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
Arachidonic acid (ARA)-Rich Oil as a Food Ingredient for Use in Infant Formula

Dr. Morissette,

On behalf of Runke Bioengineering (Fujian) Co., Ltd. (Runke Bioengineering), we are resubmitting a GRAS notification for arachidonic acid (ARA)-rich oil as a food ingredient for use in infant formula (resubmission of GRN 001162). The enclosed document provides the notice of a claim that a food ingredient, the ARA-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

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ARACHIDONIC ACID-RICH OIL DERIVED FROM *MORTIERELLA ALPINA* FJRK-MA01 AS AN INGREDIENT FOR USE IN INFANT FORMULA

Prepared for Runke Bioengineering (Fujian) Co., Ltd.
West of No. 552 Rd., Jindu Industrial Clusters Zone, Zhao'an, Zhangzhou,
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Prepared by:
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GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ARACHIDONIC ACID (ARA)-RICH OIL DERIVED FROM *MORTIERELLA ALPINA* FJRK-MA01 AS AN INGREDIENT FOR USE IN INFANT FORMULA

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List of Abbreviations

2-MCPD = 2-monochloropropane-1,3-diol
3-MCPD = 3-monochloropropane-1,2-diol
AE = adverse event
ALP = alkaline phosphatase
ALT = alanine amino transferase
AOAC = Association of Official Analytical Chemists
AOCS = American Oil Chemists´ Society
aPTT = activated partial thromboplastin time
ARA = arachidonic acid
AST = aspartate amino transferase
BAM = Bacteriological Analytical Manual
bw = body weight
CAERS = CFSAN Adverse Event Reporting System
CAS = Chemical Abstract Service
cfu = colony forming units
CFR = Code of Federal Regulations
CFSAN = Center for Food Safety and Applied Nutrition
cGMP = current Good Manufacturing Practice
COA = Certificate of Analysis
CPA = cyclophosphamide
DHA = docosahexaenoic acid
DIAMOND = DHA Intake and Measurement of Neural Development
EDI = estimated daily intake
EPA = eicosapentaenoic acid
FA = fatty acid
FCC = Food Chemicals Codex
FDA = Food and Drug Administration
FD&C = Federal Food, Drug, and Cosmetic Act
FSIS = Food Safety and Inspection Service
GD = gestation days
GGT = gamma-glutamyl transferase
GRAS = Generally Recognized as Safe
GRN = GRAS notice
h = hour
HACCP = Hazard Analysis and Critical Control Point

IMCAS = Institute of Microbiology Chinese Academy of Sciences
ISO = International Standardization Organization
LCPUFA = long-chain polyunsaturated fatty acid
LD_{50} = mean lethal dose
LDH = lactate dehydrogenase
MCHC = mean corpuscular hemoglobin concentration
MCPDs = monochloropropanediols
MCV = mean corpuscular volume
MNPCE = micronucleated polychromatic erythrocytes
MPV = mean platelet volume
NA = not available
ND = not detected
NOAEL = No Observed Adverse Effect Level
PCE = polychromatic erythrocytes
PMA = post-menstrual age
PT = prothrombin time
PUFA = polyunsaturated fatty acid
QC = quality control
RAO = refined arachidonic acid-rich oil
RBC = red blood cell
rDNA = ribosomal deoxynucleic acid
SD = standard deviation
SDH = sorbitol dehydrogenase
S.V. = seminal vesicles
TG = triglyceride
TK = thymidine kinase
U.S. = United States
USDA = United States Department of Agriculture
WBC = white blood cell
yr = years

PART 1. SIGNED STATEMENTS AND A CERTIFICATION

1.A. Submission of GRAS Notice

Pursuant to Title 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 arachidonic acid (ARA)-rich oil in infant formula, as described in Parts 2 through 7 of this GRAS notice, is not subject to the 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: marketing.usap@runke.com.cn or sales@runke.com.cn

1.C. Common or Trade Name

Arachidonic acid-rich oil from *Mortierella alpina* FJRK-MA01, ARA, ARA-rich oil, ARA-rich oil derived from *Mortierella alpina* FJRK-MA01, fungal ARA-rich oil, or fungal ARA oil.

1.D. Applicable Conditions of Use of Runke Bioengineering's ARA-rich Oil

1.D.1. Foods in Which the ARA-rich Oil will be Used

Runke Bioengineering intends to market the ARA-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 dairy such as bovine or goat milk-based; ages from birth to 12 months) in combination with a safe and suitable source of DHA. Exempt infant formula refers to formulas for pre-term infants only and does not include use in other exempt formulas (e.g., hypoallergenic formulas, and formulas for inborn errors of metabolism).

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

The intended use of ARA-rich oil is to provide a source of ARA in infant formula at a concentration consistent with that of human milk. The ARA content of human milk varies from 0.34-1.22% of total fatty acids (FAs) among different populations. Therefore, the proposed use of ARA-rich oil is to provide 0.75% and 0.50% ARA by weight of FAs in term and pre-term infant formulas, respectively, in combination with a safe and suitable source of docosahexaenoic acid (DHA). The intended use of ARA-rich oil is to deliver this concentration of ARA, which corresponds to 1.974% of total fat in non-exempt term infant formula and 1.316% of total fat in exempt pre-term infant formula because ARA-rich oil contains ≥38% ARA. The ratios of ARA to DHA are expected to be in the range of 2:1 to 1:1. Intended use levels are consistent with recommendations by Koletzko et al. (2014a; 2014b; 2020).

1.D.3. Description of the Population Expected to Consume the Substance

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

1.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. Food and Drug Administration (FDA) upon request by contacting Susan Cho at AceOne RS, Inc. (formerly NutraSource, Inc.) at the address above. The data and information will be made available to the 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 U.S.C. §552.

1.H. Certification

Runke Bioengineering certifies that, to the best of our knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information available and obtainable by Runke Bioengineering, including any favorable or

unfavorable information, pertinent to the evaluation of the safety and GRAS status of the use of ARA-rich oil.

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

Name: Sunny Tsai Date: March 17, 2024
Title: Export Manager

Address correspondence to
Susan S. Cho, Ph.D., AceOne RS, Inc., Lead Expert Panel Member
scho@aceoners.com or susanscho1@yahoo.com
(301) 875-6454

1.J. Food Safety and Inspection Service (FSIS)/USDA Statement

Runke Bioengineering does not intend to add ARA-rich oil to any meat and/or poultry products that come under the United States Department of Agriculture (USDA) jurisdiction. Therefore, 21 CFR 170.270 does not apply.

PART 2. IDENTITY, MANUFACTURING, SPECIFICATIONS, AND TECHNICAL EFFECTS OF ARA-RICH OIL

2.A.1. Identity of the Notified Substance

2.A.1.1. Common or Trade Name

Arachidonic acid-rich oil from *Mortierella alpina* FJRK-MA01, Arachidonic acid-rich oil, ARA-rich oil, arachidonic acid, ARA-rich oil from *Mortierella alpina* FJRK-MA01, fungal ARA-rich oil, or arachidonic acid-rich single-cell oil

2.A.1.2. Chemical Names

all-cis-5,8,11,14-eicosatetraenoic acid (20:4 n-6)

2.A.1.3. Chemical Abstract Service (CAS) Registry Number

ARA: 506-32-1

2.A.1.4. Empirical Formula

Molecular formula of $C_{20}H_{32}0_2$

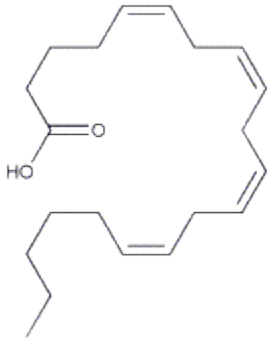
2.A.1.5. Molecular Weight

304.5

2.A.1.6. Structural Formula

Figure 1 shows the structure of ARA. In chemical structure, ARA is a carboxylic acid with a 20-carbon chain and four cis-double bonds; the first double bond is located at the sixth carbon from the omega end. Some chemistry sources define ARA to designate any eicosatetraenoic acid. However, almost all scientific literature limits the term to all-cis-5,8,11,14-eicosatetraenoic acid.

Figure 1. Chemical Structure of ARA.



2.A.1.7. Background

Because breastfeeding and human milk are the normative standards for infant feeding and nutrition, infant formula should support the nutritional needs of pre-term and term infants (Koletzko et al., 2014a, 2014b, 2020). The intended use of ARA-rich oil is to provide a source of ARA in infant formula at a concentration consistent with that of human milk. The ARA-rich oil contains approximately 40% ARA (≥38%). ARA-rich oil is a yellow to light-orange colored oil derived from the grown soil fungus, *Mortierella alpina*.

Arachidonic acid is not one of the essential FAs. However, infants, particularly pre-term infants, may have a limited ability to convert the essential precursor FAs, linoleic acid (18:2n-6) to ARA and linolenic acid (18:3n-3) to DHA, due to reduced concentrations and activity of desaturase enzymes (Hadley et al., 2016; Martin et al., 2011). Thus, the supplementation of infant formula with ARA at levels consistent with those in human milk is important because the omega-6 (n-6) and omega-3 (n-3) FAs present in human milk have critical roles in membrane structure and as precursors of eicosanoids (Hadley et al., 2016).

2.A.2. Potential Toxicants in Runke Bioengineering's ARA-rich Oil

Potential toxicants have not been identified. Residual solvent analysis showed that Runke Bioengineering's ARA-rich oil had no detectable levels of organic solvents (Table 1).

Fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPD), 2-monochloropropane-1,3-diol (2-MCPD), and glycidyl esters are heat-induced processing contaminants formed during the deodorization step of edible oil refining (Beekman et al., 2021). Because these compounds are potentially carcinogenic and/or genotoxic, their presence in refined oils and fats and foods containing these oils/fats poses possible health concerns. However,

due to the fact that the ARA-oil is not derived from vegetable sources and because there is no acid hydrolysis or use of chlorinated solutions in its production, it is not expected to have significant amounts of monochloropropanediols (MCPDs) or glycidyl esters. Analysis of 3 non-consecutive batches showed that the concentrations of MCPDs (2- and 3-MCPD; both free and ester forms) and glycidyl esters were near or below detection levels in Runke Bioengineering's ARA-rich oil. Details are presented in Table 2 and Appendix A.

Overall, no safety risk is expected in association with potential contaminants such as organic solvents, MCPDs, or glycidyl esters in Runke Bioengineering's ARA-rich oil.

