present in its DHA-rich oil, the sterols found in the subject of this notice and other *Schizochytrium* sp.-derived DHA-rich oils that are GRAS are normal components of the diet such as human milk, infant formula, and edible oils used to formulate infant formula and/or other foods.

The cholesterol content of Runke Bioengineering's DHA-rich oil is much higher than those reported in GRN 000553 and 000677, but 59% lower than that reported in GRN 000137 (0.320 vs. 0.775 wt% of fat). Cholesterol is a normal component of diet and is present in human milk, infant formula, and edible oils used to formulate infant formula and/or other foods. The safety of dietary cholesterol and phytosterols is well documented in the scientific literature (Brownawell and Falk, 2010).

In summary, FAs (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.

Table 9. 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.

Table 10. 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%).
Abbreviation: NR = not reported.

**2.D. Stability**

Three non-consecutive lots of DHA-rich oil filled with nitrogen in tightly closed original aluminum containers were stored at ≤ 25°C and -10°C for testing of DHA content, acid value, peroxide value, and anisidine value every four months. As shown in Table 11, Runke Bioengineering's DHA-rich oil was 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 11. 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 Runke Bioengineering's DHA-rich oil and powder.

**2.F. Allergens**

Raw materials used in production contain 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 Runke Bioengineering's DHA-rich oil would be allergenic.

**2.G. Intended Technical Effects**

Runke Bioengineering's DHA-rich oil will be used as a food ingredient in selected conventional foods and 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 ARA.

# PART 3. EXPOSURE ESTIMATES

## 3.A. Exposure Estimates

<u>Selected General Foods</u>

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 000137, stamped pages 10 to 12 and 27 to 28, FDA, 2004; GRN 000732, 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 daily 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 000137, FDA, 2004) received no question letters from the U.S. 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 90$^{th}$ 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/day 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.

<u>EDIs of DHA for Term Infants</u>

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 FAs, the 90th percentile intake of DHA would be 39 mg DHA/kg bw/day (7,800 mg fat/kg bw/day x 0.005 = 39 mg/kg bw/day). Since an average new infant (<1 month) 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 percentile calorie intake/kg bw/day is 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 FAs, the 90th percentile EDIs of DHA would be 30.5 mg/kg bw/day (6,111 mg fat/kg bw/day x 0.005 = 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 FAs).

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. Because 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/infant/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-rich oil). 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).

<u>EDIs of DHA for Pre-term Infants</u>

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 the calculation methods as shown below and summarized in Table 12.

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 FAs (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% DHA. 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 12. Summary of EDIs of DHA and DHA-Rich Oil

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

Abbreviations: bw = body weight; DHA = docosahexaenoic acid; EDI = estimated daily 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**

Human milk is a significant source of DHA. The worldwide mean DHA content of human milk is 0.32-0.37% of total FAs and ranges from 0.06% to 1.4% (Brenna et al., 2007; Fu et al., 2016). Fish oil and egg yolks are also 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 FAs 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 FAs 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. EDIs 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 of DHA described in Part 3.A 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*

The major sterols found in the DHA algal oil are also found in human breast milk and commercially available infant formula (Mellies et al., 1976). 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 up to 39 mg/kg bw/day for pre-term infants (please see Part 3.A. 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 were estimated to be up to 284 mg/infant/day in term infants aged 11.5 months weighing 10.2 kg (Table 12). 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 (Table 12); this level may correspond to the EDI of 1.56 mg sterols/infant/day.

In addition, the maximum EDI of cholesterol under the intended use will be approximately 0.25 mg/kg bw/day for term infants and 0.33 mg/kg bw/day for pre-term infants because cholesterol accounts for 56% of total sterols in the subject of this notice. These levels correspond to up to 2.6 mg cholesterol/infant/day for term infants and 0.87 mg cholesterol/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 months who were consuming infant formulas (Claumarchirant et al., 2015). Estimated daily cholesterol intakes ranged from 9 mg/day (0.5 month old infant) to 51 mg/day (5 month old) in infants consuming formulas (Claumarchirant et al., 2015).

In summary, the estimated intake of cholesterol and total sterols through the proposed uses of DHA-rich oil would not have an impact on the relative amount of cholesterol and total sterols already consumed via infant formulas. In addition, sterols are normal components of various foods. The presence of sterols in ARA-rich oil is not expected to pose a safety risk.

*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 n-6 DPA Under the Intended Use</u>

*Infants*

Analysis of 3 lots of DHA-rich oils indicates a mean n-6 DPA concentration of approximately 11.9% (Table 6). 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 up to 39 mg/kg bw/day for pre-term infants (please see Part 3.A for details). Thus, to calculate the EDIs of DPA for term infants, EDIs of DHA (28-39 mg/kg bw/day) were multiplied by 11.9/35.0 (or 0.34) to get EDIs of DPA. For example, 28-39 mg DHA/kg bw/day was multiplied by 0.34 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 35.0% and 11.9% of DHA and DPA, respectively. Thus, 1.5 g x 0.34 = 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 (11.9%), and n-3 DPA (0.08% total FAs). 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 ARA, and that the deficiency of n-3 essential FAs 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. Seal meat and blubber are particularly rich in DPA. For example, bearded seal oil contains 5.6% DPA. It was estimated that the Greenland Inuit population consumed 1.7 to 4.0 g DPA per day (Bang et al., 1980; 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 person 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 for term infants and up to 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 the 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 derived from *Schizochytrium* sp. 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 (U.S. 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 lipoprotein 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, the 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 U.S. FDA over the past 20 years for the proposed incorporation in food for human consumption. GRAS notifications for DHA-rich oils (derived from algae and fish) have received "no question" responses from the FDA.

As shown in Table 13, various DHA-rich oil ingredients derived from *Schizochytrium* sp. received U.S. 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 13. Regulatory Approvals for Use of DHA-Rich Oil Derived from *Schizochytrium* sp. in Foods and Infant Formulas

| GRAS Notice number, infant types (if applicable) | Year | DHA content; *Schizochytrium* sp. strain name | Intended use and EDI |
|---|---|---|---|
| Selected foods with intended uses as a direct food ingredient in the same categories as considered GRAS for menhaden oil [21CFR184.1472(a)(3)] | | | |
| 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 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, pre-term and term | 2022 | ≥45% DHA; *Schizochytrium limacinum* TKD-1 | 27-33 mg DHA/kg bw/day |

Abbreviations: ARA = arachidonic acid; bw = body weight; CFR = Code of Federal Regulations; 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, the subject of this GRAS notice, 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 toxicity studies in rats, including a 28-day subacute toxicity study, a 90-day subchronic toxicity study (Lewis et al., 2016), and developmental and reproductive toxicity study in rats (Falk et al., 2017). The data from the studies by Lewis et al. (2016) and Falk et al. (2017) provide pivotal safety data in this GRAS notice, and they are cited by many previous GRAS notices as well.

As Runke Bioengineering's DHA-rich oil, the subject of this GRAS determination, has similar specifications and chemical composition compared to those described in the previous U.S. FDA GRAS notices involving algal DHA-rich oil (Table 4), 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 (Corroborative studies: 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.

**6.B.1. Metabolic Fate of DHA** (adopted from Kremmyda et al., 2011; Kroes et al., 2003; Martin et al., 1993 and from GRN 001008, p 38-39; GRN 000731, p 27-28)

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 FAs. DHA is mainly found in the form of triglycerides, although it also occurs 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 FAs. 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 FAs.

This facilitates the absorption of polyunsaturated FAs (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 FAs 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 FAs occurs in the mitochondria following their transport across the mitochondrial membrane in the form of acylcarnitine.

FAs 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 FAs across the mitochondrial membrane is contingent upon the length of the carbon chain; FAs of 20 carbons or more are transported into the mitochondria to a lesser degree than shorter chain FAs. Therefore, long chain FAs, such as DHA, may not undergo mitochondrial beta-oxidation to the same extent (Kroes et al., 2003). Instead, they are preferentially channeled into the phospholipid pool where they are rapidly incorporated into the cell membranes of the developing brain and retina. These FAs 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 [GRN 000553, p 37-41 or stamped p 43-47, p 10-11 of 24 page section; it was cited as Fedorova-Dahms et al., 2013]; GRN 677, p 40; Yeiser et al., 2016 [cited in GRN 1008, p 60]).

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 FAs as DHA and ARA, respectively, and a higher dose provided 0.96% and 1.92% of total

FAs 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 Runke Bioengineering's DHA-Rich Oil Derived from *Schizochytrium* sp., the Subject of This GRAS Determination

Due to the abundance of literature, this review of mutagenicity and genotoxicity studies is focused on studies of Runke Bioengineering's DHA-rich oil derived from *Schizochytrium* sp. FJRK-SCH3 only instead of DHA-rich oil from various sources.

<u>Bacterial Reverse Mutation Assays for Runke Bioengineering's DHA-Rich Oil</u>

The safety of Runke Bioengineering's DHA-rich oil from *Schizochytrium* sp. FJRK-SCH3 strain was evaluated in mutagenicity and genotoxicity studies (Lewis et al., 2016; Table 14).

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 14). 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 14). 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 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 of DHA-rich oil 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 PCEs to normochromatic erythrocytes (%PCE) and individual frequencies of micronucleated (MN) PCEs 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 PCEs. 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 5,000 mg/kg bw/day under the experimental conditions.

Table 14. Summary of Pivotal Studies Showing No Mutagenicity and/or Genotoxicity of Runke Bioengineering's DHA-Rich Oil

| Test | Test system | Concentration/dose of DHA-rich oil | Previous GRN citation |
|---|---|---|---|
| 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 | GRN 000836, p.36<br>GRN 001008, p.39 |
| *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 mg/mL culture ± S9 (2%) | GRN 000776, p.23<br>GRN 000836, p.37<br>GRN 001008, p.39 |
| *In vivo* mammalian erythrocyte micronucleus test | Polychromatic erythrocytes in bone marrow of treated rats | 0, 1,000, 2,500, and 5,000 mg/kg bw/day | GRN 000776, p.23<br>GRN 000836, p.37<br>GRN 001008, p.39 |

Adapted from Lewis et al. (2016), Table 8.
Abbreviations: bw = body weight; DHA = docosahexaenoic acid; GRN = GRAS notice.

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

Due to the abundance of literature, this review of animal toxicity studies is 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 15. Of these, the pivotal toxicity studies are those by Lewis et al. (2016) and Falk et al. (2017). These studies were reviewed in previous GRAS notices (GRN 000776, p 22; GRN 000836, p 38-40, GRN 000934, p 44; GRN 001008, p 44). Corroborative studies include the published research of Abril et al. (2003), Fedorova-Dahms et al. (2011a, 2011b), Hammond et al. (2001a, 2001b, 2001c), and Schmitt et al. (2012a, 2012b).