Table 1. Residual Solvents Tested for in the ARA-rich Oil

Solvent Residues, mg/kg	Lot: 11004332	Lot: 11008334	Lot: 11012336
1,1,1,2-Tetrachloroethane	< 0.01	< 0.01	< 0.01
1,1,1-Trichloroethane	< 0.2	< 0.2	< 0.2
1,1,2-Tricholorethane	< 0.2	< 0.2	< 0.2
1,1-Dichloroethane	< 0.05	< 0.05	< 0.05
1,2-Dichloroethane	< 0.5	< 0.5	< 0.5
1,2-Dimethoxyethane	< 1.0	< 1.0	< 1.0
1-Butanol	< 1.0	< 1.0	< 1.0
2-Hexanone	< 1.0	< 1.0	< 1.0
Acetone	< 1.0	< 1.0	< 1.0
2-Butanon (Methylethylketone)	< 1	< 1	< 1
2-Methylpentane	< 1	< 1	< 1
3-Methylpentane	< 1	< 1	< 1
Benzene	< 0.10	< 0.10	< 0.10
Butyl acetate	< 0.50	< 0.50	< 0.50
Carbon tetrachloride	< 0.50	< 0.50	< 0.50
Chlorobenzene	< 0.50	< 0.50	< 0.50
Bromodichloromethane	< 0.05	< 0.05	< 0.05
Chloroform (trichloromethane)	< 0.10	< 0.10	< 0.10
Cyclohexane	< 0.20	< 0.20	< 0.20
Dichloromethane	< 0.10	< 0.10	< 0.10
Ethanol	< 1.0	< 1.0	< 1.0
cis-Dichloroethane	< 0.05	< 0.05	< 0.05
Dibromochloromethane	< 0.05	< 0.05	< 0.05
Dichloromethane	< 0.10	< 0.10	< 0.05
Ethyl Acetate	< 1.0	< 1.0	< 1.0

Ethylbenzene	< 0.01	< 0.01	< 0.01
m-/-p-Xylene	< 0.01	< 0.01	< 0.01
Methylcyclopentane	< 1	< 1	< 1
n-Heptane	< 0.20	< 0.20	< 0.20
Hexane (sum of n-hexane, iso and 3-methyl pentane)	< 0.50	< 0.50	< 0.50
Isopropanol	< 1.0	< 1.0	< 1.0
Methanol	< 1.0	< 1.0	< 1.0
Methyl Ethyl Ketone (MEK)	< 0.20	< 0.20	< 0.20
Methyl-turt-butylether (MTBE)	< 0.20	< 0.20	< 0.20
Tetralin	< 5.0	< 5.0	< 5.0
n-Pentane	< 1	< 1	< 1
Styrene	< 0.01	< 0.01	< 0.01
Sum 3 chlorinated solvents	Inapplicable	Inapplicable	Inapplicable
Technical Hexane (calculated)	Inapplicable	Inapplicable	Inapplicable
Tetrachloroethane	< 0.01	< 0.01	< 0.01
Tetrachloromethane	< 0.01	< 0.01	< 0.01
Toluene	< 0.20	< 0.20	< 0.20
trans-Dichloroethene	< 0.05	< 0.05	< 0.05
Tribromomethane	< 0.10	< 0.05	< 0.05
Trichloroethene	< 0.01	< 0.10	< 0.10
Trichloroethylene	< 0.10	< 0.10	< 0.10
Xylenes (sum)	< 0.20	< 0.20	< 0.20

Abbreviation: ARA = arachidonic acid

Table 2. Analytical Results for MCPD and Glycidol

Parameter	LOQ	Batch			Methods of Analysis
		11004332	11008334	11012336	
2-MCPD, mg/kg	0.1	< 0.10	< 0.10	< 0.10	AOCS Cd 29b-13
3-MCPD, mg/kg	0.1	0. 30	0.25	0.27	
Glycidol, mg/kg	0.1	< 0.10	< 0.10	< 0.10	

*All parameters were analyzed using validated Eurofins' internal methods.

Abbreviations: AOCS = American Oil Chemists´ Society; LOQ = Limit of Quantitation; 2-MCPD = 2-monochloropropane-1,3-diol; 3-MCPD = 3-monochloropropane-1,2-diol.

2.A.3. Particle Size

ARA-rich oil: not applicable

2.B. Method of Manufacture

ARA-rich oil is produced via a fermentation process using *Mortierella alpina* strain FJRK-MA01. The organism is grown in a pure culture heterotrophic fermentation process, recovered from the fermentation broth, and dried. The resulting dried *M. alpina* biomass is extracted with hexane to produce a crude oil that is further refined, decolorized, and deodorized using processes commonly employed in the vegetable oil industry.

a. Medium preparation and sterilization

Ingredients (glucose, yeast extract, sunflower seed oil, magnesium sulfate, potassium dihydrogen phosphate, potassium chloride, sodium hydroxide) are accurately weighed as per the ingredient mixing list. The weighed ingredients are mixed in an aqueous solution. The prepared fermentation medium is sterilized by steaming prior to inoculation and cultivation. The fermentation and cultivation of strains are carried out under bacteria-free conditions.

b. Fermentation

ARA-rich oil is produced via a heterotrophic fermentation process with *Mortierella alpina* (strain FJRK-MA01). This organism can be grown to a high cell density using a carbon-based substrate. Operating parameters such as temperature, agitation, tank pressure, ventilation capacity, aeration, and pH are controlled throughout the process to ensure that results, in terms of cell growth and oil production, are reproducible. The fermentation process is well controlled and critical control points are monitored to detect insufficient controls on the process (such as incomplete sterilization, incorrect pH or temperature ranges, insufficient FAs, etc.). If any of these 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 fermenter. The main fermentation reaction is stopped when the ARA content reaches the desired percentage above 38%.

c. Extraction

Cells (biomass) from the liquid fermentation medium are separated by pressure plate filter and cells containing oil are dried. Dried cells are extracted with hexane to produce a crude oil that is further refined, bleached, and deodorized using processes commonly employed in the vegetable oil industry. Biomass is separated from the crude oil-solvent mixture by filtration and the solvent is evaporated from the crude oil under a vacuum.

d. Refining

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

Quality Control

The ARA-rich oil is manufactured in adherence with current Good Manufacturing Practice (cGMP) to meet International Standardization Organization (ISO) 22000 standards for Hazard Analysis and Critical Control Point (HACCP).

All equipment that has direct contact with the finished ARA-rich oil or its intermediates is made of food-grade polyethylene, stainless steel, or carbon steel. All processing aids and ingredients meet Food Chemicals Codex (FCC) and/or food-grade specifications and 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. The manufacturing process includes quality control (QC) checks at every stage. Fermentation is carried out in the absence of light under axenic conditions.

All finished batches of ARA-rich oil undergo rigorous quality assurance testing to meet well-defined product specifications prior to release.

Raw Materials

The raw materials and processing aids used in the ARA-rich oil manufacturing process are summarized in Table 3.

Table 3. Raw Materials and Processing Aids Used in the Fermentation Process

Ingredient	CAS number
Fermentation medium	
Glucose [dextrose and glucose]	50-99-7
Yeast extract	8013-01-2
Sunflower seed oil	8001-21-6
Magnesium sulfate (heptahydrate)	10034-99-8
Potassium dihydrogen phosphate	7778-77-0
Potassium chloride	7447-40-7
Sodium hydroxide	1310-73-2
Processing aids	
Ascorbyl palmitate	137-66-6
Tocopherols	10191-41-0; 1406-18-4
Sodium hydroxide	1310-73-2
Sodium sulfate	7757-82-6
Activated carbon	64365-11-3
Activated clay (bentonite)	1302-78-9; 68333-91-5
Hexane	110-54-3

Abbreviation: CAS = Chemical Abstract Service

Figure 2 presents the manufacturing process of ARA-rich oil.

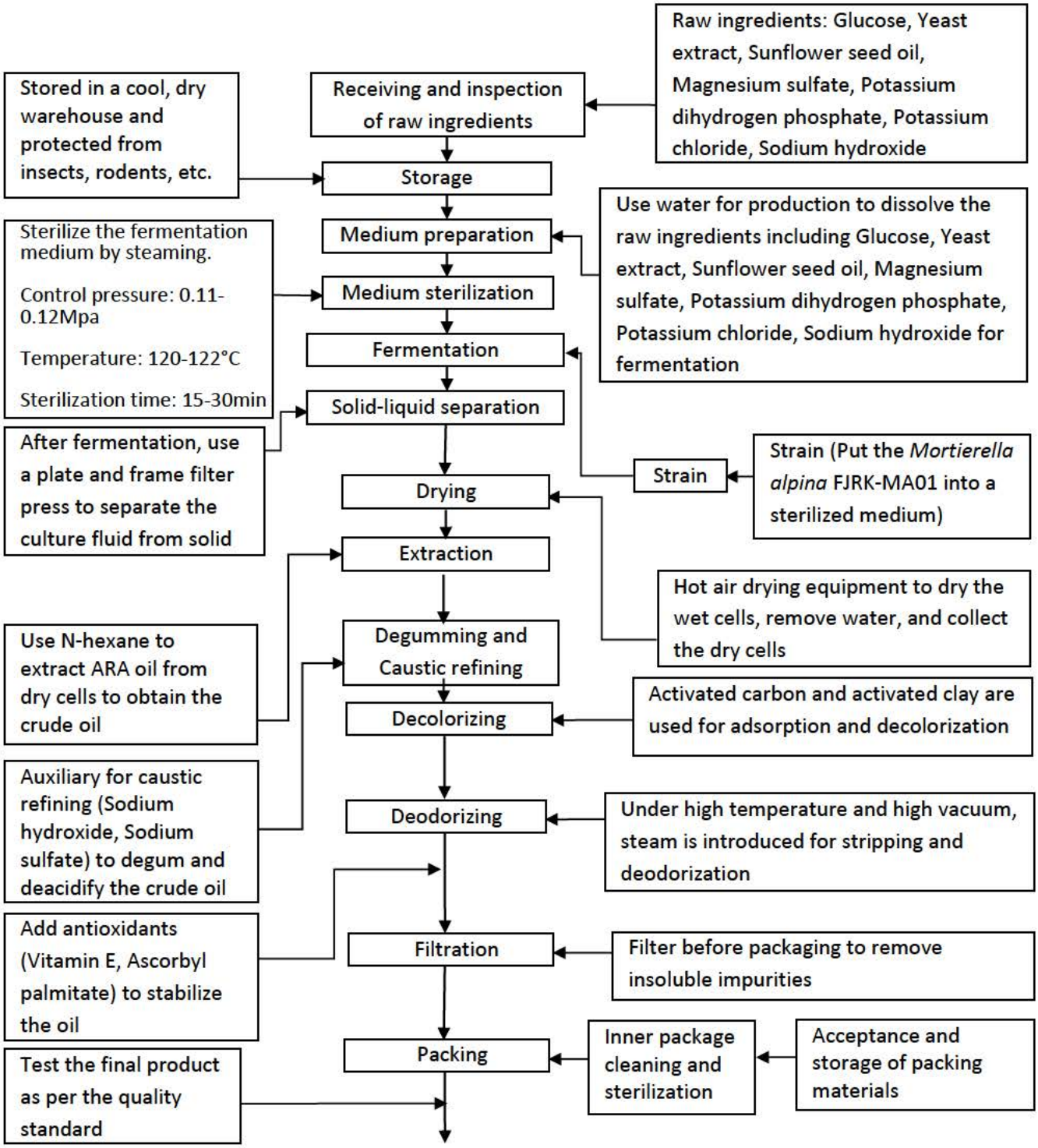


Figure 2. Manufacturing Flow Diagram of ARA-rich Oil

Characterization of the Source Organism

The principal production method (i.e., fungal production) is similar to those described by other companies whose production methods for ARA-rich oil have received no objection letters from the FDA (GRAS notices [GRNs] 000041, 000080, 000094, and 000326). ARA-rich oil is derived from the fermentation of the common soil fungus, *Mortierella alpina*. *M. alpina* is the most efficient production organism for ARA and is a common soil fungus to which humans are frequently exposed (Streekstra, 1997). Thus, it has been extensively applied to the industrial production of ARA-rich oil (Wu et al., 2015).

The genus *Mortierella* is presently classified as a member of the family Mortierellaceae within the order of the Mucorales, class Zygomycetes (Streekstra, 1997; Table 4). The Mortierellaceae are ubiquitous saprophytic fungi that are easily and frequently isolated from soil. In general, strains capable of growing at 37°C should be regarded as potentially pathogenic, whereas strains such as *M. alpina* that are unable to grow at body temperatures should be regarded as safe (Streekstra, 1997). *M. alpina* has an optimal temperature range of 26 – 28°C. On the basis of its optimal growth temperature, it is unlikely to be pathogenic. The pathogenic potential of the genus seems to be quite low.

Among the Mortierellaceae, *Mortierella wolfii,* a well-known pathogen of cattle, is the only currently recognized pathogen of the genus (Streekstra, 1997). *M. wolfii* excretes a water-soluble, heat-labile, trypsin-sensitive nephrotoxin (Davey et al., 1973). There is no evidence in the literature conveying *M. alpina* as pathogenic or toxigenic. *M. alpina* used for the production of ARA-rich oil is not a genetically modified organism.

Runke Bioengineering's production microorganism has been authenticated by morphological and rDNA-18S sequence *M. alpina* and deposited as FJRK-MA01 at the Institute of Microbiology Chinese Academy of Sciences (IMCAS). Table 4 presents taxonomic classification of *M. alpina* FJRK-MA01.

Table 4. Taxonomic Classification of *M. alpina* FJRK-MA01

Class	Scientific Classification
Kingdom	Fungi
Phylum	Zygomycota
Subdivision	Mortierellomycotina
Class	Zygomycetes
Order	Mucorales
Family	Mortierellaceae

Genus	*Mortierella*
Species	*Mortierella alpina*
Strain	*Mortierella alpina* FJRK-MA01

2.C. Specifications and Composition

Product specifications (Table 5) are set for ARA content, acid value, free FAs, unsaponifiables, anisidine value, peroxide value, residual hexane, moisture and volatiles, heavy metals, and microbiological parameters. Physical and chemical tests applied to the QC process of the oil are adapted from the Official Methods and Recommended Practices of the International Standardization Organization (ISO), the FDA Bacteriological Analytical Manual (BAM), and the American Oil Chemists' Society (AOCS). Specifications for Runke Bioengineering's ARA-rich oil are similar to those described in the previous GRAS notices and Food Chemicals Codex (FCC) (≥38% for Runke Bioengineering's; ≥40% in GRNs 000326 and 000094; 38-44% in GRNs 000080 and 000041).

Table 5. Specifications of ARA-rich Oil in Comparison with Those Specified in Previous GRAS Notices

Parameter	Current notice	GRN 000326	GRN 000094	GRNs 000080 & 000041	FCC
ARA, C 20:4n6, relative %	≥38	≥40	≥40	38-44*	≥38
Acid value, mg KOH/g	≤0.5	≤1.0	NA	NA	≤1.0
Free fatty acids, %	≤0.2	≤0.2	≤0.2	<0.4	≤0.2
Unsaponifiable matter, %	≤3.0	≤3.0	<1.0	<3.5	≤3.0
p-Anisidine value	≤20	≤20	NA	NA	≤20
Peroxide value, meq/kg	<2.0	≤2.0	<5.0	<5.0	≤2.0
Residual hexane, mg/kg	≤1.0	≤1.0	NA	NA	≤1.0
Mercury (Hg), mg/kg	≤0.05	≤0.05	<0.5	<0.2	≤0.1
Lead (Pb), mg/kg	≤0.1	⩽0.1	<0.1	<0.2	≤0.1
Arsenic (As), mg/kg	≤0.1	⩽0.1	<0.2	<0.5	≤0.1
Cadmium (Cd), mg/kg	≤0.1	⩽0.1	NA	NA	≤0.1
Moisture and volatile matter content, g/100 g	≤0.1	≤0.1	NA	NA	

Coliforms, cfu/g	≤1	≤3	NA	NA	NA
Molds, cfu/g	≤10	≤10	NA	NA	NA
Yeast, cfu/g	≤10	≤10	NA	NA	NA
Salmonella, /25 g	ND in 25 g	NA	NA	NA	NA
Enterobacteriaceae, cfu/g	<10	NA	NA	NA	NA
Cronobacter spp, /10 g	ND in 10 g	NA	NA	NA	NA
Endotoxins, EU/g	<0.109	NA	NA	NA	NA

GRN 000326, p 14 (stamped p 24); GRN 000094, p 24 (stamped p 36); GRN 000041, p 38 (stamped p 138).

*Specifications for other fatty acids are included.

Abbreviations: cfu = colony forming units; FCC = Food Chemicals Codex, 13th ed.; GRN = GRAS notice; NA = not available; ND = not detected.

Table 6 shows analytical results of 3 non-consecutive lots of ARA-rich oil. Three non-consecutive lots were analyzed for ARA, free FAs, unsaponifiable matter, anisidine value, peroxide value, residual hexane, heavy metals, and microbiological parameters to ensure that Runke Bioengineering's ARA-rich oil met the specifications and were free from contaminants. All analytical methods were validated for their intended use.

Table 6. Analytical Values for Runke Bioengineering's ARA-rich Oil

Parameters	Batch Number			Range	Method of analysis
	11004332	11008334	11012336		
ARA, C20:4n6, relative %	41.01	42.20	41.70	41.0 - 42.2	AOAC 996.06 mod.
Acid value, mg KOH/g	0.29	0.28	0.29	0.28 - 0.29	AOCS Cd 3d-63
Free fatty acids, %	0.14	0.13	0.13	0.13 - 0.14	AOCS Ca 5a-40; AOAC 940.28
Free fatty acids (as oleic acid), %	0.15	0.14	0.15	0.14 - 0.15	AOCS Cd 3d-63
Unsaponifiable matter, %	1.56	1.56	1.51	1.51 - 1.56	AOCS Ca 6a-40
p-Anisidine value	5.7	5.1	4.9	4.9 - 5.7	AOCS Cd 18-90
Peroxide value, meq/kg	0.61	0.47	0.60	0.47 - 0.61	AOCS Cd 8b-90:2017
Hexane, mg/kg	<0.50	<0.50	<0.50	<0.50	AOCS Cg 4-94

Mercury, mg/kg	<0.005	<0.005	<0.005	<0.005	BS EN 13806:2002
Lead, mg/kg	<0.05	<0.05	<0.05	<0.05	BS EN ISO 17294-2 2016 mod.
Arsenic, mg/kg	<0.005	<0.005	<0.005	<0.005	
Cadmium, mg/kg	<0.005	<0.005	<0.005	<0.005	
Moisture and volatiles, %	0.02	<0.01	0.06	0.03	AOCS Ca 2c-25
Aerobic plant count, cfu/g	<10	<10	<10	<10	US FDA BAM Ch. 3, 2001
Molds, cfu/g	<10	<10	<10	<10	US FDA BAM Ch. 18, 2001
Yeast, cfu/g	<10	<10	<10	<10	
Salmonella, /25 g	ND in 25 g	ND in 25 g	ND in 25 g	ND in 25 g	US FDA BAM Ch. 5, 2021
Enterobacteriaceae, cfu/g	<10	<10	<10	<10	ISO 21528-2-2017
Cronobacter spp, /10 g	ND in 10 g	ND	ND	ND	ISO 22964:2017
Endotoxins, EU/g	<0.109 or <LOQ	<0.109	<0.109	<0.109	USP 43<85>

Abbreviations: AOAC = Association of Official Analytical Chemists; AOCS = American Oil Chemists' Society; BAM = Bacteriological Analytical Manual; cfu = colony forming units; ch= chapter; ISO = International Standardization Organization; LOQ = limit of quantitation; ND = not detected.

Table 7 presents FA profiles of ARA-rich oil. As shown in Table 8, the FA profile of Runke Bioengineering's ARA-rich oil is similar to those described in previous GRAS notices, in particular those of GRNs 000326 and 000041.

ARA-rich oil is composed predominantly of triglycerides (TGs; approximately 93%) with some diglycerides (5.5%), monoglycerides (approximately 1.8%), and unsaponifiable matter (<3%) as is typical for food-grade vegetable oil products (Appendix A). The specification and composition data indicate that Runke Bioengineering's ARA-rich oil is substantially equivalent to existing ARA-rich oil ingredients that have been the subject of previous GRAS determinations (GRNs 000326, 000094, 000080, and 000041).

Table 7. Fatty Acid Profiles of Runke Bioengineering's ARA-rich Oil

Parameters, %	Batch Number			Range	Mean
	1100 4332	1100 8334	1101 2336		
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	<0.02	<0.02	<0.02	<0.02	<0.02
C14:0 Myristic acid	0.29	0.31	0.30	0.29 - 0.31	0.30
C14:1 Myristoleic acid	<0.02	<0.02	<0.02	<0.02	<0.02
C15:0 Pentadecanoic acid	0.10	0.09	0.10	0.09 - 0.10	0.10
C15:1 Pentadecenoic acid	<0.02	<0.02	<0.02	<0.02	<0.02
C16:0 Palmitic acid	7.10	7.21	7.06	7.06 - 7.21	7.12
C16:1 Omega 7	0.17	0.18	0.17	0.17 - 0.18	0.17
C16:1 Total (Palmitoleic acid + isomers)	0.23	0.23	0.22	0.22 - 0.23	0.23
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	0.25	0.26	0.26	0.25 - 0.26	0.26
C17:1 Heptadecenoic acid	0.03	0.03	0.03	0.03	0.03
C18:0 Stearic acid	7.26	7.73	7.43	7.26 - 7.73	7.47
C18:1 Vaccenic acid	0.35	0.37	0.35	0.35 - 0.37	0.36
C18:1 Omega 9 (oleic acid)	8.78	9.36	8.67	8.67 - 9.36	8.94
C18:1 Total (oleic acid + isomers)	9.24	9.87	9.14	9.14 - 9.87	9.42
C18:2 Omega 6 (linoleic acid)	12.18	13.34	11.91	11.91 - 13.34	12.48
C18:2 Total (linoleic acid + isomers)	12.54	13.79	12.26	12.26 - 13.79	12.86
C18:3 Omega 3 (alpha linolenic acid)	0.05	0.05	0.05	0.05	0.05
C18:3 Omega 6 (gamma linolenic acid)	2.25	2.18	2.18	2.18 - 2.25	2.20
C18:3 Total (linolenic acid + isomers)	2.29	2.24	2.23	2.23 - 2.29	2.25
C18:4 Omega 3 (octadecatetraenoic acid)	<0.02	<0.02	<0.02	<0.02	<0.02
C18:4 Total (octadecatetraenoic acid)	<0.02	<0.02	<0.02	<0.02	<0.02
C20:0 Arachidic acid	0.72	0.75	0.74	0.72 - 0.75	0.74
C20:1 Omega 9 (gondoic acid)	0.36	0.36	0.35	0.35 - 0.36	0.36

C20:1 Total (gondoic acid + isomers)	0.39	0.39	0.40	0.39 - 0.40	0.39
C20:2 Omega 6	0.50	0.52	0.49	0.49 - 0.52	0.50
C20:2 Total (eicosadienoic acid)	0.50	0.52	0.49	0.49 - 0.52	0.50
C20:3 Omega 3	0.14	0.15	0.12	0.12 - 0.15	0.14
C20:3 Omega 6	1.92	1.90	1.87	1.87 - 1.92	1.90
C20:3 Total (eicosatrienoic acid)	2.07	2.04	1.99	1.99 - 2.07	2.03
C20:4 Omega 3	<0.02	<0.02	<0.02	<0.02	<0.02
C20:4 Omega 6 (arachidonic acid)	41.01	42.20	41.70	41.01 - 42.20	41.64
C20:4 Total (eicosatetraenoic acid)	41.03	42.20	41.71	41.03 - 42.20	41.65
C20:5 Omega 3 (eicosapentaenoic acid)	0.06	0.06	0.06	0.06	0.06
C21:5 Omega 3 (heneicosapentaenoic acid)	<0.02	<0.02	<0.02	<0.02	<0.02
C22:0 Behenic acid	0.06	1.49	0.06	0.06 - 1.49	0.54
C22:1 Omega 9 (erucic acid)	<0.02	<0.02	<0.02	<0.02	<0.02
C22:1 Total (erucic acid + isomers)	<0.02	<0.02	<0.02	<0.02	<0.02
C22:2 Docosadienoic omega 6	0.03	0.04	0.03	0.03 - 0.04	0.03
C22:3 Docosatrienoic, omega 3	0.02	0.02	0.03	0.02 - 0.03	0.02
C22:4 Docosatetraenoic omega 6	0.20	0.22	0.21	0.20 - 0.22	0.21
C22:5 Docosapentaenoic omega 3	<0.02	<0.02	<0.02	<0.02	<0.02
C22:5 Docosapentaenoic omega 6	0.10	0.06	0.08	0.06 - 0.10	0.08
C22:5 Total (docosapentaenoic acid)	0.10	0.06	0.08	0.06 - 0.10	0.08
C22:6 Docosahexaenoic omega 3	0.32	0.20	0.25	0.20 - 0.32	0.26
C24:0 Lignoceric acid	1.16	1.22	1.19	1.16 - 1.22	1.19
C24:1 Omega 9 (nervonic acid)	0.19	0.20	0.19	0.19 - 0.20	0.19
C24:1 Total (nervonic acid + isomers)	0.19	0.20	0.25	0.19 - 0.25	0.21
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	89.95	95.15	90.29	89.95 - 95.15	91.80

Total fatty acids	86.20	91.20	86.54	86.20 - 91.20	87.98
Total monounsaturated fatty acids	9.97	10.60	9.93	9.93 - 10.60	10.17
Total omega 3 isomers	0.60	0.49	0.52	0.49 - 0.60	0.54
Total omega 6 isomers	58.20	60.46	58.47	58.20 - 60.46	59.04
Total polyunsaturated fatty acids	59.09	61.34	59.29	59.09 - 61.34	59.91
Total saturated fatty acids	16.96	19.07	17.14	16.96 - 19.07	17.72
Total trans fatty acids	0.18	0.18	0.18	0.18	0.18

Method of analysis: AOAC 996.06 mod.