Table 15. 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 | Previous GRN citation |
|---|---|---|---|---|---|---|---|
| Pivotal Toxicity Studies of Runke Bioengineering's DHA-Rich Oil Derived from *Schizochytrium* sp. FJRK-SCH3 Strain, the Subject of This GRAS Determination | | | | | | | |
| 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 | GRN 000776, p 22<br>GRN 000836, p 38<br>GRN 000934, p 44<br>GRN 001008, p 44 |
| 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 | GRN 000776, p 22<br>GRN 000836, p 38<br>GRN 000934, p 44<br>GRN 001008, p 44 |
| 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)<br>5,000 (F) | Lewis et al., 2016 | GRN 000776, p 22<br>GRN 000836, p 39<br>GRN 000860, p 36<br>GRN 000934, p 44<br>GRN 001008, p 44 |
| Maternal/ paternal repro-ductive 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 | Falk et al., 2017 | GRN 000776, p 24<br>GRN 000836, p 40<br>GRN 000860, p 37<br>GRN 000934, p 44<br>GRN 001008, p 44 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | treatment-related reproductive toxicity | | |
| Corroborative Studies: 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 | GRN 000677, p 35<br>GRN 001008, p 43 |
| 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)<br>3,343 (F) | Fedorova-Dahms et al., 2011a | GRN 000677, p 37<br>GRN 001008, p 43 |
| 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)<br>3,679 (F) | Schmitt et al., 2012a | GRN 000677, p 35<br>GRN 001008, p 43 |
| Repro-ductive 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;<br>$F_1$: 90 d with | Rat | No treatment-related adverse effects | $F_0$ premating: 3,466 (M), 4,013 (F);<br>$F_0$ gestation: 3,469 (F);<br>$F_0$ lactation: 8,322 (F).<br>$F_1$ 90-day with *in utero* exposure phase: | Fedorova-Dahms et al., 2011b | GRN 000553, p 32 (stamped p 38)<br>GRN 001008, p 43 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *in utero* phase, followed by a 4 wk recovery phase | | | 4,122 (M), 4,399 (F) | | |
| 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 | GRN 000677, p 35 |
| Repro-ductive 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 | GRN 000677, p 33-35<br>GRN 001008, p 43 |
| | | $F_1$ 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 | $F_{1:}$ 5% in diet (both M + F); $F_1$: 3,526 (M), 4,138 (F); Systematic toxicity- 3,526 (M), 2,069 (F) | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | consumption, body weight, and body weight gain in the 5% $F_1$ female group | | | |
| Corroborative Studies: DRM Studies Reviewed in Previous GRAS Notices | | | | | | | |
| Subchronic 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 experiment period) | No feed consumption data on a mg/kg bw basis; no NOAEL was reported | Abril et al., 2003 | GRN 000137, p 15<br>GRN 000677, p 40<br>GRN 001008, p 45 |
| Subchronic toxicity (diet) | 400, 1,500, or 4,000 mg/kg bw/d (8.7% DHA on a dry wt basis) | 13 wk | Rat | No treatment-related adverse effects | 4,000 DRM (correspond-ing to 348 DHA**) | Hammond et al., 2001a | GRN 000137, p 10-11<br>GRN 000553, p 33 (stamped p 39)<br>GRN 000677, p 37<br>GRN 001008, p 45 |
| Repro-ductive 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 develop-mental toxicity - 22,000 DRM (correspond-ing to 1,914 DHA**) | Hammond et al., 2001b | GRN 000137, p 11-12<br>GRN 000553, p 33 (stamped p 39)<br>GRN 000677, p 38<br>GRN 001008, p 45 |
| Single-generation repro-duction | | M- 15 wk;<br>F- 2 weeks prior to mating, during | Rat | No treatment-related adverse effects | 17,847 DRM (correspond-ing to 1,512 DHA**) (M); | Hammond et al., 2001c | GRN 000137, p 12<br>GRN 000553, p 33 (stamped p 39)<br>GRN 000677, p 38 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| toxicity (diet) | | mating, and throughout gestation and lactation (10 wk) | | | 20,669 DRM (corresponding to 1,680 DHA**) (F) | | GRN 001008, p 45 |
| Reproductive and developmental 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). No significant effect on post-implantation loss, mean fetal bw/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 + F) | Hammond et al., 2001b | GRN 000137, p 12<br>GRN 000553, p 33 (stamped p 39)<br>GRN 000677, p 38<br>GRN 001008, p 45 |

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

## Animal Toxicity Studies of Runke Bioengineering's DHA-Rich Oil, The Subject of This GRAS Determination

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 5 g/kg bw of DHA-rich oil (41.37% DHA) at a maximum dose volume of 10 mL/kg bw. 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 occurred during the 14-day observation period. Thus, an additional group of 5 rats received 5 g/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 determined to be >5 g/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 the Organisation for Economic Co-operation and Development (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 abnormalities were noted in feed consumption, mean body weights, clinical signs or symptoms, ophthalmological examination parameters, hematology, blood chemistry, urinalysis, and microscopic and histopathological examination parameters. There were no significant adverse effects at DHA doses up to 5,000 mg/kg bw/day. The no-observed-adverse-effect-level (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; recovery groups) were treated with vehicle control (corn oil) or 5,000 mg/kg bw/day DHA-rich oil for an 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 and clinical signs. 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 (MPV), prothrombin time (PT), activated partial thromboplastin time (aPTT), and neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts (Table 16). Statistically significant hematological changes included small changes in RBC counts (males, water control vs. corn oil control vs. low-dose vs. mid-dose: 7.7 vs. 7.4 vs. 7.5 vs. 7.6 $x10^6$ /µL, $P<0.05$ for corn oil control and 2 test groups compared to water control, but not significant compared to corn oil control), hematocrit (males- corn oil control vs. mid dose: 43.3 vs. 4.5%, $P<0.05$; females- corn oil control vs. low-dose vs. mid-dose: 44 vs. 45 vs. 46%, $P<0.05$ for 2 test groups), neutrophil counts (males, oil control vs. test groups: 12 vs. 13-14, $P<0.05$); however, these changes were not considered to be adverse because differences were small in magnitude and resolved during the recovery period.

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 17). Small increases were noted in cholesterol (by 22-26% in males and 12-18% in females) and triglycerides (18-26% in males and 16-21% in females) in all DHA-rich oil doses for both sexes. Triglycerides for the female DHA-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; Table 17). However, the differences were small in magnitude, were resolved by the end of the recovery, and were not accompanied by changes in 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 mg/dL, females, 0.26 vs. 0.34 mg/dL; albumin, males and females, 4.2-4.3 vs. 4.5 g/dL; 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 urinalysis 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.

Organ weights (Table 18), gross pathological analyses, and physical and microscopic examination parameters 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 16. Hematology and Coagulation Parameters for Wistar Rats Administered DHA-Rich Oil for 90 Days

| Parameter | 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, $\mu m^3$ | 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, $\mu m^3$ | 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± standard deviation for groups of 20 rats treated for 90 days prior to sacrifice. [a]*P*<0.05 vs water control; [b]*P*<0.05 vs vehicle control.
Abbreviations: 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 17. Blood Biochemistry for Wistar Rats Administered DHA-Rich Oil for 90 Days

| Parameter | 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] |
| SDH, IU/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] |
| T. protein, g/dL | 6.8±0.4 | 6.7±0.4 | 6.6±0.3 | 7.0±0.4 | 7.0±0.5 |
| T. 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] |
| SDH, IU/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± standard deviation. [a]*P*<0.05 vs water control; [b]*P*<0.05 vs. vehicle control.
Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; bw = body weight; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; SDH = sorbitol dehydrogenase; T. = total.

Table 18. Organ Weights for Wistar Rats Administered DHA-Rich Oil for 90 Days

| Parameter | 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 ± standard deviation.
Abbreviations: bw = body weight; S.V. = seminal vesicles.

Reproductive and Developmental Toxicity Study of Runke Bioengineering's DHA-Rich Oil
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 (Tables 19 and 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 19).

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, bilobed centra, and incomplete or delayed ossification in the cranial bones were all within historical control ranges.

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 effects 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 cohabited 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.

$F_0$ generation

No treatment-related mortality was observed in the parental or pup generation during the course of the study. For the $F_0$ 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 $F_0$ 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 21) 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 22). 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 $F_1$ 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 atelectasis 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 19. Changes in Fetal Development in the Prenatal Developmental Toxicity Study

| Parameter | Dose (mg/kg bw/day) | | | | |
|---|---|---|---|---|---|
| | 0 (untreated) | 0 (corn oil) | 1,000 | 2,500 | 5,000 |
| 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).

Table 20. Summary of Major Malformations and Minor Skeletal Variations in the Prenatal Developmental Toxicity Study

| Parameter | Dose (mg/kg bw/day) | | | | |
|---|---|---|---|---|---|
| | 0 (untreated) | 0 (corn oil) | 1,000 | 2,500 | 5,000 |
| 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).

Table 21. $F_0$ Fertility and Reproductive Performance in the Reproductive Toxicity Study

| Fertility Indices | Dose (mg/kg bw/day) | | | |
|---|---|---|---|---|
| | 0 (corn oil) | 1,000 | 2,500 | 5,000 |
| 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

Table 22. Physical Observations and Gross Necropsy Findings of $F_1$ Newborn Pups in the Reproductive Toxicity Study

| Physical Observations – Mean days ± SD | Dose (mg/kg bw/day) | | | |
|---|---|---|---|---|
| | 0 (corn oil) | 1,000 | 2,500 | 5,000 |
| 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: Atelectasis | 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).

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

In GRNs 000553 (stamped pages 37-47, 40-54), 000677 (p 33-41), 000731 (p 30-34), 000732 (p 33-37), 000776 (p 17-24), 000777 (p 15-22), 000836 (p 32-34, 38-45), 000843 (p 19-25), 000844 (p 18-25), 000862 (p 29-38), 000933 (p 34-40), 000934 (p 35-44), and 001008 (p 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. Abril et al. (2003; 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 15.

2) In a subchronic toxicity study on another source of DRM, which contains 8.7% DHA on a dry weight basis (p 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).

3) In a reproductive and developmental toxicity study in rabbits by Hammond et al. (2001b), both the high-dose (1,800 mg/kg bw/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 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 gestational days (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. Although 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, 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.

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 developmental toxicity. This level corresponds to 1,914 mg DHA/kg bw/day, assuming the DHA content in DRM was 8.7%.

4) 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 most human studies were designed to investigate the efficacy of DHA-rich oil on various health parameters, some studies evaluated several safety-related endpoints during the experiments. Therefore, these studies including safety parameters are reviewed below as additional supporting information. The absence of adverse effects provides some evidence of the safe use of DHA-rich oils. Our review is focused on the studies which include safety parameters in their measurement endpoints. This review will focus on the safety and will not discuss health benefits of DHA-rich oil. Publications that are not relevant to assessing the safety of DHA in infant formula (such as those that employed different food forms including supplements or enteral feeding) were not included in this review.

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 U.S. 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 23 to 25). The DHA-rich oil in this GRAS determination has similar specifications compared to the those in the previous GRAS notices (Table 6), and 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 (MacDonald and Sieving, 2018 [GRN 000933, p 41, 44]; Sanders et al., 2006 [GRN 001008, p 61-62]). Thus, this notice incorporates, by reference, the above mentioned studies discussed in the previous GRAS notices, and will not discuss previously reviewed references in detail.

Briefly, the study by 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 8 adverse events reported by 4 participants, and all 8 events were considered not related to the DHA supplementation.

In the 2006 study by Sanders et al., effects of DHA-rich oil supplementation on cardiovascular risk factors were evaluated in 79 healthy men and women (mean ages, 30-35 years) in a placebo-controlled, randomized, double-blind study. Subjects received 4 g oil (providing 1.5 g DHA and 0.6 g DPA; derived from *Schizochytrium* sp. (DHA-S; test group) or 4 g refined olive oil (placebo group) each day for 4 weeks. Compared to the placebo group, the test group had significantly higher serum concentrations of total, LDL- and HDL-cholesterol, and Factor VII (FVII) coagulant activity (increase by 0.33 mmol/l [7.3%], 0.26 mmol/l [10.4 %] and 0.14 mmol/l [9.0 %], and 12%, respectively; $P<0.01$ for all parameters). However, there were no significant differences between treatments in LDL size, blood pressure, plasma glucose, serum C-reactive protein, plasma FVII antigen, FVII activated, fibrinogen, von Willebrand factor, tocopherol or carotenoid concentrations, plasminogen activator inhibitor-1, creatine kinase or troponin-I activities, hematology, or liver function tests or self-reported adverse effects. Although an LDL-cholesterol increasing effect was noted, neither the LDL:HDL ratio nor LDL size were significantly different between the groups. Thus, the authors concluded that the lipoprotein changes induced by the *Schizochytrium* sp. supplement have a net neutral effect on cardiovascular risk factors. Overall, the authors concluded that the DHA-rich oil derived from *Schizochytrium* sp. was well tolerated and did not adversely affect cardiovascular risk.

*An Efficacy Study Not Considered for Safety Evaluation*

The study by Smith et al. (2018) included in a previous GRAS notice (GRN 001008, p 61-62) was not considered for the safety review in this GRAS determination. In this prospective 8-week open label study with 28 patients (18-65 years) with major depression disorder who were non-responsive to medication or psychotherapy, with a Hamilton Depression Rating Scale score of greater than 17, measurement endpoints included depression, clinical severity, and daytime sleepiness. Although no adverse effects of DHA supplementation (260 mg or 520 mg per day DHA) for 8 weeks were reported on measured outcomes, no safety parameters were evaluated in this study.

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

**Studies in Children**

Since January 2022, no new studies of DHA in children have been published.

GRN 001008 included the study by Ingol et al., which was published in June 2019 (GRN 1008, p 63; Table 23). 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* (Martek 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, one new study of DHA derived from *Schizochytrium* sp. in pregnant women has been published (Garmendia et al., 2021; see Table 23).

From the Maternal obesIty/overweight control throuGh Healthy nuTrition (MIGHT) study, Garmendia et al. (2021) evaluated the effects of DHA supplementation on 1002 obese and overweight pregnant women (<15 weeks of gestation; BMI ≥25 kg/m$^2$ at first prenatal visit) 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. Primary endpoint was the overall incidence of gestational diabetes mellitus (fasting glucose ≥92 mg/dL and/or 2 h after ≥153 mg/dL) diagnosed by a 75-g oral glucose tolerance test (OGTT) at 24–28 weeks of gestation. The secondary endpoints included the incidence of macrosomia (birthweight >4000 g) and neonatal insulin resistance (cord blood Homeostasis Model Assessment for Insulin Resistance [HOMA-IR]), glucose concentrations, and adverse events. No significant differences were found among groups with regard to measurement outcomes. The overall incidence of gestational diabetes mellitus was 20.2% (Group 1: 21.0%, Group 2: 20.1%, Group 3: 18.9%, and Group 4: 20.9%). Mean birth weight was 3,403.0 g, and the incidence of macrosomia was 11.9% (Group 1: 13.2%, Group 2: 10.8%, Group 3: 11.5%, and Group 4: 12.1%). Median cord blood HOMA-IR was 0.9 (interquartile range, 0.6–1.7), and 10.2% showed cord blood insulin resistance (Group 1:

12.0%, Group 2: 12.0%, Group 3: 9.7%, and Group 4: 5.1%). The only exception was the glucose concentrations in the cord blood samples that were lower in those adherents to the DHA supplementation (*P*< 0.05). The authors concluded that for women who were overweight or obese at the beginning of pregnancy, this combined intervention with DHA and counseling did not reduce the risk of gestational diabetes in mothers or macrosomia and insulin resistance in neonates. 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, 000933, and 001008) that intake of DHA is safe as long as the daily intake does not exceed 1.5 g/person/day.

Table 23. Human Studies Reporting No Adverse Effects of DHA from Algal Sources in Children and Women During Pregnancy and/or through Postpartum*

| Objective | Subject | Dose | Duration | Measurements | Reference | Previous GRN citation |
|---|---|---|---|---|---|---|
| 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 wk gestation) who were 10-16 mo in corrected age | 2 groups:<br>1) DHA (200 mg/d; *Schizochytrium* sp.; source: Martek Biosciences Corp/DSM,) plus ARA (200 mg/d)<br>2) corn oil placebo | 180 d | Growth and adiposity; adherence and adverse events<br><u>Adverse events:</u> Mainly minor gastrointestinal illness and respiratory infections; not treatment-related | Ingol et al., 2019 | GRN 001008, p.63 |
| 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-weight control throuGh Healthy nuTrition (MIGHT) study | 4 groups:<br>1) Home-based dietary counseling + 800 mg/d DHA (source: *Schizochytrium* sp., DSM)<br>2) 800 mg/d DHA only<br>3) Home-based dietary counseling + 200 mg/d DHA<br>4) 200 mg/d DHA only | From <15 wk of gestation until delivery | The overall incidence of gestational diabetes mellitus, the incidence of macrosomia, and cord blood Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and glucose concentrations. | Garmendia et al., 2021 | New study not cited in previous GRN |

*Excluding studies of DHA from fish oil source or DHA-ethyl ether; none of these studies reported adverse effects of DHA on measured outcomes.

Abbreviations: ARA = arachidonic acid; d = days; DHA = docosahexaenoic acid; GRN = GRAS notice; mo = months; wk = weeks.

**Studies of DHA in Term Infants**

No new studies published since January 2022 have been identified from the literature. However, this review includes a few key term infant studies related to safety of DHA-rich oils in term infants (Table 24). Our review includes the studies testing the safety of DHA levels at or higher than 0.5% of FAs of the infant formulas. Due to the fact that Runke Bioengineering's intended use is up to 0.5% of FAs as DHA, the studies employing DHA doses of lower than 0.5% of the fat component of the infant formulas were not included in this review. It is assumed that the safety of DHA at up to 0.5% of FAs is justified if the studies using higher doses reported no adverse effects of DHA supplementation (at 0.5-0.96% of the fat component of the infant formulas).

Based on the established bioequivalence between DHA oils from *C. cohnii* and *Schizochytrium* sp. (Fedorova-Dahms et al., 2014; Yeiser et al., 2016), studies of DHA-rich oils derived from *C. cohnii* were included as long as those studies included safety parameters and the use levels were at or higher than 0.5% of FAs as DHA.

<u>Infant Formula Studies Including Safety Parameters</u>

Three studies evaluating safety parameters have been identified for term infants with formulas supplemented with algal DHA oils (from *C. cohnii* or *Schizochytrium* sp.) at or higher than 0.5% of FAs as DHA: Birch et al., 2010 (up to 0.96% FAs as DHA derived from *C. cohnii* or up to 61 mg DHA/kg bw/d), Currie et al., 2015 (up to 0.96% of FAs as DHA from *C. cohnii*), and Chase et al., 2015 (61.2 mg DHA/kg bw/day derived from *Schizochytrium* sp.). The studies are summarized in previous GRAS notices: Birch et al., 2010, p 51 of GRN 000553; Currie et al., 2015, p 30 of GRN 000677 and p 37 of GRN 731; Chase et al., 2015, p 35 of GRN 000731 and p 60 of GRN 001008. Thus, the summaries in those GRNs are incorporated by reference and will not be discussed in detail. In these studies, daily doses up to DHA at 0.96% of FAs or 61 mg/kg bw/day for up to 1 year were well-tolerated in term infants. A brief summary of these studies is presented in Table 24.

From the DHA Intake and Measurement of Neural Development (DIAMOND) study (USA), Birch et al. (2010) determined the effect of varying amounts of DHA supplementation on the visual acuity as well as visual acuity maturation, RBC FAs, 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% (low-dose DHA), 0.64% (mid-dose DHA), or 0.96% (high-dose DHA) of FAs (or 17, 34, or 51 mg DHA/100 kcal) with the fixed amount of ARA (*M. alpina* source) at 0.64% of total FAs (or 34 mg ARA/100 kcal). 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 mid-dose DHA group; mid-dose 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 mid-dose DHA group and the formula was not determined. The amounts of formula consumed in the 24 h before study visits at 1.5, 6, 9, or 12 months of age were not significantly different among the formula groups (data not shown). In addition, no differences were observed between formula groups in the number of bowel movements occurring in the 24 h before study visits, consistency or color of bowel movements, frequency of diarrhea or constipation, or frequency of unusual gas or fussiness throughout the study (data not shown). 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 (USA) described above, Currie et al. (2015) evaluated the effects of feeding DHA-ARA supplemented formula throughout infancy on growth from birth to 6 years of age. One hundred fifty-nine healthy, term infants were enrolled at 1-9 days of age and were randomly assigned to be fed one of the following 4 infant formulas containing equivalent nutrient amounts for 12 months: control (0% DHA), 0.32, 0.64, or 0.96% of FAs as algal DHA derived from *C. cohnii*. All 3 DHA-supplemented formulas also provided 0.64% ARA derived from *M. alpina*. Compared to children fed control formula, children who consumed DHA-ARA- supplemented formula had higher stature-for-age (59.1 vs. 46.5 percentile; $P=0.001$) and weight-for-age percentiles (68.0 ± 10.8 vs. 49.8 ± 12.0 percentile; $P=0.02$) but not body mass index (BMI) from birth to 6 years. The authors concluded that DHA-ARA supplementation during infancy had no adverse effects on child growth or weight status.

Chase et al. (2015) investigated the effect of supplementation of algal DHA derived from *Schizochytrium* sp. (DHASCO-5 from Martek) on stimulated inflammatory cytokine production in WBCs from infants with a high genetic risk for type 1 diabetes. This was a multicenter, two-arm, randomized, double-blind pilot trial (USA) of DHA-rich oil supplementation, beginning either in the last trimester of pregnancy (41 infants) or in the first

5 months after birth (57 infants). Mothers of infants in Group A (41 infants) were enrolled in the last trimester of pregnancy. If the Group A neonate did not have high-risk Type I diabetes genes at birth (21 infants), they were discontinued from the study. Group A mothers were randomized to receive DHA (800 mg/day) or corn/soy oil (800 mg/day) in the last trimester of pregnancy and continued on this same dose after delivery if breast-feeding. Formula-fed infants received formula with 10.2 mg DHA/ounce (treatment; or 61 mg/kg bw/day) or 3.4 mg DHA/ounce (control). Formula-fed infants and infants of breast-feeding mothers in Group B (57 infants) were randomized in the first five postnatal months to receive similar dosages of DHA or corn/soy oil as their counterparts in Group A. Starting 12 months, all infants received 400 mg DHA/day (DHASCO-5 from Martek) or corn/soy oil until 36 months (approx. 40.9 mg DHA/kg bw/day). Formula fed infants in the group entering in the first 5 postnatal months had their initial follow-up blood draw at the age of 6 months (not 6 months after entry). Their mean age of entry was 4.0 months. Measurements included levels of DHA in infant, inflammatory cytokines, biochemical islet autoantibodies, and maternal and infant levels of RBC DHA and DPA at 6, 12, 18, 24, 30, and 36 months of age. The levels of RBC DHA were increased by 61–100% in treated compared to control infants at ages 6 to 36 months. The inflammatory marker, high-sensitivity C-reactive protein (hsCRP), was significantly lower in breast-fed DHA-treated infants compared to all formula-fed infants at age 12 months, although no significant differences were noted in the blood levels of inflammatory cytokines (such as interleukin [IL]-1β, tumor necrosis factor alpha [TNFα] or IL-12p40) among the groups at any time points measured. Three infants (two in the test group and one in the control group) were removed from the study as a result of developing ≥ two persistently positive biochemical islet autoantibodies. The authors concluded that supplementation of infant diets with DHA-rich oil was safe. No adverse effects on measured outcomes were noted.

*The Studies Employing Doses Lower Than 0.5% of FAs Were Not Considered for Safety Evaluation*

The studies employing the DHA level of lower than 0.5% of FAs are not included in this review. Although the studies listed below reported no adverse effects of DHA supplementation at lower levels of DHA (<0.36% of FAs as DHA), they do not support the safety of DHA at levels higher than 0.37% of FAs as DHA. Examples of such studies include the following, but are not limited to:

1) Study by Birch et al. (2005) evaluating sweep visual evoked potential acuity, random dot stereoacuity, blood lipid profile, growth, and tolerance in 103 term infants; use of 0.32% of FAs as DHA and 0.72% of FAs as ARA.
2) Study bv Birch et al. (2007) evaluating visual and cognitive outcomes at 4 years of age after 17 weeks of supplementation of formula with 0.35-0.36% of FAs as DHA. In this study, study diets were formula with iron, formula with iron supplemented with 0.35%

of FAs as DHA, or formula with iron supplemented with 0.36% of FAs as DHA and 0.72% FAs as ARA.