Table 8. Comparison of Fatty Acid Profiles of ARA-rich Oil

Fatty Acid, g/100 g	Current notice	GRN 000326	GRN 000094	GRN 000041	FCC standards
C 6:0 (Caproic acid)	<0.02				
C 8:0 (Caprylic acid)	<0.02	<0.01			
C 10:0 (Capric acid)	<0.02	0.03			
C 12:0 (Lauric acid)	<0.02	0.01			
C 14:0 (Myristic acid)	0.30	0.26	0.46	0.44	0.1-0.5
C 14:1 (Myristoleic acid)	<0.02	0.01			
C 15:0 (Pentadecanoic acid)	0.10	0.09	0.17		
C 15:1 (Pentadecenoic acid)	<0.02				
C 16:0 (Palmitic acid)	7.12	6.02	13.35	8.13	4.3-8.1
C 16:1 (Palmitoleic acid)	0.17	0.18	0.15		0-0.4
C 17:0 (Margaric acid)	0.26	0.18	0.35	0.39	
C 17:1 (Heptadecenoic acid)	0.03				
C 18:0 (Stearic acid)	7.47	5.11	7.70	9.04	4.2-7.6
C 18:1 (Oleic acid)	8.94	4.97	6.45	19.69	3.4-9.5
C 18:1n7 (Vaccenic acid)	0.36	0.24	0.40	0.28	
C 18:2n6 (Linoleic acid)	12.48	7.87	10.69	6.78	3.8-15.2
C 18:3n3 (alpha-Linolenic acid)	0.05	0.04	0.54		
C 18:3n6 (gamma-Linolenic acid)	2.20	2.10	2.35	2.77	1.7-2.7
C 20:0 (Arachidic acid)	0.74	0.76	0.76	0.91	0.6-1.0
C 20:1n9 (Eicosenoic or gondoic acid)	0.39	0.22	0.49	0.40	
C 20:2n6 (Eicosadienoic acid)	0.50	0.44	0.63	0.63	
C 20:3n3 (Eicosatrienoic acid)	0.14	0.03			
C 20:3n6 (homo-gamma-Linolenic acid)	1.90	3.69	3.26	1.96	3.0-5.0

C 20:4n6 (Arachidonic acid)	**41.64**	**43.30**	**40.63**	**43.26**	**38.0-48.5**
C 20:5n3 (Eicosapentaenoic acid)	0.06	0.14	0.20		
C 21:0 (Heneicosanoic acid)		0.10	ND		
C 22:0 (Behenic acid)	0.54	3.11	2.58	2.01	2.5-4.1
C 22:1n9 (Erucic acid)	<0.02	0.17	0.1		
C 22:2n6 (Docosadienoic acid)	0.03	0.02			
C 22:6n3 (Docosahexaenoic acid)	0.26	0.04			
C 22-5n3 (Docosapentaenoic acid)	<0.02				
C 22-5n6 (Docosapentaenoic acid)	0.08			<0.01	
C 23:0 (Tricosanoic acid)					
C 24:0 (Lignoceric acid)	1.19	10.12	6.88	1.93	7.8-12.6
C 24:1 (Nervonic acid)	0.19	0.49	0.22	0.17	
C 26:0		1.36			
Saturated fat	17.7	27.50	32.3	22.8	
Total fat	91.8	95.1	99.9	98.7	

GRN 000041, ARASCO®, available from Martek/DSM; from Table 7 (page 30, stamped page 130)
GRN 000094, SUNTGA40S, available from Mead Johnson Nutritionals; from Table II-3 (pages 26-27, stamped pages 38-39).
GRN 000326, RAO, available from Cargill; Table 18 (pages 40-42, stamped pages 50-52).
Abbreviations: FCC = Food Chemicals Codex; GRN = GRAS notice.

Sterol Profile

Sterols form the main part of the unsaponifiable fraction of ARA-rich oil (Hempenius et al., 1997). Table 9 presents the sterol profile of Runke Bioengineering's ARA. The analysis was done at two independent laboratories (i.e., Eurofins and the Institute for Advanced Study, Shenzhen University, China). The difference in analytical methods resulted in different values for the same samples. A mean of 6 analytical values from 3 non-consecutive lots was calculated for each sterol. The major sterols associated with *M. alpina* oil include desmosterol and 24-methyl sterols. Brassicasterol (24-methyl cholest-5,22-dien-3β-ol) is the most abundant phytosterol (1.21 g/100 g oil), followed by desmosterol (0.734 g/100 g oil).

The major sterols of some *Mortierella* species include ergosterol, desmosterol, 24-methylenecholesterol, 22-dihydroergosterol, and 24,25-methylenecholesterol (Volkman, 2003; Weete and Gandhi, 1999). However, *M. alpina* is known to have desmosterol as the major sterol with no ergosterol (Weete and Gandhi, 1997).

A few scientific papers reported that the main sterols present in infant formulas are cholesterol (0.03-2.58 %wt/v) and desmosterol (0.05-0.31 g/100 mL) (Claumarchirant et

al., 2015). These sterols are also present in human milk (cholesterol, 0.065-2.92 %wt/v). In infant formulas, total plant sterols (%wt/v) ranged from 0.31 to 0.50 g/100 mL. β-Sitosterol, the most abundant phytosterol, ranged from 0.18 to 0.30, followed by campesterol (0.072−0 .115), stigmasterol (0.027−0.053), and brassicasterol (0.014−0.028) (Claumarchirant et al., 2015).

Table 9. Sterol Profile of Runke Bioengineering's ARA-rich Oil

Parameters, g/100 g	Batch Number (Appendix B; Eurofins)			Batch Number (Appendix C; Sterol report)			Mean
	11004 332	11008 334	11012 336	11004 332	11008 334	11012 336	
24-methyl cholest-5,22-dien-3β-ol (Brassicasterol)	1.218	1.196	1.227				1.214
24-methyl cholesta-5,24(25)-dien-3β-ol				0.008	0.008	0.009	0.008
24-Methylene cholesterol				0.004	0.004	0.004	0.004
Cholesterol	0.008	0.005	0.006				0.006
Campesterol	0.081	0.073	0.079	0.007	0.007	0.006	0.042
Desmosterol				0.629	0.745	0.828	0.734
Campestanol	0.003	0.002	0.003				0.003
Stigmasterol	0.011	0.011	0.011				0.011
Unidentified sterols	0.146	0.127	0.139				0.137
Sitosterol	0.062	0.062	0.062	0.028	0.026	0.017	0.043
Sitostanol + delta-5-avenasterol	0.018	0.019	0.020				0.019
Delta-5,24-stigmastadienol	0.003	0.003	0.003				0.003
Delta-7-stigmastenol	0.010	0.011	0.010				0.010
Delta-7-Avenasterol	0.002	0.003	0.002				0.002
Cycloartenol	0.004	0.004	0.004				0.004
24-Methylenecycloartanol	0.003	0.003	0.002				0.003
Citrostadienol	0.006	0.007	0.006				0.006
Lanosterol				0.015	0.014	0.012	0.014
Total sterols							2.263

Table 10 presents the sterol content of Runke Bioengineering's ARA-rich oil in comparison with those described in GRN 000080 (pages 21-22, stamped pages 27-28), GRN 000094

(page 21), GRN 000326 (pages 44, stamped page 54), and GRN 000963 (page 18). Total plant sterol and stanol (%wt/v) content in Runke Bioengineering's ARA-rich oil was approximately 2.26 g/100 g oil. This level is somewhat higher than the values reported in GRNs 000041 and 000080 for ARASCO (1.42 g/kg), GRN 000094 (0.98 g/kg) and GRN 000963 (1.71 g/kg). However, the unsaponifiable content specification (i.e., not more than 3.0%) for the subject of the current notice is consistent with the specifications of other ARA-rich oils described in other GRAS notices (GRNs 000041, 000080, and 000326).

Major sterols associated with *M. alpina* oils include desmosterol and 24-methyl sterols. The desmosterol content in Runke's ARA-rich oil is comparable to those reported in GRNs 000041/000080 and 000963. It is noteworthy that the desmosterol content was reported in all GRAS notices. However, Certificates of Analysis (COAs) from Eurofins only (Appendix B) included the content of brassicasterol (24-methyl cholesta-5,22-dien-3β-ol). The difference in analytical methods may partly be responsible. It appears that the analytical condition that can quantify 24-methyl cholest-5,22-dien-3β-ol does not analyze the desmosterol content as demonstrated in the reports issued by Eurofins (i.e., COAs in Appendix B) and vice versa. It is not impossible that the sterol content reported in other GRAS notices (i.e., GRNs 000041/000080, 000094, and 000326) may have been underestimated.

Sterols are normal components in the diet, and the sterols identified in Runke's ARA-rich oil do not pose any safety concern. In addition, the safety of sterols present in Runke Bioengineering's ARA-rich oil can be justified based on the estimated daily intakes (EDIs) of sterols under the intended use relative to total sterols already consumed via the diet (details are described in Part 3.D.).