3) Study by Burks et al. (2008) evaluating growth, formula acceptance, tolerance, AEs, and allergy with the amino acid-based formula supplemented with DHA at the level of 0.32% of total FAs (17 mg/100 kcal) in combination with ARA (0.64% of total FAs; 34 mg/100 kcal) from 14 ± 2 through 120 ± 4 days of age in 164 healthy term infants.
4) Study by Burks et al. (2008) evaluating the effect of DHA-supplemented formula on allergy parameters with the formula supplemented with DHA at 0.32% of total FAs (17 mg/100 kcal) in combination with ARA (0.64% of total FAs; 34 mg/100 kcal) after double-blind and open challenges, followed by a 7-day home feeding period in 32 healthy term infants and children.
5) Study by Hoffman et al. (2008) evaluating the effects of DHA-supplemented formulas on anthropometric measurements, atopic dermatitis, gastrointestinal tolerance, and adverse effects in all infants and clinical chemistry parameters in subset infants. In this study, 244 term infants were fed control, soy formula with and without supplementation of DHA + ARA (0.32% of total FAs or 17 mg DHA/100 kcal from algal oil and 0.64% of total FAs or 34 mg ARA/100 kcal from fungal oil) from 14 to 120 days of age.
6) Study by Fleddermann et al. (2014) evaluating the effects of DHA-supplemented formulas on growth, gastrointestinal tolerance, and adverse effects. In this study, 213 healthy term infants were fed either 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.

*The Studies Evaluating Efficacy Only were Not Considered for Safety Evaluation*

The studies evaluating the efficacy of DHA in improving health parameters without including safety parameters were not included in this review. Examples of such studies include, but are not limited to:

(1) Birch et al. (2007) evaluating the effects of DHA supplementation on cognition and visual acuity with no safety parameters,
(2) Colombo et al. (2011; DIAMOND trial) evaluating cognitive performance, and
(3) Columbo et al. (2017; DIAMOND trial) evaluating the effects of DHA and ARA supplementation on DHA/ARA concentrations in the RBC phospholipids and cognition parameters (including memory, executive function and problem solving, and verbal and composite intellectual quotient). In this DIAMOND trial, test infant formulas provided 0.64% of FAs as ARA (a fixed level) in combination with a varied concentration of DHA (0.32, 0.64 or 0.96% of FAs). The control formula had no added DHA/ARA. This study showed that blood DHA levels generally rose with increased DHA supplementation, although those levels tended to plateau as the DHA-supplemented level exceeded 0.64% of FAs. ARA levels showed a strong inverted-U

function in response to increased DHA supplementation, and that infants assigned to the formula with the highest dose of DHA showed a reduction in blood ARA and reduced benefits in improved attention, executive function and problem solving, and verbal and composite intellectual quotient scores relative to lower DHA doses (0.32 or 0.64% of FAs as DHA). However, the highest dose (0.96% FAs as DHA) was not different from the control group in the cognition performance tested in this study. This study demonstrated the benefits of DHA supplementation at low- or mid-dose (0.32 or 0.64% of FAs as DHA with the fixed amount of ARA at 0.64% FAs as ARA), rather than an increased risk or actual harm at the highest DHA dose (0.96% of FAs as DHA and 0.64% of FAs as ARA). Thus, this study is considered as an efficacy study demonstrating health benefits of DHA supplementation instead of evaluating safety.

Overall Conclusion for Infant Formula Applications for Term Infants

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

Table 24. Human Studies Reporting No Adverse Effects of DHA-Rich Oil in Term Infants Consuming >0.5% Total FAs as DHA (or >34 mg DHA/kg bw/day)*

| Objective | Subject | Dose | Duration | Measurements/safety-related outcomes | Reference | Previous GRN citation |
|---|---|---|---|---|---|---|
| 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 FAs 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 d of life) through age 52 wk | Tolerance, anthropometric measures visual acuity and its maturation, RBC FAs over the 52-wk period/ No adverse effects on measured outcomes | Birch et al. (2010) | GRN 000553, p 51 |
| To evaluate the effects of feeding DHA-ARA supplemented formula throughout infancy on growth from birth to 6 y | 159 term infants | | Formula fed for 12 mo; follow-up from birth to 6 y | Growth/ No adverse effects on child growth or weight status. | Currie et al. (2015) | GRN 000677, p 30; GRN 000731, p 37 |
| To investigate the effect of DHA supplementation on stimulated inflammatory cytokine production in WBCs from | 41 mother and 57 infant pairs at high genetic risk for type 1 diabetes | Control, 3.4 mg DHA/oz infant formula (~20.4 mg DHA/kg bw/d); Test group, 10.2 mg DHA from *Schizochytrium* sp./oz infant formula (approx. 61.2 mg | Intervention – the first 5 mo after birth; follow up – up to 36 mo of age | Infant WBC stimulated inflammatory cytokine production (IL-1β, TNFα, or IL-12p40); the inflammatory marker (hsCRP); biochemical islet | Chase et al. (2015) | GRN 000731, p 37; GRN 001008, p 60 |

| | | | | | | |
|---|---|---|---|---|---|---|
| infants with a high genetic risk for type 1 diabetes | | DHA/kg bw/d**) the first 5 mo after birth. Starting 12 months, all infants received 400 mg DHA/d (derived from microalgae; approx. 40.9 mg DHA/kg bw/d) or corn/soy oil until 36 mo | | autoantibodies; maternal and infant levels of RBC DHA and DPA/ No adverse effects on measured outcomes. | | |

* Assuming that infants consume 6.7 g fat/kg bw/day; **Assuming that infant formulas contain 20 kcal/ounce, that infants consume 120 kcal/kg bw/day, and that the average weight of an infant is 9.76 kg.

Abbreviations: ARA = arachidonic acid; bw = body weight; d = days; DHA = docosahexaenoic acid; DIAMOND = DHA Intake and Measurement of Neural Development; DPA = docosapentaenoic acid; FAs = fatty acids; GRN = GRAS notice; hsCRP = high-sensitivity C-reactive protein; IL = interleukin; mo = months; RBC = red blood cell; TNFα = tumor necrosis factor-alpha; WBC = white blood cell; wk = weeks; y = years.

Previous GRAS citations focused on GRN 000553, 000677, 000731, and 001008.

**Studies of DHA in Pre-term Infants**

This review includes studies published until December 2023 that report on safety parameters and employ DHA levels at 0.5% or higher in infant formulas in pre-term infants (Table 25). Four studies were identified (Carnielli et al., 2007; Clandinin et al., 1997; Sauerwald et al., 2012; Fewtrell et al., 2004). These studies were reviewed in previous GRAS notices (Carnielli et al., 2007, reviewed in GRN 001008, p 54; Clandinin et al., 1997, reviewed in GRN 000379, p 38, 52; Sauerwald et al., 2012, GRN 000553, p 50; GRN 001008, p 56; Fewtrell et al., 2004, GRN 000379, p 31, 36; GRN 001008, p 49). Thus, the summaries in those GRNs are incorporated by reference and will not be discussed in detail. In these studies, daily doses up to DHA at 0.64% of FAs for the first 7 months of age (Carnielli et al., 2007), at 0.76% of FAs for the first 6 weeks of life (Clandinin et al., 1997), and at 0.5% of FAs for 9 months after term (Fewtrell et al., 2004) were well-tolerated in pre-term infants. These studies are briefly summarized in Table 25 and below.

Carnielli et al. (2007; reviewed in GRN 001008, p 54) used 22 healthy, non-breast-fed, pre-term infants (n=22) who were randomly assigned equally to control (standard formula) and test groups (standard formula supplemented with 0.64% of FAs as algal DHA [DHASCO, algal type was not specified, but probably derived from *C. cohnii*, DSM] and 0.84% of FAs as fungal ARA [ARASCO, DSM]). Infants were exclusively fed control and test formulas for 7 months before weaning to local food diets. Measurements included growth, plasma phospholipid FAs, and estimation of endogenous synthesis of long-chain PUFAs. The concentrations of ARA and DHA in plasma phospholipids of infants fed the DHA/ARA formula were significantly higher ($P<0.01$) than those in the control group. The synthesis of ARA was significantly higher than that of DHA, and both decreased with age. All infants grew normally during the first 7 months of life, and no significant difference between groups was found in weight gain at any of the study time points. No adverse effects were observed on measured outcomes.

In the 1997 study by Clandinin et al. (1997; reviewed in GRN 000379, p 38, 52), pre-term infants (<2,300 g) were randomized to one of the following 5 treatments in their first 6 weeks of life: the commercially available control formula (Preemie SMA®, Wyeth Nutritionals International); test formulas supplemented with 0.32% of FAs as fungal ARA (ARASCO, DSM) plus 0.24% of FAs as algal DHA (DHASCO, DSM), 0.49% of FAs as ARA plus 0.35% of FAs as DHA, or 1.1% of FAs as ARA plus 0.76% of FAs as DHA, or breast milk (reference group). Ninety-one infants completed the study. Blood samples were taken at 2 and 6 weeks of age and analyzed for fatty acid composition of erythrocyte membrane phospholipids, lymphocyte membrane phospholipids, and plasma lipoprotein. In addition, hematology (RBC, WBC, and platelet counts, hemoglobin, hematocrit, MCV, MCHC, WBC differential count) and urinalysis parameters, and serum creatinine were routinely monitored. All values were within normal ranges. Length and head circumference were measured weekly and weight was measured daily. At 2 weeks, growth was similar in all groups. However, by 6 weeks, formula-fed infants showed greater growth (weight and length) than breastfed infants regardless of supplementation with no differences among infants in the 4 control and test formula groups. Blood lipid profile (fatty acid composition of erythrocyte membrane phospholipids, lymphocyte membrane phospholipids, and plasma lipoprotein) were comparable among the groups and were within the normal range, although a dose-response was observed with increasing levels of ARA and DHA supplementation. The authors suggested that approximately 0.6% of FAs as ARA and 0.4% of FAs as DHA provide sufficient (and perhaps optimum) levels of these FAs and that ARA and DHA supplementation to infant formula did not result in any adverse effects on measured outcomes including growth or clinical parameters (hematology, urinalysis parameters, and serum creatine). No adverse effects were observed at the highest levels of DHA and ARA (i.e., 0.76% DHA and 1.1% ARA in the fat component of the formula).

In a randomized double-blind study by Sauerwald et al. (2012; reviewed in GRN 000553, p 50; GRN 001008, p 56), 42 pre-term infants with birth weights ranging from 1,000 to 2,200 g were randomized to receive one of the following 3 formulas with fixed amounts of gamma-linolenic acid (0.4%) and ARA (0.1%) with varying DHA contents (0.04%, 0.33%, or 0.52% of FAs). A group of additional 24 infants received human milk (0.51% of FAs as ARA, 0.38% of FAs as DHA, non-randomized) and served as a reference group. Among 66 enrolled infants, 42 completed the study. Measurements included growth (length, head circumference, and body weights), adverse events, DHA/ARA synthesis, and plasma and RBC concentrations of FAs. Z scores for weight, length, and head circumference did not differ among groups at any time point (data not shown). No treatment-related adverse events were recorded. The authors concluded that DHA supplementation to formulas did not inhibit DHA or ARA synthesis.

In a randomized placebo-controlled study by Fewtrell et al. (2004; reviewed in GRN 000379, p 31, 36; GRN 001008, p 49), 238 pre-term (<35 weeks, <2,000 g birth weight) infants were randomly assigned to unsupplemented (control group) or long-chain PUFA (0.5% FAs as DHA, from tuna oil plus gamma-linolenic acid from borage oil)-supplemented formula (test group) to 9 months after term. The primary endpoint was neurodevelopment scores as measured by the Bayley Mental and Psychomotor Indices at 18 months after term. Safety outcome measures included growth (9 and 18 months), gastrointestinal tolerance, infection, and adverse events. By 9 months, 25 and 9 infants in the control and test groups, respectively, dropped the study by non-treatment related reasons. By 18 months, 93 and 106 children in the control and test groups, respectively, were assessed. At 9 months, long chain PUFA-supplemented infants showed significantly greater weight gain (by a mean of 310 g, $P<0.05$) and length gain (by a mean of 1.0 cm, $P = 0.05$), with greater effects in boys: the mean weight and length were higher by 510 g and 1.8 cm (all *P*<0.05). At 18 months, no differences were observed in the incidence of adverse events, tolerance, growth, and neurodevelopment scores. In addition, no group differences were noted in the incidence of intraventricular hemorrhage, periventricular leukomalacia, patent ductus arteriosus requiring treatment, retinopathy of prematurity, pulmonary hemorrhage, and skin infection as well as in the proportion of infants requiring ventilation or therapy with >30% oxygen. The authors concluded that supplementation of infant formula with long-chain PUFA from tuna oil and borage oil up to 9 months after term is safe.

**Pre-term Infant Studies not Considered for Safety Review**

The following studies were not included in the safety review for various reasons listed below.

*Studies Employing Doses Lower Than 0.5% of FAs Were Not Included in This Review*

The studies employing DHA levels of lower than 0.5% of FAs are not included in this review because these studies do not support the safety of DHA at up to 0.5% of FAs. An example includes Clandinin et al. (2005): the use level of DHA was of 0.32% of FAs.

*Studies Employing Efficacy Parameters only*

An example includes Clandinin et al. (1999). This study is a continuation of the 1997 study by Clanindin et al., but did not include a safety parameter.

*Studies Employing Capsule Supplements, Human Milk by Enteral Feeding, or Intravenous Administration were not Included in the Safety Evaluation in This Review*

The studies administering DHA via supplement capsules, enteral feeding, human milk fed by enteral feeding, or intravenous administration are not included in this review because food forms or routes of administration may impact the safety of the test substance. Thus, the studies employing different food forms or different administration methods may not accurately reflect the safety of DHA-rich oil administered in an infant formula form.

*Emulsified supplement via the nasogastric tube:*

Study by Frost et al. (2021): In this study, the DHA supplement was administered via the nasogastric tube to 192 very low birth weight infants with a mean birth weight of 1,040 g (mean gestational age of 28 weeks) for 8 weeks or until discharged, whichever came first. If the infant was not being fed enterally, the supplement could be flushed with sterile water via the nasogastric tube. Pre-term infants received 1 of the following 3 treatments: 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 long-chain PUFA levels were measured.

Study by Hewawasam et al. (2021): In this study, 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.

*Examples of DHA-rich oil via enteral dose:*

Study by Bernabe-García et al. (2021): In this study, 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 algal DHA/kg bw 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. Measurements included the incidence of necrotizing enterocolitis (NEC), an inflammatory bowel disease based on Bell's scale from stage IIa and IIb, and adverse effects (including death, median platelet counts, bleeding events such as periventricular/intraventricular hemorrhage grade ≥II and upper gastrointestinal tract and/or pulmonary bleeding), and FA profile of erythrocyte membranes.

*Examples of supplementation to human milk and fed by enteral feeding:*

Studies by Almaas et al. (2015, 2016), Henriksen et al. (2008), and Westerberg et al. (2011): In these studies, human milk supplemented with 32 mg DHA (0.86% of total FAs as DHA; source not specified) and 31 mg ARA (0.91% of total FAs per 100 mL) was fed to pre-term infants each day for 9 weeks after birth with an 8-year follow-up.

*Examples of human milk with high DHA concentrations (mothers taking tuna oil or soy oil capsules to achieve a breast milk DHA concentration that was 1% or 0.35% of total FAs without altering the naturally occurring concentration of ARA in breast milk):*

DHA for the Improvement of Neurodevelopmental Outcome (DINO) Trial such as Gunaratne et al. (2019) evaluating the incidence and the severity of eczema symptoms and Manley et al. (2011) evaluating allergic and respiratory parameters. In the DINO trial: 657 pre-term infants of <33 weeks of gestation 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 FAs 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% 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 and the median duration of treatment was 9.4 weeks.

*Examples of parenteral/intravenous administration of fish oil-based fat emulsion*: Such studies include Pawlick et al. (2011, 2014).

In summary, DHA-rich oil derived from *Schizochytrium* sp. at the use level of up to 0.5-0.76% of total FAs as DHA is not expected to adversely impact the pre-term infants who would be consuming infant formulas.

**Meta-analysis**

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

Table 25. Human Studies Reporting No Adverse Effects of DHA-Rich Oil in Pre-Term Infants Consuming >0.5% of FAs as DHA

| Subjects | Intervention | Duration | Measurements | Reference | GRN Ref |
|---|---|---|---|---|---|
| Algal DHA | | | | | |
| 22 pre-term infants with gestational ages of approximately 31 wk | Control: Infant formula; or<br>Infant formula supplemented with algal DHA (0.64% of FAs**;** DHASCO; approx. 42.8 mg DHA/kg bw/d and ARA (0.84% of FAs; ARASCO) | From birth to 7 mo of age | Growth; plasma phospholipid FAs; estimation of endogenous synthesis of long-chain<br>PUFAs | Carnielli et al., 2007 | GRN 001008, p 54 |
| 117 pre-term infants <2,300 g | 5 groups:<br>1) breast milk;<br>2) unsupplemented formula;<br>formulas supplemented with;<br>3) DHA, 0.24% FAs/ARA, 0.32% FAs;<br>4) DHA, 0.35% FAs/ARA, 0.49% FAs;<br>5) DHA, 0.76% FAs/ARA, 1.1% FAs;<br>Algal DHA and fungal ARA were from Martek (now DSM);<br>91 completed the study | First 6 wk of life | Growth, blood values including hematology; blood lipid profile (FA composition of erythrocyte membrane phospholipids, lymphocyte membrane phospholipids, and plasma lipoprotein) | Clandinin et al., 1997 | GRN 000379, p 38, 52 |
| Source not identified | | | | | |
| 66 pre-term infants with birth weights between 1000 and 2200 g | 4 Groups:<br>1) human milk (0.38% FAs as DHA; 25.4 mg/kg bw/d; reference)<br>Or formulas with:<br>2) low-dose DHA, 0.04% of FAs, 2.7 mg/kg bw/d<br>3) mid-dose DHA, 0.33% of FAs, 22.1 mg/kg bw/d<br>4) high-dose DHA, 0.52% of FAs, 34.8 mg/kg bw/d | Until the postconceptional age of 48 wk or 28 d | Growth; intake during the study period; DHA content in the plasma phospholipids; no adverse effects were reported | Sauerwald et al., 2012 | GRN 000553, p 50; GRN 001008, p 56 |
| DHA from fish oil | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 238 pre-term infants (<35 wk, ≤2000 g birth weight) | Unsupplemented formula or formula supplemented with 0.5% DHA (approx. 33.5 mg DHA/kg bw/d, assuming that infants consume 6.7 g fat/kg bw/d), 0.04% ARA, and 0.1% EPA. *The source of DHA was tuna oil. | Subjects consumed the formulas to 9 mo after term. | Neurodevelopment scores (Bayley Mental and Psychomotor Indices) at 18 mo after term; Safety: growth (9 and 18 mo), tolerance, infection, and clinical complications. | Fewtrell et al., 2004 | GRN 000379, p 31, 36; GRN 001008, p 49 |

*Assuming that infants consume 6.7 g fat/kg bw/day; Our review focused on GRNs 000379, 000553, 000677, 000933, and 001008.
Abbreviations: ARA = arachidonic acid; bw = body weight; d = days; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid; GRN = GRAS notice; mo = months; PUFAs = polyunsaturated fatty acids; Ref = reference; wk = weeks; y = years.

### 6.B.5. Potential Adverse Effects

The U.S. 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 U.S. 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 FAs 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 FAs (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 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 a *Schizochytrium* sp. source at a dose of 0.30 g/kg in the 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 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 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 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 Runke Bioengineering's 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 U.S. FDA GRAS notices, it is concluded that Runke Bioengineering's DHA-rich oil is substantially chemically equivalent to those described in GRNs 000137, 000553, 000677, 000731, and 001008. Thus, the information and data presented or reviewed in these previous GRAS notices are pertinent when evaluating the safety of the DHA-rich oil in this GRAS notice. As noted above, the U.S. 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 000137 and 000732) received U.S. 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;

001008). 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 U.S. 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 humans. The NOAEL was determined to be 2,069 mg/kg bw/day in a subchronic toxicity study with an *in utero* phase 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 U.S. 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 (U.S. FDA, 2004), 000553 (U.S. FDA, 2015), 000677 (U.S. FDA, 2017), 000731/000732 (U.S. FDA, 2018a, 2018b), 000776/000777 (U.S. FDA, 2018c, 2018d), 000836 (U.S. FDA, 2019a), 000843/000844 (U.S. FDA, 2019b, 2019c), 000862 (U.S. FDA, 2020a), 000933 (U.S. FDA, 2020b), 000934 (U.S. FDA, 2021), and 001008 (U.S. 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 U.S. FDA '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 the 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% and 0.76% of total FAs were reported in term and pre-term infants, respectively.

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

### 7.A. References That Are Generally Available

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

Altug T. Introduction to toxicology and food. CRC Press, Boca Raton, FL. 2003.

Andersson SW, Skinner J, Ellegård L, Welch AA, Bingham S, Mulligan A, Andersson H, Khaw KT. Intake of dietary plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: a cross-sectional study. Eur J Clin Nutr. 2004;58:1378-85.

Bang HO, Dyerberg J, Sinclair HM. The composition of the Eskimo food in north western Greenland. Am J Clin Nutr. 1980; 3:2657-61.

Birch EE, Carlson SE, Hoffman DR, Fitzgerald-Gustafson KM, Fu VL, Drover JR, Castañeda YS, Minns L, Wheaton DK, Mundy D, Marunycz J, Diersen-Schade DA. The DIAMOND (DHA Intake And Measurement Of Neural Development) Study: A double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid. Am J Clin Nutr. 2010;91:848-59.

Birch EE, Castañeda YS, Wheaton DH, Birch DG, Uauy RD, Hoffman DR. Visual maturation of term infants fed long-chain polyunsaturated fatty acid-supplemented or control formula for 12 mo. Am J Clin Nutr. 2005;81(4):871-79.

Birch EE, Garfield S, Castañeda Y, Hughbanks-Wheaton D, Uauy R, Hoffman D. Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. Early Hum Dev. 2007;83(5):279-84.

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

Brownawell AM, Falk MC. Cholesterol: Where science and public health policy intersect. Nutr Rev. 2010;68:355-364.

Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated FAs in coronary heart disease: A meta-analysis of randomized controlled trials. Am J Med. 2002;112:298-304.

Buckley R, Shewring B, Turner R, Yaqoob P, Minihane AM. Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. Br J Nutr. 2004;92:477-83.

Byelashov OA, Sinclair AJ, Kaur G. Dietary sources, current intakes, and nutritional role of omega-3 docosapentaenoic acid. Lipid Technol. 2015;27:79-82.

Carnielli VP, Simonato M, Verlato G, Luijendijk I, De Curtis M, Sauer PJ, Cogo PE. Synthesis of long-chain polyunsaturated FAs in pre-term newborns fed formula with long-chain polyunsaturated FAs. Am J Clin Nutr. 2007;86:1323-1330.

Chase HP, Boulware D, Rodriguez H, Donaldson D, Chritton S, Rafkin-Mervis L, Krischer J, Skyler JS, Clare-Salzler M; Type 1 Diabetes Trial Net Nutritional Intervention to Prevent (NIP) Type 1 Diabetes Study Group. Effect of docosahexaenoic acid supplementation on inflammatory cytokine levels in infants at high genetic risk for type 1 diabetes. Pediatr Diabetes. 2015;16(4):271-79.