Table 10. The Content of Sterols Reported in Various GRAS Notices

Compound	Average sterol content (g/100 g oil)				
	Current notice	GRN 41* & 80	GRN 94*	GRN 326*	GRN 963*
5α-cholestra-8,14 diene-3beta-ol	-	-	-	-	0.042
4α-Methyl zymosterol (4α-Methyl cholesta-8,24-dienol)	-	-	-	0.018	-
24-Methyl cholesta-5,24(25 or 28)-dien-3β-ol	-	0.108	-	-	-
24-methyl cholesta-5,24(25)-dien-3β-ol	0.008		-	0.533	-
24-methyl choesta-5,25-dien-3β-ol	-	0.109	-	-	-

24-methyl cholesta-5(25)27-dien-3β-ol	-		-	0.111	-
Brassicasterol (24-methyl cholesta-5,22-dien-3β-ol)	1.214	-	-	-	-
24-Methyl desmosterol	-	-	-	-	0.0032
24-Methyl lanosterol	-	-	-	-	-
24-Methylene cholesterol	0.004	-	-	0.061	-
24,25-methylene cholesta-5-en-3β-ol	-	ND	0.025	-	-
Desmosterol (Cholesta-5,24-dien-3β-ol)	0.734	0.528	0.138	0.083	0.800
31-Norlanosterol	-	-	-	0.029	-
β-sitosterol	0.043	-	-	-	0.018
Campestanol	0.003	-	-	-	-
Campesterol	0.042	-	-	0.013	0.009
Cholesta-5,25-dien-3β-ol	-	0.012	-	-	-
Cholesta-7,24-dien-3β-ol	-	-	-	-	0.016
Cholesterol	-	-	-	-	0.001
Delta-5,24-Stigmastadienol	0.003	-	-	-	<0.001
Delta-7-campesterol	-	-	-	-	A total of 4 compounds, ~0.31
Delta-5-Avenasterol	-	-	-	-	
Delta-7-Avenasterol	0.002	-	-	-	
Delta-7-Stigmastenol	0.010	-	-	-	
Ergosterol	-	-	-	-	0.040
Fucosterol	-	-	-	-	0.001
Iso fucosterol	-	-	-	-	0.054
Lanosterol (4α,4β,14-trimethyl-8,24-dien-3β-ol, PubChem 246983)	-	0.015	-	0.038	-
Stigma-5-ene-3β-ol	-	-	-	-	0.001
Sitostanol+Delta-5-Avenasterol		-	-	-	-
Sitosterol (β-sitosterol, PubChem ID 222284)	-	-	-	0.034	-
Stigmasterol	-	-	-	-	0.003
Zymosterol	-	-	-	0.012	0.0102
Unidentified Sterols or others	0.19	-	-	0.045	0.157
Total Sterols (g/100 g oil) (number of batches indicated)	2.26** (n=3)	0.79 (n=1)	0.21 (n=3)	0.98 (n=5)	1.57 (n=6)

*Sources: GRN00041 (ARASCO®), Table 8: N=2 for individual sterols, but N=1 for total sterols (page 31, stamped page 131). GRN000094 (SUNTGA40S), Table VI-2 (page 80, stamped page 92). GRN 000326 (RAO), Table 19 (page 44, stamped page 54). GRN 000963, Table 7 (page 18).

**Total Sterol value for the current notice represents the combined values from two independent laboratories.

2.D. Stability

ARA-rich oil is sensitive to oxidative degradation upon exposure to air, heat, and light, and should be stored at temperatures under -10°C after opening. The stability of Runke Bioengineering's ARA-rich oil was evaluated at -10°C and ≤25°C. As shown in Table 11, ARA-rich oil is stable for at least 12 months at -10°C and ≤25°C. Based on commercial experience with a similar oil derived from *M. alpina* (GRN 000326, pages 13 and 15; GRN 000963, pages 20-21), a shelf life of a minimum of 12-18 and 36 months is expected under refrigerated and frozen conditions, respectively. The oil should be stored (after opening) in tightly closed original packaging in a cool and dry place under inert atmosphere.

Table 11. Stability Testing for ARA-rich Oil

Batch	Parameters	Time of Storage (months)			
		0	4	8	12
Storage at ≤ 25°C					
11004332	Acid value	0.40	0.31	0.37	0.38
	Peroxide value	< 0.1	0.6	1.9	2.8
	Anisidine value	5.4	7.5	8.2	8.3
	ARA%	44.2	43.7	43.9	43.8
11008334	Acid value	0.25	0.27	0.30	0.23
	Peroxide value	< 0.1	0.5	1.6	2.8
	Anisidine value	4.3	4.3	4.8	8.8
	ARA%	43.8	43.8	43.5	43.8
11012336	Acid value	0.26	0.23	0.25	0.23
	Peroxide value	< 0.1	0.6	2.3	3.0
	Anisidine value	4.2	4.2	9.7	9.8
	ARA%	45.5	45.7	45.9	45.8
Storage at -10°C					
11004332	Acid value	0.4	0.36	0.37	0.37
	Peroxide value	< 0.1	< 0.1	< 0.1	< 0.1
	Anisidine value	5.4	5.5	5.2	5.3
	ARA%	44.2	43.6	43.7	43.6
11008334	Acid value	0.25	0.26	0.29	0.26
	Peroxide value	< 0.1	< 0.1	< 0.1	0.3
	Anisidine value	4.3	4.7	4.8	5.8
	ARA%	43.8	43.9	43.6	43.7

11012336	Acid value	0.26	0.25	0.25	0.25
	Peroxide value	< 0.1	< 0.1	< 0.1	0.7
	Anisidine value	4.2	4.2	4.7	4.8
	ARA%	45.5	45.7	45.6	45.6

ARA = Arachidonic acid; (test method= ISO 660-2009; ISO 3960-2007).
Acid values met the specification (≤0.5 mg KOH/g).
Peroxide values met the specification (<5.0 meq/kg oil).
Anisidine values met the specification (≤ 20.0).

2.E. Intended Technical Effects

ARA-rich oil can be used as a food ingredient in infant formula as a source of long-chain polyunsaturated fatty acids (LCPUFAs) at concentrations consistent with cGMP.

PART 3. DIETARY EXPOSURE

3.A. Estimated Daily Intakes (EDIs) of ARA

Because breastfeeding and human milk are the normative standards for infant feeding and nutrition, infant formula should support the nutritional needs of pre-term and term infants (Koletzko et al., 2014a, 2014b, 2020).

The intended use of ARA-rich oil is to provide a source of ARA in infant formula at a concentration consistent with that of human milk. The ARA content of human milk varies from 0.34-1.22% of total FAs among different populations. Therefore, the proposed use of ARA-rich oil is to provide 0.75% and 0.50% ARA by weight of FAs in term and pre-term infant formulas, respectively. These ARA levels correspond to 1.97% of ARA-rich oil in non-exempt term infant formula and 1.32% of ARA-rich oil in exempt pre-term infant formula because ARA-rich oil contains ≥38% ARA. The ratios of ARA:DHA are expected to be in the range of 2:1-1:1.

For EDI calculations, the following assumptions were made: (1) pre-term and term infants consume 120 kcal/kg body weight (bw)/day and 100 kcal/kg bw/day, respectively, (2) FAs comprise 50% of the available energy in breast milk or infant formula, and (3) 1 g of fat contains 9 kcal. These assumptions upon which this estimation was made are the same as those cited in GRN 000326 (term and pre-term infants, page 60, FDA, 2010). An estimate of exposure to ARA from its addition to infant formula is based on mean target ARA concentrations of 0.75% and 0.50% of total fat for term and pre-term infants, respectively, and ARA-rich oil contains at least 38% ARA.

Assuming human infants consume about 100 kcal/kg bw/day (term infants aged 56 days or older) to 120 kcal/kg bw/day (pre-term infants), of which fat comprises about 50% of those calories, an infant will consume about 5.56 g (term infants aged 56 days or older) to 6.67 g (pre-term infants) of fat/kg bw/day (1 g fat = 9 kcal). These correspond to intakes of ARA of 42 mg and 33.4 mg ARA/kg bw/day (for example, 5.56 g fat/kg bw/day x 7.5 mg ARA/g fat = 41.7 mg ARA/kg bw/day for term infants). Because ARA-rich oil contains at least 38% ARA, daily intake of ARA-rich oil is estimated at 110 and 88 mg of ARA-rich oil/kg bw/day for term infants and pre-term infants, respectively (41.7 mg ARA/0.38 = 109.7 mg ARA-rich oil/kg bw/day for term infants; 33.4 mg ARA/0.38 = 87.9 mg ARA-rich oil/kg bw/day for pre-term infants).

After considering body weights, it is expected that the maximum EDIs of ARA in terms of per person per day would be 83, 50, and 33 mg ARA/person/day in pre-term low-, very low-, and extremely low- birth weight infants, respectively (Table 12). For example, daily ARA intake/person/day in pre-term low-birth weight infants would be 83.5 mg ARA/person/day (33.4 mg ARA/kg bw/day x 2.5 kg bw/person).

In summary, the daily intakes of ARA were estimated to be 42 mg/kg bw/day in term infants and 33 mg/kg bw/day in pre-term infants. These EDIs are within the range found in human milk. In addition, these EDIs are consistent with current ARA recommendations: 18–45 mg/kg bw/day, preferably higher intakes of 35–45 mg/kg bw/day (~ 0.6–0.75% of total FAs intake; Koletzko et al., 2014a) for pre-term infants; infant formula contents of ARA should be in quantities equal to at least those of added DHA (Koletzko et al., 2014b, 2020).

Table 12. Summary of Maximum EDIs of ARA and ARA-rich Oil

Infants	mg ARA/ kg bw/day	mg ARA-rich oil/ kg bw/day	mg ARA/ infant/day
Term infants	42	110	
Pre-term infants			
Low-birth weight, 2.5 kg	33.4	88	83
Very low-birth weight, 1.5 kg	33.4	88	50
Extremely low-birth weight, 1 kg	33.4	88	33

Abbreviations: ARA = arachidonic acid; bw = body weight; EDIs = estimated daily intakes.

In summary, Runke Bioengineering's ARA-rich oil is intended for use in infant formula in a manner similar to the currently approved ARA-rich oil ingredients, although the use level for pre-term infants will be higher than that of the currently approved oils. Runke Bioengineering's ARA-rich oil is expected to be used as an alternative to existing ARA-rich oils, thus, cumulative EDIs are not expected to be changed. The EDIs of 42 and 33.4 mg ARA/kg bw/day for term and pre-term infants, respectively, are consistent with current ARA recommendations: 18–45 mg/kg bw/day, preferably high intakes of 35–45 mg ARA/kg bw/day (approximately 0.6–0.75% of the total FAs) for very low birth weight pre-term infants (Koletzko et al., 2014b).

3.B. Food Sources of ARA

Human milk provides small quantities of ARA and DHA, usually less than 1% of total FAs (Agostoni et al., 1999; Bahrami and Rahimi, 2005; Brenna et al., 2007). The mean ARA content of American women's milk ranged from 0.40 to 0.67% of total FAs (Bopp et al., 2005; Brenna et al., 2007; Jensen et al., 2005). Arachidonic acid content in colostrum tends to be higher (usually by 50%) than that of mature milk. Asian mothers tend to have higher ARA concentrations in their milk than their Western counterparts, and ARA concentrations ranged from 0.30 to 1.22% of total FAs (Brenna et al., 2007).

3.C. EDIs of ARA from the Diet

It is not expected that infants will consume ARA from other foods while consuming infant formulas.

3.D. EDIs of Sterols Under the Intended Use

The EDIs of sterols under the intended use were calculated using the EDI values of ARA described in Part 3.A of this GRAS determination and the ratio of total sterols to ARA present in Runke Bioengineering's ARA-rich oil.

To calculate EDIs of sterols/person/day, The maximum EDIs of sterols/kg bw/day were calculated first. EDIs of sterols were calculated as 2.5 mg/kg bw/day for term infants and 2.0 mg/kg bw/day for pre-term infants using the following formulas: 1) Total sterols and ARA content present in 1 gram of Runke Bioengineering's ARA-rich oil (22.6 mg and 380 mg, respectively), thus, the ratio of total sterols to ARA is approximately 1:16.8; and 2) the maximum EDIs of ARA are 42 mg and 33.4 mg/kg bw/day for term and pre-term infants, respectively (please see details in Part 3.A). Thus, to calculate the EDIs of sterols, EDIs of ARA (33.4 to 42 mg/kg bw/day) were divided by 16.8. For example, 33.4−42 mg ARA/kg bw/day were divided by 16.8 to get 1.99−2.5 mg sterols/kg bw/day.

Then, in consideration of the body weight of infants, daily intakes of sterols under the intended use were estimated to be up to 25.5 mg/infant/day in term infants aged 11.5 months weighing 10.2 kg (2.5 mg sterols/kg bw/day x 10.2 kg = 25.5 mg/infant/day). These intakes are well below the amounts of sterols already consumed as natural constituents in the infant formulas as 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).

Thus, the estimated intake of sterols under the proposed uses of ARA-rich oil would not have a significant impact on the relative amount of 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.