Chen J, Jiao R, Jiang Y, Bi Y, Chen ZY. Algal sterols are as effective as β-sitosterol in reducing plasma cholesterol concentration. J Agric Food Chem. 2014;62:675-81.

Clandinin MT, Van Aerde JE, Parrott A, Field CJ, Euler AR, Lien EL. Assessment of the efficacious dose of arachidonic and docosahexaenoic acids in pre-term infant formulas: fatty acid composition of erythrocyte membrane lipids. Pediatr Res. 1997;42:819-25.

Claumarchirant L, Matencio E, Sanchez-Siles LM, Alegría A, Lagarda MJ. Sterol composition in infant formulas and estimated intake. J Agri Food Chem. 2015;63(32):7245-51.

Colombo J, Shaddy DJ, Kerling EH, Gustafson KM, Carlson SE. Docosahexaenoic acid (DHA) and arachidonic acid (ARA) balance in developmental outcomes. Prostaglandins, Leukotrienes and Essential FAs. 2017;121:52-6.

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

EFSA, 2016. Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidal fatty acid esters in food, Question Number: EFSA-Q2014-00535. EFSA Journal 14, 4426.

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

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

Fedorova-Dahms I, Marone PA, Bauter M, Ryan AS. Safety evaluation of DHA-rich algal oil from *Schizochytrium* sp. Food Chem Toxicol. 2011b;49:3310-18.

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

Fewtrell MS, Abbott RA, Kennedy K, Singhal A, Morley R, Caine E, Jamieson C, Cockburn F, Lucas A. Randomized, double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in pre-term infants. J Pediatrics. 2004;144(4):471-79.

Fomon SJ. 1993. Nutrition of Normal Infants. (Baltimore: Mosby-Year Book, Inc.).

Food Chemicals Codex (FCC) online, 2023. 13th ed. https://www.foodchemicalscodex.org/.

Food and Drug Administration (FDA)**,** 1997. Substances affirmed as Generally Recognized as Safe: Menhaden oil; Final rule. Federal Reg. 62:30751.

FDA, 2004. Agency Response Letter. GRAS Notice No. GRN000137. Algal oil (Schizochytrium sp.). February 12, 2004. https://wayback.archive-it.org/7993/20171031032149/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm153961.htm.

FDA, 2005. 21CFR 184.1472. Menhaden oil final rule (21 CFR, Part 184, Fed Reg (U.S.) 70 (55):14530-2.

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

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

FDA, 2018a. Agency Response Letter. GRAS Notice No. GRN000731. Docosahexaenoic oil produced in *Schizochytrium* sp.

https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM630673.pdf.

FDA, 2018b. Agency Response Letter. GRAS Notice No. GRN 000732. Docosahexaenoic acid oil produced in *Schizochytrium* sp. April 6, 2018. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM630674.pdf.

FDA, 2018c. Agency Response Letter. GRAS Notice No. GRN000776. Algal oil (35% docosahexaenoic acid) from *Schizochytrium* sp. strain FCC-1324. October 26, 2018. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625983.pdf.

FDA, 2018d. Agency Response Letter. GRAS Notice No. GRN000777. Algal oil (55% docosahexaenoic acid) from *Schizochytrium* sp. strain FCC-3204. October 26, 2018. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625998.pdf.

FDA, 2019a. Agency Response Letter. GRAS Notice No. GRN000836. Algal oil (50-60% docosahexaenoic acid) from *Schizochytrium* sp. HS01. Sep 3, 2019. https://www.fda.gov/media/132031/download.

FDA, 2019b. Agency Response Letter. GRAS Notice No. GRN000843. Algal oil (35% docosahexaenoic acid) from *Schizochytrium* sp. strain FCC-1324. Oct 18, 2019. https://www.fda.gov/media/133204/download.

FDA, 2019c. Agency Response Letter. GRAS Notice No. GRN000844. Algal oil (55% docosahexaenoic acid) from *Schizochytrium* sp. strain FCC-3204. Oct 18, 2019. https://www.fda.gov/media/133205/download.

FDA, 2020a. Agency Response Letter. GRAS Notice No. GRN 000862; Algal oil (40% docosahexaenoic acid) from Schizochytrium sp. strain ONC-T18. June 15, 2020. https://www.fda.gov/media/139607/download.

FDA, 2020b. Agency Response Letter. GRAS Notice No. GRN 000933; Algal oil (≥36% docosahexaenoic acid) from Schizochytrium sp. strain DHF. Nov 13, 2020. https://www.fda.gov/media/144599/download.

FDA, 2021. Agency Response Letter. GRAS Notice No. GRN 000934; Algal oil (≥35% docosahexaenoic acid) from Schizochytrium sp. strain CABIO-A-2. July 20, 2021. https://www.fda.gov/media/153270/download.

FDA, 2022. Agency Response Letter. GRAS Notice No. GRN 001008; Algal oil (≥45% docosahexaenoic acid) from *Aurantiochytrium limacinum* TKD-1 (or *Schizochytrium limacinum* TKD-1). Feb 25, 2022. https://www.fda.gov/media/156854/download.

Fu Y, Liu X, Zhou B, Jiang AC, Cha, L. An updated review of worldwide levels of docosahexaenoic and arachidonic acid in human breast milk by region. Public Health Nutr. 2016;19:2675-87.

Garmendia ML, Casanello P, Flores M, Kusanovic JP, Uauy R. The effects of a combined intervention (docosahexaenoic acid supplementation and home-based dietary counseling) on metabolic control in obese and overweight pregnant women:the MIGHT study. Am J Obstet Gynecol. 2021 May;224(5):526.e1-526.e25. doi:10.1016/j.ajog.2020.10.048. Epub 2020 Nov 2. PMID: 33152314.

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

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

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

Ingol TT, Li R, Boone KM, Rausch J, Klebanoff MA, Turner AN, Yeates KO, Nelin MA, Sheppard KW, Keim SA. Docosahexaenoic and arachidonic acid supplementation of toddlers born pre-term does not affect short-term growth or adiposity. J Nutr. 2019;149:2182-90.

Joint FAO/WHO 1996. Biotechnology and food safety. Report of a Joint FAO/WHO Consultation. Rome, Italy, 30 September - 4 October 1996. Page 4.

Koletzko B, Bergmann K, Brenna JT, Calder PC, Campoy C, Clandinin MT, Colombo J, Daly M, Decsi T, Demmelmair H, Domellöf M, FidlerMis N, Gonzalez-Casanova I, van Goudoever JB, Hadjipanayis A, Hernell O, Lapillonne A, Mader S, Martin CR, Matthäus V, Ramakrishan U, Smuts CM, Strain SJJ, Tanjung C, Tounian P, Carlson SE. Should formula for infants provide arachidonic acid along with DHA? A position paper of the European Academy of Paediatrics and the Child Health Foundation. Am J Clin Nutr. 2020;111:10-6.

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

Koletzko B, Poindexter B, Uauy R. Recommended nutrient intake levels for stable, fully enterally fed very low birth weight infants. World Rev Nutr Diet. 2014a;110:297-9.

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

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

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

MacDonald IM, Sieving PA. Investigation of the effect of dietary docosahexaenoic acid (DHA) supplementation on macular function in subjects with autosomal recessive Stargardt macular dystrophy. Ophthalmic Genet. 2018;39:477-86.

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

Mellies M, Glueck CJ, Sweeney C, Fallat RW, Tsang RC, Ishikawa TT. Plasma and dietary phytosterols in children. Pediatrics. 1976;57:60-7.

Mioso R, Toledo Marante FJ, González JE, Rodríguez JJ, de Laguna IH. Metabolite profiling of Schizochytrium sp. by GC-MS, an oleaginous microbial source of biodiesel. Braz J Microbiol. 2014;45:403-9.

Sanders TA, Gleason K, Griffin B, Miller GJ. Influence of an algal triacylglycerol containing docosahexaenoic acid (22 : 6n-3) and docosapentaenoic acid (22 : 5n-6) on cardiovascular risk factors in healthy men and women. Br J Nutr. 2006;95:525-31.

Sauerwald UC, Fink MM, Demmelmair H, Schoenaich PV, Rauh-Pfeiffer AA, Koletzko B. Effect of different levels of docosahexaenoic acid supply on fatty acid status and linoleic and α-linolenic acid conversion in pre-term infants. J Pediatr Gastroenterol Nutr. 2012;54:353-63.

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

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

Tam PS, Umeda-Sawada R, Yaguchi T, Akimoto K, Kiso Y, Igarashi O. The metabolism and distribution of docosapentaenoic acid (n-6) in rats and rat hepatocytes. Lipids. 2000;35:71-5.

Tanaka K, Tanaka S, Shah N, Ota E, Namba F. Docosahexaenoic acid and bronchopulmonary dysplasia in pre-term infants: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2022;35:1730-8.

Wang R, Feng Y, Chen J, Chen Y, Ma F. Association between polyunsaturated fatty acid intake and infertility among American women aged 20-44 years. Front Public Health. 2022;10:938343.

Yeiser M, Harris CL, Kirchoff AL, Patterson AC, Wampler JL, Zissman EN, Berseth CL. Growth and tolerance of infants fed formula with a new algal source of docosahexaenoic acid: Double-blind, randomized, controlled trial. Prostaglandins Leukot Essent FAs. 2016;115:89-96.

Zhou Z, Li S, Yang Q, Yang X, Liu Y, Hao K, Xu S, Zhao N, Zheng P. Association of n-3 polyunsaturated fatty acid intakes with juvenile myopia: A cross-sectional study based on the NHANES database. Front Pediatr. 2023;11:1122773.

**Studies not included in the safety review of DHA-rich oil for the intended use**

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

Almaas AN, Tamnes CK, Nakstad B, Henriksen C, Grydeland H, Walhovd KB, Fjell AM, Iversen PO, Drevon CA. Diffusion tensor imaging and behavior in premature infants at 8 years of age, a randomized controlled trial with long-chain polyunsaturated FAs. Early Hum Dev. 2016;95:41-46.

Bernabe-García M, Calder PC, Villegas-Silva R, Rodríguez-Cruz M, Chávez-Sánchez L, Cruz-Reynoso L, Mateos-Sánchez L, Lara-Flores G, Aguilera-Joaquín AR, Sánchez-García L. Efficacy of docosahexaenoic acid for the prevention of necrotizing enterocolitis in pre-term infants: A Randomized Clinical Trial. Nutrients. 2021;13:648.

Burks W, Jones SM, Berseth CL, Harris C, Sampson HA, Scalabrin DM. Hypoallergenicity and effects on growth and tolerance of a new amino acid-based formula with docosahexaenoic acid and arachidonic acid. J Pediatr. 2008;153:266-71.

Clandinin MT, Van Aerde JE, Merkel KL, Harris CL, Springer MA, Hansen JW, Diersen-Schade DA. Growth and development of pre-term infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. J Pediatr. 2005;146:461-68.

Clandinin MT, Van Aerde JE, Parrott A, Field CJ, Euler AR, Lien E. Assessment of feeding different amounts of arachidonic and docosahexaenoic acids in pre-term infant formulas on the fatty acid content of lipoprotein lipids. Acta Paediatr. 1999;88:890-96.

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

Fleddermann M, Demmelmair H, Grote V, Nikolic T, Trisic B, Koletzko B. Infant formula composition affects energetic efficiency for growth: The BeMIM study, a randomized controlled trial. Clin Nutr. 2014;33:588-95.

Frost BL, Patel AL, Robinson DT, Berseth CL, Cooper T, Caplan M. Randomized controlled trial of early docosahexaenoic acid and arachidonic acid enteral supplementation in very low birth weight infants. J Pediatr. 2021;232:23-30.

Gunaratne AW, Makrides M, Collins CT, Gibson RA, McPhee AJ, Sullivan TR, Gould JF, Green TJ, Doyle LW, Davis PG, French NP, Colditz PB, Simmer K, Morris SA, Best KP. Docosahexaenoic acid supplementation of pre-term infants and parent-reported symptoms of allergic disease at 7 years corrected age: follow-up of a randomized controlled trial. Am J Clin Nutr. 2019;109(6):1600-10.

Henriksen C, Haugholt K, Lindgren M, Aurvåg AK, Rønnestad A, Grønn M, Solberg R, Moen A, Nakstad B, Berge RK, Smith L, Iversen PO, Drevon CA. Improved cognitive development among pre-term infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. Pediatrics. 2008;121:1137-45.

Hewawasam E, Collins CT, Muhlhausler BS, Yelland LN, Smithers LG, Colombo J, Makrides M, McPhee AJ, Gould JF. DHA supplementation in infants born pre-term and the effect on attention at 18 months' corrected age: Follow-up of a subset of the N3RO randomised controlled trial. Br J Nutr. 2021;125:420-31.

Hoffman D, Ziegler E, Mitmesser SH, Harris CL, Diersen-Schade DA. Soy-based infant formula supplemented with DHA and ARA supports growth and increases circulating concentrations of these FAs in infants. Lipids. 2008;43:29-35.

Manley BJ, Makrides M, Collins CT, McPhee AJ, Gibson RA, Ryan P, Sullivan TR, Davis PG; DINO Steering Committee. High-dose docosahexaenoic acid supplementation of pre-term infants: respiratory and allergy outcomes. Pediatrics. 2011;128(1):e71-7.

Pawlik D, Lauterbach R, Hurkala J. The efficacy of fish-oil based fat emulsion administered from the first day of life in very low birth weight newborns. Med Wieku Rozwoj 2011;15:312-17.

Pawlik D, Lauterbach R, Walczak M, Hurkala J, Sherman MP. Fish-oil fat emulsion supplementation reduces the risk of retinopathy in very low birth weight infants : a prospective, randomized study. Journal of Parenteral and Enteral Nutrition 2014;38(6):711-16.

Smith DJ, Sarris J, Dowling N, O'Connor M, Ng CH. Adjunctive low-dose docosahexaenoic acid (DHA) for major depression: An open-label pilot trial. Nutr Neurosci. 2018;21:224-28.

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

**7.B. References That Are Not Generally Available**

Not applicable.

## Appendix A. Certificates of Analysis

eurofins



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<br>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.<br>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.<br>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.<br>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<br>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<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |
| | Salmonella | Not Detected | /25 ml | | |
| ^# SU1A7 | Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001<br>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<br>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<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China
Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

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

eurofins



| | Results | Unit | LOQ | LOD |
|---|---|---|---|---|
| Peroxide value | 0.36 | 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 | 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 | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



| | 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 | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



| | | 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
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation.
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



# 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<br>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<br>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<br>Accreditation: ISO/IEC 17025:2017 A2LA 2993.01 | | | | |
| | Glycidol (calculated) | <0.10 | mg/kg | 0.1 | |

**SIGNATURE**

Claire Wang
Authorized Signatory

**EXPLANATORY NOTE**

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
^ CNAS # DAkkS ¤CMA
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence. Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



CNAS 中国认可 检测 TESTING CNAS L3788

## 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<br>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<br>Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788 | | | | |
| | Aerobic Plate Count | <10 | cfu/g | | |
| + SU1A4 | Salmonella Method: US FDA BAM Chapter 5, 2021<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |
| | Salmonella | Not Detected | /25 g | | |
| +# SU1A7 | Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001<br>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<br>Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788 | | | | |
| | E. coli | Not Detected | /25 g | | |

SIGNATURE

Tracy Li
Authorized Signatory

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

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

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
+ CNAS # DAkkS =CMA
☆ means the test is subcontracted within Eurofins group
● means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



## 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**

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
△ CNAS # DAkkS □CMA
☆ means the test is subcontracted within Eurofins group
• means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

eurofins



CNAS 中国认可 检测 TESTING CNAS L3788

## 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<br>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<br>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<br>Authorized Signatory | Jack He<br>Authorized Signatory |

**EXPLANATORY NOTE**

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
^ CNAS # DAkkS □CMA
☆ means the test is subcontracted within Eurofins group
● means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
www.eurofins.cn

report

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

The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
www.eurofins.cn

Scan QR code to view report

eurofins

Page 1/1
AR-22-SU-047148-02

# 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<br>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**

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
+ CNAS # DAkkS =CMA
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



CNAS 中国认可 检测 TESTING CNAS L3788

## Analytical Report

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

Runke Bioengineering (Fujian) Co.,Ltd.
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<br>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.<br>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.<br>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.<br>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<br>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<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |
| | Salmonella | Not Detected | /25 ml | | |
| ^# SU1A7 | Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001<br>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<br>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<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

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

eurofins



| | | 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 | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

| | 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 | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



| | 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 idenfitications 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
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group

Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



# 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<br>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 (℃) | 14.0 | Sample Weight | 140g*2 |

| | | Results | Unit | LOQ | LOD |
|---|---|---|---|---|---|
| ☆ QA04G | Monochloropropanediols (sum of free and esters) Method: AOCS Cd 29b-13<br>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<br>Accreditation: ISO/IEC 17025:2017 A2LA 2993.01 | | | | |
| | Glycidol (calculated) | <0.10 | mg/kg | 0.1 | |

SIGNATURE

Claire Wang
Authorized Signatory

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
⋆ CNAS # DAkkS ¤CMA
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence. Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins

Page 1/2
AR-22-SU-033314-02

CNAS
中国认可
检测
TESTING
CNAS L3788

# 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：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: | 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<br>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<br>Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788 | | | | |
| | Aerobic Plate Count | <10 | cfu/g | | |
| + SU1A4 | Salmonella Method: US FDA BAM Chapter 5, 2021<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |
| | Salmonella | Not Detected | /25 g | | |
| +# SU1A7 | Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001<br>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<br>Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788 | | | | |
| | E. coli | Not Detected | /25 g | | |

SIGNATURE

Tracy Li
Authorized Signatory

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

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

EXPLANATORY NOTE

| | |
|---|---|
| LOQ: Limit of Quantification | + CNAS # DAkkS ＝CMA |
| < LOQ: Below Limit of Quantification | ☆ means the test is subcontracted within Eurofins group |
| N/A means Not applicable | ᵒ means the test is subcontracted outside Eurofins group |

Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins

Page 1/1
AR-22-SU-056886-02

## 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**

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
▲ CNAS # DAkkS □CMA
☆ means the test is subcontracted within Eurofins group
• means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

eurofins



CNAS 中国认可 检测 TESTING CNAS L3788

## 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<br>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<br>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**

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
△ CNAS # DAkkS □CMA
☆ means the test is subcontracted within Eurofins group
● means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.

**Eurofins Tech. Service (Suzhou) Co., Ltd**
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
www.eurofins.cn

Scan QR code to view report

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

The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
www.eurofins.cn

Scan QR code to view report

eurofins

Page 1/1
AR-22-SU-047149-02

# 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：11027716<br>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 | |

**SIGNATURE**

Shine Xie
Authorized Signatory

**EXPLANATORY NOTE**

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
^ CNAS # DAkkS ¤CMA
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicity available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



中国认可
检测
TESTING
CNAS L3788

## Analytical Report

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

Runke Bioengineering (Fujian) Co.,Ltd.
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<br>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.<br>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.<br>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.<br>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<br>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<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |
| | Salmonella | Not Detected | /25 ml | | |
| ^# SU1A7 | Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001<br>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<br>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<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

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

eurofins



| | 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 | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



| | 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 | |

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China
Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



| | 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 idenfitications 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
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group

Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins

Page 1/1
AR-22-SU-007860-02

# 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<br>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 (°C) | 14.0 | Sample Weight | 140g*2 |

| | Results | Unit | LOQ | LOD |
|---|---|---|---|---|
| ☆ QA04G Monochloropropanediols (sum of free and esters) Method: AOCS Cd 29b-13<br>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<br>Accreditation: ISO/IEC 17025:2017 A2LA 2993.01 | | | | |
| Glycidol (calculated) | <0.10 | mg/kg | 0.1 | |

SIGNATURE

Claire Wang
Authorized Signatory

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
^ CNAS # DAkkS ¤CMA
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



CNAS 中国认可 检测 TESTING CNAS L3788

## 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<br>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<br>Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788 | | | | |
| | Aerobic Plate Count | <10 | cfu/g | | |
| + SU1A4 | Salmonella Method: US FDA BAM Chapter 5, 2021<br>Accreditation: ISO/IEC 17025:2017 CNAS L3788 | | | | |
| | Salmonella | Not Detected | /25 g | | |
| +# SU1A7 | Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001<br>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<br>Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788 | | | | |
| | E. coli | Not Detected | /25 g | | |

SIGNATURE

Tracy Li
Authorized Signatory

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

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

eurofins



EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
+ CNAS # DAkkS =CMA
☆ means the test is subcontracted within Eurofins group
° means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicity available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

eurofins



## 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**

LOQ: Limit of Quantification

< LOQ: Below Limit of Quantification

N/A means Not applicable

^ CNAS # DAkkS ¤CMA

☆ means the test is subcontracted within Eurofins group

• means the test is subcontracted outside Eurofins group

Sum compounds results are calculated from the results of each quantified compound as set by regulation

The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.

The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.

The analytical result herein is applicable for the sample(s) tested only.

This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.

The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.

The Eurofins General Terms and Conditions apply to this analytical report.

For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

eurofins

Page 1/2
AR-23-SU-007405-02

CNAS 中国认可 检测 TESTING CNAS L3788

## 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<br>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<br>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<br>Authorized Signatory | Jack He<br>Authorized Signatory |

**EXPLANATORY NOTE**

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
△ CNAS # DAkkS □CMA
☆ means the test is subcontracted within Eurofins group
● means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
www.eurofins.cn

Scan QR code to view report

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

eurofins

Page 2/2
AR-23-SU-007405-02

The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

**Eurofins Tech. Service (Suzhou) Co., Ltd**
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
www.eurofins.cn

Scan QR code to view report

eurofins



# 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-047150-01

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<br>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

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
\- CNAS # DAkkS =CMA
☆ means the test is subcontracted within Eurofins group
* means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
The sample description and information are provided by the Client. Eurofins is not responsible for verifying the accuracy, relevancy, adequacy and/or completeness of the information provided by the Client.
The analytical result herein is applicable for the sample(s) tested only.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
The Eurofins General Terms and Conditions apply to this analytical report.
For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China
Phone +86 400 828 5088
Fax
www.eurofins.cn

Scan QR code to view report

## 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

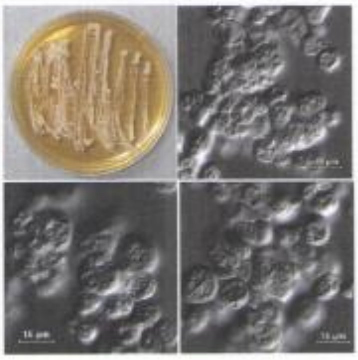
IMCAS Report No. 2023JB157

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.