PART 4. SELF-LIMITING LEVELS OF USE

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

PART 5. HISTORY OF CONSUMPTION

The statutory basis for the GRAS status of ARA-rich oil derived from *M. alpina* in this document is not based on common use in food before 1958. The GRAS determination is based on scientific procedures.

PART 6. NARRATIVES

6.A. Current Regulatory Status

Currently, ARA-rich oil has an established GRAS notice status with the FDA. Table 13 summarizes the maximum ARA use concentrations in infant formulas described in many GRAS notices for which the FDA had no questions on the safety under their intended uses. The ARA concentrations in infant formula supplementation ranged from 0.4 to 0.75% of total FAs. Intended use levels in this GRAS determination are up to 0.75 and 0.5% in non-exempt and exempt infant formulas, respectively. The use level in non-exempt formulas in this GRAS determination is slightly higher than that described in previous GRAS notices (0.5 vs. 0.4%).

Table 13. Maximum ARA Use Concentrations in Infant Formulas

	ARA source	Infants	% of total fat as ARA	Maximum estimated intake (mg/kg bw/day)
GRN 000041 (US FDA, 2001a)	*M. alpina*	Term	0.5	30
GRN 000080 (US FDA, 2001b)	*M. alpina*	Term	0.75	45
GRN 000094 (US FDA, 2006)	*M. alpina*	Term	0.40	26.3
		Pre-term, hospitalized	0.40	32.4
		Pre-term, post-discharge	0.40	27.7
GRN 000326 (US FDA, 2010)	*M. alpina*	Pre-term	0.40	27
		Term	0.75	42
GRN 000730 (US FDA, 2018)	*M. alpina*	Pre-term	0.40	27
		Term	0.75	42
GRN 000963 (US FDA, 2021)	*M. alpina*	Pre-term	0.40	27
		Term	0.75	42
GRN 001115 (US FDA, 2023)	*M. alpina*	Pre-term	0.40	27
		Term	0.75	42
Current notice	*M. alpina*	Pre-term	0.5	33
		Term	0.75	42

In the European Community, ARA-rich oil, produced by the *M. alpina* strain 1S-4, is authorized as a novel food (EFSA, 2008).

6.B. Review of Safety Data

As noted above, the FDA has issued 'no question' letters on previous GRAS notices (GRNs 000041, 000080, 000094, 000326, 000730, 000963, and 001115) related to food uses of ARA-rich oil derived from *M. alpina* for infant formula applications. Based on a comparison of the specifications and composition of these products, it is concluded that the specifications and composition of the subject of this GRAS determination are substantially equivalent to those of other ARA-rich oil products described in the FDA GRAS notices; thus, it is recognized that the information and data in the other GRAS notices are pertinent to the evaluation of the safety of the ARA-rich oil in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in previous GRNs. Page numbers of specific studies are presented in summary tables, and we will not discuss previously reviewed references in detail. Additionally, this notice discusses newly published meta analysis data that have been published since the FDA's last review in 2022-2023 (or in the period of May 2022 and December 2023). The subject of the present GRAS assessment is Runke Bioengineering's ARA-rich oil.

6.B.1. Metabolic Fate of ARA

(Adopted from Kremmyda et al., 2011; Kroes et al., 2003; Martin et al., 1993; 2011; GRN 730, page 29)

In breast milk, ARA and DHA are mainly found in the form of triglycerides (TGs), although they also occur in phospholipids (Martin et al., 1993). In general, dietary TGs undergo enzymatic hydrolysis in the upper intestine to free FAs and 2-monoglycerides. These products then are integrated into bile acid micelles for diffusion into the interior of the intestinal epithelial cells for subsequent incorporation into new or reconstituted TGs (Kroes et al., 2003). These reconstructed TGs enter the lymph in the form of chylomicrons for transport to the blood, which allows distribution and incorporation into plasma lipids, erythrocyte membranes, platelets, and adipose tissue. The chylomicron-contained TGs are hydrolyzed by lipoprotein lipase during passage through the capillaries of adipose tissue and the liver to release free FAs to the tissues for metabolism or for cellular uptake, with subsequent re-esterification into TGs and phospholipids for storage as energy or as structural components of cell membranes. Following their transport across the mitochondrial membrane, the metabolism of FAs occurs in the mitochondria in the form of acylcarnitine. FAs are metabolized predominantly via beta-oxidation, a process that involves a shortening of the FA carbon chain and the production of acetic acid and acetyl

CoA, which combines with oxaloacetic acid and enters the citric acid cycle for energy production.

The degree of transport of FAs across the mitochondrial membrane is contingent upon the length of the carbon chain; FAs of 20 carbons or more are transported into the mitochondria to a lesser degree than shorter chain FAs. Therefore, long-chain FAs, such as ARA, 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. Arachidonic acid may be metabolized by cyclooxygenase to form prostaglandin E2, prostacyclin I2, and thromboxane A2 (Needleman et al., 1986).

Arachidonic acid is a long-chain polyunsaturated fatty acid (PUFA) present in phospholipids in membranes of body cells, and is abundant in the brain, muscles, and liver. Arachidonic acid is one of the most abundant FAs in the brain and is present in similar quantities to DHA. The two account for approximately 20% of its FA content.

In pre-term infants, approximately 80% of ingested ARA (either from breast milk or fungal ARA-supplemented formula) is absorbed. Non-absorbed ARA is excreted via the feces. In general, long-chain PUFA concentrations travel from maternal tissues to fetal circulation to fetal tissues. Placenta FA composition can be indicative of maternal FA status and reflects FAs that are selectively transferred to the fetus. During the last trimester of pregnancy, the placenta provides the fetus with ARA and DHA.

These FAs may be conditionally essential depending on the availability of essential FAs (linoleic and linolenic acids). Studies indicate that infants may not synthesize sufficient amounts of ARA and DHA *de novo* from their precursors to cover the high demand during this period of rapid accretion for normal growth and development. It is known that pre-term birth, which curtails maternal supply of ARA and DHA to the fetus, is associated with sub-optimal neural and visual development, which can be improved by providing exogenous ARA and DHA (Kremmyda et al., 2011). After delivery, the premature infant becomes dependent on external sources for its nutritional requirements due to the shorter period and lesser extent of intrauterine long-chain PUFA accumulation.

In summary, infants may have a limited ability to convert essential precursor FAs, linoleic acid (18:2n-6) to ARA and linolenic acid (18:3n-3) to DHA, due to reduced concentrations

and activity of desaturase enzymes (Martin et al., 2011). Supplementation of these precursor FAs may not provide normal concentrations of downstream FAs. Thus, ARA supplementation can benefit both term and pre-term infants.

6.B.2. Studies on Mutagenicity and Genotoxicity of ARA-rich Oil (from *M. alpina*)

Pivotal Studies of Runke Bioengineering's ARA-rich Oil, the Subject of This GRAS Determination

In a study by Lewis et al. (2016), the safety of ARA-rich oil from *M. alpina* (ARA, 40.34%) was evaluated by testing for gene mutations and genotoxicity. The results of all mutagenicity and genotoxicity tests were negative under the experimental conditions (Table 14-1).

Bacterial Reverse Mutation Assay

The mutagenic potential of Runke Bioengineering's ARA-rich oil was evaluated at concentrations of 0.1, 0.5, 1.25, 2.5, 3.75, and 5 mg/plate in histidine-requiring *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and a tryptophan-requiring *E. coli* strain (WP2 uvrA) in the presence or absence of metabolic activation (Lewis et al., 2016). The positive controls were the following: 2-nitrofluorene in the absence of S9 for the TA98 strain; 2-aminoanthracene in the presence of S9 for the TA98, TA100, TA1535, and TA1537 strains; sodium azide in the absence of S9 for the TA100 and TA1535 strains; 4-nitroquinoline1-oxide in the absence of S9 for *E. coli*; 9-aminoacridine in the absence of S9 for the TA1537 strain; and 2-aminoanthracene in the presence of S9 for *E. coli* WP2 uvrA. None of the revertant colonies exceeded three times the mean of the solvent control in the presence or absence of metabolic activation when treated with ARA-rich oil or DHA-rich oil. There was no dose-related increase observed for any of the five tester strains used. The results indicate that ARA-rich oil doses up to 5 mg/plate were not mutagenic under the test conditions.

In-vitro Mammalian Chromosome Aberration Assay

Human peripheral blood lymphocyte cultures were used to evaluate the chromosomal aberration induction potential of Runke Bioengineering's ARA-rich oil in an in-vitro mammalian chromosomal aberration assay (Lewis et al., 2016). Prior to the chromosomal aberration assay, the cytotoxicity of ARA-rich oil was assessed using ARA-rich oil concentrations of 1.25, 2.5, and 5.0 mg/mL of culture media in the presence and absence

of metabolic activation. There was no significant change in pH and no significant dose-dependent decrease in mean mitotic index in the presence and absence of metabolic activation. The highest dose that did not reduce the mitotic index by more than 50% was 5 mg/mL. The 5 mg/mL concentration was chosen for further study of ARA-rich oil.

For the main test, two phases were performed. In Phase 1, the cultures were treated for 4 hours (h) with ARA-rich oil and the mean percentage of aberrant cells was determined in the presence and absence of metabolic activation for concentrations of 0.00 (water control), 0.00 (vehicle control), 1.25, 2.5, and 5.0 mg ARA-rich oil/mL and positive controls, respectively. The recovery and harvest periods were approximately 20 and 25 h, respectively. Phase 2 was conducted to confirm the negative results of Phase 1. In Phase 2, the cells were exposed to 1.25, 2.5, and 5.0 mg/mL. The exposure period was set to 4 h with harvest time of 24 h and no recovery period in the absence of S9. In the presence of S9, the exposure period was 4 h, and the recovery and harvest periods were 20.5 and 24 h, respectively. The number of metaphase cells, percentage of aberrant cells, and type, numbers, and frequency of chromosomal aberrations were recorded. Treatment with positive controls (600 mg/mL ethyl methanesulfonate in the absence of metabolic activation, and 30 mg/mL cyclophosphamide [CPA] in the presence of metabolic activation) resulted in a significant increase in the percentage of aberrant cells. The analysis did not reveal any statistically significant results for ARA-rich oil. Under these experimental conditions, ARA-rich oil did not induce chromosomal aberration and was not genotoxic in the presence or absence of metabolic activation.

In-vivo Mammalian Erythrocyte Micronucleus Test in Wistar Rats

ARA-rich oil was tested for the ability to induce micronuclei in polychromatic erythrocytes (PCE) of the bone marrow of treated Wistar rats (Lewis et al., 2016). In this study, the doses of ARA-rich oil were 0 (the vehicle corn oil), 1,000, 2,500, and 5,000 mg/kg bw/day. Groups of five male and five female rats were treated twice via oral gavage. Five male and five female rats were treated once with the positive control (CPA, 100 mg/kg in saline) on the second day of dosing. Bone marrow smears were prepared from sacrificed animals approximately 24 h following the final administration. All doses were well tolerated, and no clinical signs were observed. There were no differences in the mean %PCE (mean frequency of PCE to normochromatic erythrocytes) and individual frequencies of micronucleated polychromatic erythrocytes (MNPCE) between the test and the vehicle control groups. Increased numbers of MNPCE and %PCE are indicators of bone marrow toxicity. Positive control animals exhibited significantly increased numbers of MNPCE and

%PCE. Thus, the assay system was considered valid. ARA-rich oil doses up to 5,000 mg/kg bw/day were not clastogenic in rats under the test conditions.

Table 14-1. Summary of Studies Showing No Mutagenicity and Genotoxicity of Runke Bioengineering's ARA-rich Oil, the Subject of This GRAS Determination

Test	Test system	Concentration/ dose of ARA-rich oil	Previous GRN Citations
Bacterial reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. coli* WP2 uvrA	0.1, 0.5, 1.25, 2.5, 3.75 and 5.0 mg/plate, plate incorporation and preincubation ± S9	GRN 730, p.30-31 GRN 1115, p.39
In-vitro chromosomal aberration test using human blood peripheral lymphocyte	Human blood peripheral lymphocytes	Main tests: Concentration of 0.0, 1.25, 2.5, and 5 mg/mL culture ± S9	GRN 730, p.30-31 GRN 1115, p.39
Mammalian erythrocyte micronucleus test	PCE in bone marrow of treated rats; 2 consecutive days for ARA-rich oil and the 2nd day dosing for the CPA control; bone marrows were collected at 24 h following the final dosing	0, 1,000, 2,500, and 5,000 mg/kg bw/day	GRN 730, p.30-31 GRN 1115, p.39

Reference, Lewis et al. (2016; GRN 730, p.30-31; GRN 1115, p.39)
Abbreviations: ARA = arachidonic acid; CPA = cyclophosphamide; PCE = polychromatic erythrocytes.

Corroborative Studies Reviewed in Previous GRAS Notices

Due to an abundance of literature, studies published since 2000 are summarized in this review. Table 14-2 summarizes corroborative studies reporting no mutagenicity and genotoxicity of ARA-rich oils. Arterburn et al. (2000a) and Casterton et al. (2009) evaluated the mutagenic and genotoxic potential of ARA-rich oil ingredients derived from *M. alpina* containing 48.5% and 43.3%, respectively (Table 14-2). These studies are

summarized in previous GRAS notices. Therefore, this notice incorporates, by reference, the mutagenicity and genotoxicity studies discussed in previous GRAS notices and will not discuss previously reviewed references in detail. Page numbers of specific studies summarized in previous GRAS notices are presented in Table 14-2.

Table 14-2. Corroborative Studies Reporting No Mutagenicity and Genotoxicity of ARA-rich Oils

Test	Reference	Previous GRN Citations
Bacterial reverse mutation assay	Arterburn et al. (2000a)	GRN 326, p.124; GRN 1115, p.38
	Casterton et al. (2009)	GRN 326, p.123-124, 135; GRN 1115, p.38
Mouse Lymphoma Forward Mutation Assay (Gene Mutation in the TK-locus)	Arterburn et al. (2000a)	GRN 326, p.149
	Casterton et al. (2009)	GRN 326, p.127-128, 135; GRN 1115, p.38
In-vitro chromosomal aberration test using human blood peripheral lymphocyte	Arterburn et al. (2000a)	GRN 326, p.149
	Casterton et al. (2009)	GRN 326, p.125-126, 135; GRN 1115, p.38

Abbreviations: GRN = GRAS notice; TK = thymidine kinase.

6.B.3. Animal Toxicity Studies of ARA-rich Oil Derived from *M. alpina*

This review covers animal toxicity studies using ARA-rich oil derived from *M. alpina* (Table 15).

6.B.3.1. Oral Toxicity Study of Runke Bioengineering's ARA-rich Oil, the Subject of this GRAS Determination

Pivotal oral toxicity studies of Runke Bioengineering's ARA-rich oil, the subject of this GRAS determination, include acute toxicity study, a 28-day toxicity study, a 90-day subchronic toxicity study (Lewis et al., 2016), and reproductive and developmental studies (Falk et al., 2017). As shown in Table 15, these pivotal oral toxicity studies of Runke Bioengineering's ARA-rich oil are summarized in previous GRAS notices also.

Table 15. Summary of Animal Toxicity Studies of the Subject of This GRAS Determination

Species	Test substance	Dose	Duration	NOAEL	Reference	Previous GRN Citations
Studies of Runke Bioengineering's ARA, the Subject of This GRAS Determination						
Rat, Wistar	ARA-rich oil from *M. alpina* (40.3% ARA)	0, 1,000, 2,500, or 5,000 mg/kg bw ARA-rich oil	4 wk 13 wk	ARA-rich oil:5,000 mg/kg bw/day	Lewis et al., 2016	GRN 730, p.32; GRN 963, p.30-31; GRN 1115, p.44
			Develop-mental toxicity, GD 6-20	Both develop-mental and reproductive toxicity; ARA-rich oil: 5,000 mg/kg bw/day	Falk et al., 2017	GRN 730, p.32; GRN 963; p.32 GRN 1115, p.45

Abbreviations: ARA = arachidonic acid; bw = body weight; GD = gestation days; GRN = GRAS notice; NOAEL = no-observed-adverse-effect-level; wk = weeks.

Acute Oral Toxicity Study of Runke Bioengineering's ARA-rich Oil

Lewis et al. (2016) studied the acute toxicity of ARA-rich oil (40.34% ARA) in 8- to 10-week-old female Wistar rats (body weights, 180-189 g) prior to dosing. The rats were fasted for 16–18 h before dosing and for 3 to 4 h after dosing. Ten rats were orally gavaged either 5,000 mg/kg bw of the ARA-rich oil or DHA-rich oil and were observed twice daily for mortality and clinical signs for 14 days. Because no unscheduled mortalities occurred in the treatment group, additional groups of 5 rats each were gavaged 5,000 mg/kg bw of ARA-rich oil and were observed for 14 days for morbidity and mortality. At the conclusion of the observation period, surviving rats were sacrificed and subjected to gross pathological examinations.

No unscheduled mortality occurred. In addition, no treatment-related abnormalities in clinical signs or body weights were observed in treated animals. Under the conditions of the study, the acute mean lethal dose (LD_{50}) for ARA-rich oil was above 5,000 mg/kg bw/day in both male and female rats.

A 28-Day Oral Toxicity Study of Runke Bioengineering's ARA-rich Oil

Lewis et al. (2016) evaluated the oral toxicity of Runke Bioengineering's ARA-rich oil from *M. alpina* containing 40.34% ARA. Male and female Wistar rats aged 6-8 weeks old (n=10/sex/group) were orally gavaged 1,000, 2,500, or 5,000 mg/kg bw/day ARA-rich oil, control (distilled water), or vehicle control (corn oil) once a day for 28 days. Body weight, morbidity, mortality, clinical examinations, detailed clinical observations, food and water consumption, clinical pathology examinations, hematology, clinical biochemistry, urine chemistry, and histopathological parameters were assessed. No mortality was observed. In the female rats, body weights were decreased by 6-10% on day 7 in all the ARA groups but was quickly regained and there were no differences for the remainder of the study compared to the control. There were no differences in body weight among the male rats. No treatment-related abnormalities were observed in clinical signs or symptoms, food consumption, hematology, blood chemistry, urinalysis, and ophthalmological parameters. The No Observed Adverse Effect Level (NOAEL) for ARA-rich oil was set at 5,000 mg/kg bw/day, the highest dose tested.

A 90-Day Subchronic Oral Toxicity Study of Runke Bioengineering's ARA-rich Oil

Lewis et al. (2016) conducted a 90-day repeated oral dose toxicity study of Runke Bioengineering's ARA-rich oil from *M. alpina* containing 40.34% ARA. Male and female Wistar rats received control (water), vehicle control (corn oil), 1,000, 2,500, or 5,000 mg/kg bw/day ARA-rich oil by oral gavage for 90 days (n=10/sex/group). On day 91, all surviving animals except those in the recovery groups were subjected to necropsy. Two recovery groups of animals (vehicle control [corn oil] or 5,000 mg/kg bw/day ARA-rich oil; n=10/sex/group) were observed for an additional 14 days after a 90-day treatment of ARA or corn oil. Animals in the recovery groups underwent necropsy and detailed gross pathological evaluation on day 105. Body weight, feed consumption, clinical pathology of blood and serum, water intake, urine analysis, necropsy, detailed gross pathological evaluation, microscopic examination, and histopathological examination were conducted.

No unscheduled deaths occurred during the study. There were no treatment-related clinical signs or symptoms. The ophthalmological examinations, detailed physical examinations, home cage observations, handheld examinations, open field observations, and sensory reactivity tests revealed no treatment-related abnormalities. In the corn oil and low-dose groups, the body weight and body weight gain were significantly lower than in the water control group on days 1 to 50. After day 50, no differences in body weights were noted among all ARA-treated and control groups. Additionally, no differences in

body weights were recorded among control or ARA-rich oil treated rats during the recovery period.

The male mid- and high-dose groups consumed 2-4% more food compared to the water control group during the first 9 weeks. The male high-dose group consumed more food than the corn oil control group during weeks 1-4. After 9 weeks, there were no differences compared to the control groups. In females, all ARA-rich oil groups consumed 5-7% more food than the water control group. The female mid- and high-dose groups consumed more food than the corn oil control group throughout the study.

Hematological parameters were comparable among the groups (Table 16). Small changes were observed in some parameters (for example, mean corpuscular hemoglobin concentration [MCHC], 35 g/dL in oil vehicle control vs. 36 g/dL in male low-dose rats, $P<0.05$; white blood cells (WBCs), 8.6 $x10^3$ μL in oil control vs. 8.0-9.1 $x10^3$ μL in 3 male test groups, $P<0.05$). These changes were observed only in one sex, were not dose-dependent, were not of a clinically relevant magnitude, and did not persist through the recovery period; thus, these changes were considered non-adverse.

Some changes in clinical chemistry parameters were comparable to the controls, biologically insignificant, and not correlated with other toxicological findings (Table 17). The small increases in cholesterol and TGs in all ARA-rich oil groups of both sexes (averages of water and vehicle controls vs. all treated groups: males, total cholesterol, 64-65 vs. 68-71 mg/dL, $P<0.05$; TGs, 63 vs. 68-73 mg/dL, $P<0.05$; females, total cholesterol, 62-64 mg/dL vs. 66-71 mg/dL, $P<0.05$; an average of water and vehicle controls vs. mid- and high-dose females: TGs, 66 mg/dL vs. 67-69 mg/dL; $P<0.05$) were related to the consumption of a high-fat diet, and were considered non-adverse because the differences were not of clinically relevant magnitude and resolved during the recovery period. In females in the recovery group, TGs remained slightly elevated after discontinuation of the treatment compared to the water control but were equivalent to the corn oil control group. Likewise, small increases in alanine amino transferase (ALT; 6-9% increase in male test groups and 6-14% increase in female test groups, $P<0.05$ compared to both control groups), aspartate amino transferase (AST; 6% increase in high-dose males only; 6-11% increase in all female test groups, $P<0.05$), and alkaline phosphatase (ALP; 3% increase in high-dose males only and 3-5% increase in all female test groups, $P<0.05$) were not of clinically relevant magnitude, resolved during the recovery period, and were not supported by histopathology; thus, these increases were considered non-adverse. In

addition, the small increases in sorbitol dehydrogenase (SDH) were not clinically significant; thus, the changes were considered non-adverse (although the authors did not present explanations in the article). Changes in bilirubin, albumin, total protein, phosphorus, globulin, and lactate dehydrogenase were small, not clinically relevant, found only in one sex, and resolved during the recovery period; thus, the changes were considered non-adverse.

Most urinalysis parameters were not significantly different and were comparable to the controls (data not shown). The low-dose groups of male and female rats had differences in volume and specific gravity compared to the water control group. The pH was decreased compared to the water control group. The changes were not dose-dependent, did not persist during the recovery period, and were not different from the vehicle control; thus, they were considered non-adverse.

Gross pathology, physical parameters, and microscopic examinations revealed no differences among the groups. Prostate weights were significantly decreased compared to the vehicle control (Table 18; 0.72-0.74 g in both controls vs. 0.70-0.71 g in test groups, $P<0.05$). Spleen weight was increased in all female ARA-rich oil groups (0.73-0.75 g in water and oil controls vs. 0.79-0.80 g in test groups, $P<0.05$) and decreased in the male high-dose group (0.82-0.85 g in water and oil controls vs. 0.81 g in high-dose males, $P<0.05$). Increased testes weight was observed in the high-dose group (4.21-4.26 g in controls vs. 4.35 g in high-dose males, $P<0.05$). These few changes were not dose related, were not associated with notable clinical chemistry or histopathological changes, and were resolved during the recovery period; thus, they were considered incidental.