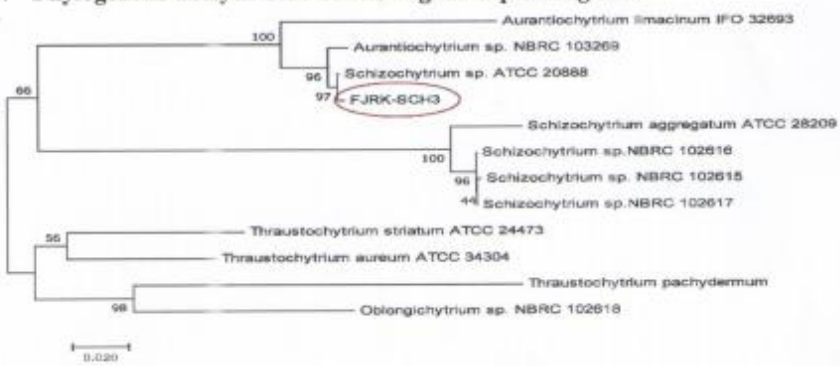


**2. Partial sequence of 18S rRNA gene**

**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 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 U.S. FDA 'no question' letters for infant formula applications (GRN 000553 – U.S. FDA, 2015; GRN 000677 – U.S. FDA, 2017; GRN 000731 – U.S. FDA, 2018a, GRNs 000776/000777 – U.S. FDA, 2018c, 2018d; GRN 000862 – U.S. FDA, 2020a; GRN 000933 – U.S. FDA, 2020b; GRN 000934 – U.S. FDA, 2021; GRN 001008 – U.S. FDA, 2022) and selected conventional food applications (GRN 000137 – U.S. FDA, 2004; GRN 000732 – U.S. FDA, 2018b; GRN 000836 – U.S. FDA 2019a; GRN 000843/000844 – U.S. FDA, 2019b, 2019c; GRN 000862 – U.S. FDA, 2020a; GRN 000933 – U.S. FDA, 2020b; GRN 000934 – U.S. FDA, 2021; GRN 001008 – U.S. 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, the U.S. FDA issued a final rule on menhaden oil ensuring daily intakes of eicosapentaenoic acid (EPA) and DHA do not exceed 3 g/person/day (U.S. 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 FAs 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 (U.S. 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).

<u>Animal Toxicity Studies</u>
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 U.S. 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 an *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., 2012) to 4,399 mg/kg bw/day (females - Fedorova-Dahms et al., 2011) 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., 2012) to 8,322 mg/kg bw/day ($F_0$ females during lactation) in rats (Fedorova-Dahms et al., 2011).

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 the 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., 2012) to 4,399 mg/kg bw/day (females - Fedorova-Dahms et al., 2011) in rats.

<u>Human Clinical Studies</u>

Human clinical studies reported daily doses of DHA instead of DHA-rich oil. This review includes studies published between January 2022 and December 2023.

*Studies of DHA in Adults*

Since January 2022, 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 (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 2022, one study of DHA-rich oil derived from *Schizochytrium* sp. in pregnant women was published (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 2022 have been identified from the literature relating to algal DHA intake in term infants. Previous GRAS notices stated that algal DHA, up to 0.96% of total FAs (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 FAs, 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 FAs (Birch et al., 2010; Chase et al., 2015; Currie et al., 2015). Thus, it is concluded that the literature supports the intended use of DHA at 0.5% of total FAs 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 to formulas in pre-term infants (Carnielli et al., 2007; Clandinin et al., 1997; Fewtrell et al., 2004; Sauerwald et al., 2012).

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 (U.S. FDA, 2015, 2017, 2018a, 2018c, 2018d, 2020a, 2020b, 2021, 2022, respectively). 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.

<u>Conclusion</u>

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:

March 18, 2024

Joanne Slavin, Ph.D., R.D.
Professor, University of Minnesota

Date

March 18, 2024

George C. Fahey, Jr, Ph.D.
Professor Emeritus, University of Illinois at Urbana-Champaign

Date

March 19, 2024

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

Date

## References

Birch EE, Khoury JC, Berseth CL, Castañeda YS, Couch JM, Bean J, Tamer R, Harris CL, Mitmesser SH, Scalabrin DM. The impact of early nutrition on incidence of allergic manifestations and common respiratory illnesses in children. J Pediatr. 2010;156:902-06.e1.

Carnielli VP, Simonato M, Verlato G, Luijendijk I, De Curtis M, Sauer PJ, Cogo PE. Synthesis of long-chain polyunsaturated FAs in pre-term newborns fed formula with long-chain polyunsaturated FAs. Am J Clin Nutr. 2007;86:1323-1330.

Chase HP, Boulware D, Rodriguez H, Donaldson D, Chritton S, Rafkin-Mervis L, Krischer J, Skyler JS, Clare-Salzler M; Type 1 Diabetes Trial Net Nutritional Intervention to Prevent (NIP) Type 1 Diabetes Study Group. Effect of docosahexaenoic acid supplementation on inflammatory cytokine levels in infants at high genetic risk for type 1 diabetes. Pediatr Diabetes. 2015;16(4):271-79.

Clandinin MT, Van Aerde JE, Parrott A, Field CJ, Euler AR, Lien EL. Assessment of the efficacious dose of arachidonic and docosahexaenoic acids in pre-term infant formulas: fatty acid composition of erythrocyte membrane lipids. Pediatr Res. 1997;42:819-25.

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

Falk MC, Zheng X, Chen D, Jiang Y, Liu Z, Lewis KD. Developmental and reproductive toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-Rich oil. Food Chem Toxicol. 2017;103:270-78.

Fedorova-Dahms I, Marone PA, Bauter M, Ryan AS. Safety evaluation of DHA-rich algal oil from *Schizochytrium* sp. Food Chem Toxicol. 2011;49:3310-18.

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

Fewtrell MS, Abbott RA, Kennedy K, Singhal A, Morley R, Caine E, Jamieson C, Cockburn F, Lucas A. Randomized, double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in pre-term infants. J Pediatrics. 2004;144:471-9.

Garmendia ML, Casanello P, Flores M, Kusanovic JP, Uauy R. The effects of a combined intervention (docosahexaenoic acid supplementation and home-based dietary counseling) on metabolic control in obese and overweight pregnant women:the MIGHT study. Am J Obstet Gynecol. 2021 May;224(5):526.e1-526.e25. doi:10.1016/j.ajog.2020.10.048. Epub 2020 Nov 2. PMID: 33152314.

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

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

MacDonald IM, Sieving PA. Investigation of the effect of dietary docosahexaenoic acid (DHA) supplementation on macular function in subjects with autosomal recessive Stargardt macular dystrophy. Ophthalmic Genet. 2018;39:477-486.

Sanders TA, Gleason K, Griffin B, Miller GJ. Influence of an algal triacylglycerol containing docosahexaenoic acid (22 : 6n-3) and docosapentaenoic acid (22 : 5n-6) on cardiovascular risk factors in healthy men and women. Br J Nutr. 2006;95:525-31.

Sauerwald UC, Fink MM, Demmelmair H, Schoenaich PV, Rauh-Pfeiffer AA, Koletzko B. Effect of different levels of docosahexaenoic acid supply on fatty acid status and linoleic and α-linolenic acid conversion in pre-term infants. J Pediatr Gastroenterol Nutr. 2012;54:353-63.

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

Smith DJ, Sarris J, Dowling N, O'Connor M, Ng CH. Adjunctive low-dose docosahexaenoic acid (DHA) for major depression: An open-label pilot trial. Nutr Neurosci. 2018;21:224-28.

United States Food and Drug Administration (U.S. FDA), 2004. Agency Response Letter. GRAS Notice No. GRN000137. Algal oil (Schizochytrium sp.). February 12, 2004. http://www.fda.gov/Food/FoodIngredientsPackanina/GenerallyRecognizedasSafe GRAS/GRAS Listingducm 153961.htm.

U.S. FDA, 2005. 21CFR 184.1472. Menhaden oil final rule (21 CFR, Part 184, Fed Reg (U.S.) 70 (55):14530-2.

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

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

U.S. FDA, 2018a. Agency Response Letter. GRAS Notice No. GRN000731. Docosahexaenoic acid oil produced in *Schizochytrium* sp. April 6, 2018. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM630673.pdf.

U.S. FDA, 2018b. Agency Response Letter. GRAS Notice No. GRN 000732. Docosahexaenoic acid oil produced in *Schizochytrium* sp. April 6, 2018. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM630674.pdf.

U.S. FDA, 2018c. Agency Response Letter. GRAS Notice No. GRN000776. Algal oil (35% docosahexaenoic acid) from *Schizochytrium* sp. strain FCC-1324. October 26, 2018. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625983.pdf.

U.S. FDA, 2018d. Agency Response Letter. GRAS Notice No. GRN000777. Algal oil (55% docosahexaenoic acid) from *Schizochytrium* sp. strain FCC-3204. October 26, 2018. https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm625998.pdf.

U.S. FDA, 2019a. Agency Response Letter. GRAS Notice No. GRN000836. Algal oil (50-60% docosahexaenoic acid) from *Schizochytrium* sp. HS01. Sep 3, 2019. https://www.fda.gov/media/132031/download.

U.S. FDA, 2019b. Agency Response Letter. GRAS Notice No. GRN000843. Algal oil (35% docosahexaenoic acid) from *Schizochytrium* sp. strain FCC-1324. Oct 18, 2019. https://www.fda.gov/media/133204/download.

U.S. FDA, 2019c. Agency Response Letter. GRAS Notice No. GRN000844. Algal oil (55% docosahexaenoic acid) from *Schizochytrium* sp. strain FCC-3204. Oct 18, 2019. https://www.fda.gov/media/133205/download.

U.S. FDA, 2020a Agency Response Letter. GRAS Notice No. GRN 000862; Algal oil (40% docosahexaenoic acid) from Schizochytrium sp. strain ONC-T18. June 15, 2020.https://www.fda.gov/media/139607/download.

U.S. FDA, 2020b. Agency Response Letter. GRAS Notice No. GRN 000933; Algal oil (≥36% docosahexaenoic acid) from Schizochytrium sp. strain DHF. Nov 13, 2020. https://www.fda.gov/media/144599/download.

U.S. FDA, 2021. Agency Response Letter. GRAS Notice No. GRN 000934; Algal oil (≥35% docosahexaenoic acid) from Schizochytrium sp. strain CABIO-A-2. July 20, 2021. https://www.fda.gov/media/153270/download.

U.S. FDA, 2022. Agency Response Letter. GRAS Notice No. GRN 001008; Algal oil (≥45% docosahexaenoic acid) from *Aurantiochytrium limacinum* TKD-1 (or *Schizochytrium limacinum* TKD-1). Feb 25, 2022. https://www.fda.gov/media/156854/download

Yeiser M, Harris CL, Kirchoff AL, Patterson AC, Wampler JL, Zissman EN, Berseth CL. Growth and tolerance of infants fed formula with a new algal source of docosahexaenoic acid: Double-blind, randomized, controlled trial. Prostaglandins Leukot Essent FAs. 2016;115:89-96.

Form Approved: OMB No. 0910-0342; Expiration Date: 08/31/2025
*(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 |
|---|---|
| 001185 | Apr 3, 2024 |
| 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)*: 2024-02-26

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 | | sales@runke.com.cn |

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

| Name of Contact Person | Position or Title |
|---|---|
| Susan Cho | Chief Sceince Officer |

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

Mailing Address *(number and street)*: #313, 14631 Route 29

| City | State or Province | Zip Code/Postal Code | Country |
|---|---|---|---|
| Centreville | Virginia | 20121 | United States of America |

| Telephone Number | Fax Number | E-Mail Address |
|---|---|---|
| 301-875-6454 | | susanscho1@yahoo.com |

## SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Docosahexaenoic acid (DHA)-rich oil from Schizochytrium sp. FJRK-SCH3

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.

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.
Infant formulas: 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

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-rich oil from Schizochytrium sp. FJRK-SCH3
*(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.

AceOne RS, Inc. Suite 313, 14631 Route 29, Centreville, VA 20121
*(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 Digitally signed by Susan Cho Date: 2024.04.03 08:51:03 -04'00' | Susan Cho | 04/02/2024 |

## 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 |
| | COSM_3667_19028_RunkeBioengineeringF.pdf | Administrative |
| | DHA-GRASFinalsubmittedtoFDA4-2-24.pdf | Administrative |
| | DHAcoverletter4-2-24r.pdf | Administrative |