Histopathological examination demonstrated no treatment-related changes. In the ARA-rich oil groups, some changes in tissues and organs were observed. Congestion was found in the spleen. Foci of inflammation, hemorrhage, and tubular dilation were observed in the kidney. The liver showed small foci of necrosis, inflammation, bile duct hyperplasia, and sinusoidal hemorrhage. Tubular degeneration was found in the testes and vacuolar degeneration in the adrenal glands. The lungs exhibited alveolar and bronchiolar inflammation and hemorrhage. The non-specific histopathological changes were not dose dependent and these effects were observed in no more than one animal per sex per treatment group. They occurred in both treatment and control groups with no dose-response relationship; therefore, they were not considered to be treatment-related. It was concluded that ARA did not induce pathological changes.

Table 16. Hematology and Coagulation Parameters for Wistar Rats Administered ARA-rich Oil for 90 Days

Item	Dose (mg/kg bw/day)				
	0 (water)	0 (corn)	1,000	2,500	5,000
Males					
RBC x 10^6 µL	7.6±0.3	7.6±0.4	7.6±0.3	7.6±0.4	7.4±0.3
Hematocrit, %	42±2	42±1	42±1	44±2[a,b]	44±2[a,b]
MCV, μm^3	52±9	54±2	54±2	54±2	54±2
Hemoglobin, g/dL	15±1	15±1	15±0	16±0	16±0
MCH, pg	17±1	18±1	18±1	18±1[a]	18±1
MCHC, g/dL	36±2	35±1[a]	36±2[b]	36±1	36±1
Platelets	969±29	958±50	956±28	952±34	949±43
MPV	55±4	54±1	54±2	54±2	54±2
WBC x 10^3 µL	8.4±0.7	8.6±0.7	8.0±0.7[b]	9.1±0.8[a]	8.9±0.7
Neutrophil	16±13	13±2	13±2	14±2	14±2
Lymphocyte	84±2	84±2	83±29	84±2	84±2
Monocyte	2.8±0.9	2.5±0.8	2.7±0.8	2.9±0.7	2.5±0.8
Eosinophil	1.6±1.0	1.8±1.1	1.9±1.0	1.8±0.9	1.9±0.8
Basophil	0±0	0±0	0±0	0±0	0±0
PT	13±1[b]	11±1	13±1[b]	13±1[b]	14±1[b]
aPTT	16±1	16±1	16±1	16±1	15±1
Females					
RBC x 10^6 µL	7.4±0.3	7.4±0.2	7.5±0.3	7.5±0.3	7.6±03
Hematocrit, %	43±2	44±2	45±1	44±2	44±2
MCV, μm^3	53±2	54±2	53±2	54±2	54±2
Hemoglobin, g/dL	22±32	16±1	16±0	16±0	16±0
MCH, pg	18±0	18±1	18±0	18±0	18±1
MCHC, g/dL	35±1	36±1	35±2	35±1	35±1
Platelets	958±32	960±26	944±33	945±36	954±37
MPV	54±2	54±2	54±2	54±2	54±2
WBC x 10^3 µL	9.4±0.9	9.6±0.5	9.5±0.7	9.5±0.4	9.3±0.7
Neutrophil	12±3	12±2	13±1	13±2	14±1[a,b]
Lymphocyte	84±2	84±2	83±2	84±2	83±2
Monocyte	2.4±0.7	2.4±0.8	2.7±0.7	2.6±0.8	2.5±0.7
Eosinophil	1.8±0.8	1.8±1.0	2.0±0.9	1.6±0.8	1.9±0.8
Basophil	0±0	0±0	0±0	0±0	0±0
PT	11±1[b]	12±2[a]	12±1	12±3	12±1
aPTT	16±1	15±1	16±1[b]	165±1 (may be a typo; It should have been 16.5)	16±1

From Lewis et al., 2016. Values are mean ± SD 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; ARA = arachidonic acid; bw = body weight; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; PT = prothrombin time; RBC = red blood cell; SD = standard deviation; WBC = white blood cell.

Table 17. Blood Biochemistry for Wistar Rats Administered ARA-rich Oil for 90 Days

Item	Dose (mg/kg bw/day)				
	0 (water)	0 (corn)	1,000	2,500	5,000
Males					
Glucose, mg/dL	117±6	114±6	114±6	116±6	116±5
Cholesterol, mg/dL	64±3	65±3	68±4[a,b]	69±4[a,b]	71±4[a,b]
Triglyceride, mg/dL	63±3	63±3	68±4[a,b]	70±4[a,b]	73±3[a,b]
ALT, IU/L	61±4	64±3	66±4[a,b]	68±4[a,b]	68±4[a,b]
AST, IU/L	113±4	112±5	115±3	114±5	119±5[a,b]
ALP, IU/L	152±4	150±4	152±4	152±3[b]	155±5[a,b]
SDH IU/L	16±2	16±2	16±2	17±3	18±3[a,b]
Calcium, mg/dL	14±1	14±1	14±1	15±1[a]	15±1
Urea, mg/dL	15±1	15±1	15±1	16±1	16±2[a,b]
Phosphorus, mg/dL	6.1±0.6	6.2±0.7	6.7±0.6[a,b]	6.7±0.5[a,b]	6.8±0.6[a,b]
Albumin, g/dL	4.3±0.3	4.4±0.3	6.6±0.3[a,b]	6.5±0.2[a,b]	4.4±0.3
Total protein, g/dL	6.6±0.3	6.6±0.4	6.6±0.3	7.0±0.4[a,b]	6.7±0.3
Bilirubin, mg/dL	0.30±0.15	0.34±0.15	0.30±0.17	0.36±0.18	0.29±0.16
Creatinine, mg/dL	0.31±0.13	0.31±0.10	0.33±0.15	0.30±0.10	0.31±0.12
Globulin, g/dL	3.7±0.4	3.7±0.5	3.8±0.5	4.0±0.5	3.7±0.4
LDH, IU/L	76±5	73±6	73±3	74±6	78±8[b]
GGT, IU/L	0.3±0.04	0.3±0.03	0.3±0.04	0.3±0.06	0.3±0.4
Sodium, mmol/L	146±3	146±3	148±3	148±3	153±4[a,b]
Potassium mmol/L	5.5±0.5	5.5±0.4	5.6±0.3	5.5±0.3	5.6±0.3
Chloride, mmol/L	105±1	104±1	104±2	105±1	104±1

Females					
Glucose, mg/dL	111±6	112±5	111±6	111±5	110±6
Cholesterol, mg/dL	64±2	62±3	66±3[b]	71±5[a,b]	70±4[a,b]
Triglyceride, mg/dL	66±4	66±3	67±4	69±4[b]	69±3[a,b]
ALT, IU/L	61±4	63±2	67±3[a,b]	68±4[a,b]	71±3[a,b]
AST, IU/L	103±22	109±5	112±5[a]	117±4[a,b]	117±4[a,b]
ALP, IU/L	147±5	147±4	151±4[a,b]	151±3[a,b]	154±5[a,b]
SDH, IU/L	15±2	14±2	16±3[b]	19±4[a,b]	19±4[a,b]
Calcium, mg/dL	13±1	13±1	13±1	15±1[a,b]	13±1
Urea, mg/dL	14±1	14±1	15±1	15±2	16±2[a,b]
Phosphorus, mg/dL	6.1±0.5	6.4±0.4	5.8±0.4	5.7±0.4[b]	6.1±0.7[b]
Albumin, g/dL	4.4±0.3	6.6±9.7	4.4±0.2	4.4±0.2	6.4±8.4
Total protein, g/dL	6.4±0.3	6.4±0.3	6.5±0.3	6.4±0.3	6.440
Bilirubin, mg/dL	0.29±0.14	0.29±0.14	0.28±0.13	0.33±0.15	0.37±0.15
Creatinine, mg/dL	0.33±0.15	0.31±0.14	0.28±0.12	0.30±0.13	0.30±0.11
Globulin, g/dL	3.4±0.2	3.4±0.3	3.4±0.2	3.5±0.3	3.5±0.3
LDH, IU/L	70±8	70±6	82±8[a,b]	74±8	81±10[a,b]
GGT, IU/L	0.3±0.05	0.3±0.06	0.2±0.0[a,b]	0.2±0.0[b]	0.2±0.0
Sodium, mmol/L	143±2	144±3	145±2	146±3[b]	146±2
Potassium mmol/L	5.1±0.5	5.0±0.6	5.4±0.5	5.5±0.4[b]	5.3±0.4[b]
Chloride, mmol/L	104±1	104±1	104±1	104±1	104±1

From Lewis et al., 2016. Values are mean ± SD 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: ALP = alkaline phosphatase; ALT = alanine amino transferase; ARA = arachidonic acid; AST = aspartate amino transferase; bw = body weight; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; SD = standard deviation; SDH = sorbitol dehydrogenase.

Table 18. Organ Weights for Wistar Rats Administered ARA-rich Oil for 90 Days

Organ weight, g	Dose (mg/kg bw/day)				
	0 (water)	0 (corn)	1,000	2,500	5,000
Males					
Brain	2.46±0.21	2.60±0.26	2.61±0.22	2.64±0.18	2.75±0.15
Adrenals	0.093±0.02	0.098±0.01	0.095±0.01	0.099±0.01	0.098±0.01
Pituitary	0.013±0.001	0.013±0.001	0.013±0.001	0.013±0.001	0.014±0.002
Prostate/ S.V.	1.76±0.03	1.77±0.04	1.75±0.05	1.77±0.06	1.74±0.50
Prostate	0.72±0.05	0.74±0.05	0.70±0.06[b]	0.70±0.08	0.71±0.05[b]
Testes	4.26±0.13	4.21±0.11	4.31±0.17	4.21±0.16	4.35±0.24[b]
Epididymis	1.79±0.05	1.80±0.07	1.78±0.06	1.77±0.78	1.77±0.90
Heart	1.56±0.07	1.54±0.22	1.53±0.07	1.54±0.08	1.50±0.09
Liver	12.6±0.43	12.7±0.63	12.6±0.51	12.5±0.57	12.7±0.23
Kidneys	2.70±0.14	2.69±0.15	2.68±0.16	2.67±0.15	2.78±0.16
Spleen	0.82±0.06	0.85±0.06	0.83±0.04	0.84±0.05	0.81±0.04[b]
Thymus	0.54±0.07	0.55±0.04	0.55±0.03	0.55±0.03	0.55±0.03
Females					
Brain	2.14±0.12	2.07±0.10	2.12±0.11	2.12±0.12	2.12±0.10
Adrenals	0.063±0.01	0.063±0.01	0.063±0.01	0.061±0.01	0.059±0.01
Pituitary	0.013±0.001	0.014±0.001	0.013±0.001	0.013±0.001	0.014±0.002
Uterus	0.80±0.04	0.78±0.06	0.77±0.04	0.77±0.05	0.76±0.05
Ovaries	0.27±0.02	0.27±0.01	0.27±0.02	0.27±0.02	0.27±0.02
Heart	1.06±0.10	1.05±0.11	1.10±0.12	1.05±0.08	1.05±0.10
Liver	9.4±0.59	9.5±0.56	9.6±0.58	9.2±2.0	9.6±0.50
Kidneys	1.57±0.08	1.55±0.05	1.56±0.05	1.58±0.12	1.59±0.06
Spleen	0.75±0.06	0.73±0.08	0.79±0.06[b]	0.80±0.04[b]	0.80±0.06[b]
Thymus	0.51±0.04	0.52±0.04	0.51±0.05	0.50±0.1	0.52±0.03

From Lewis et al., 2016. Values are mean±SD 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: ARA = arachidonic acid; bw = body weight; S.V. = seminal vesicles

Reproductive and Developmental Toxicity Study of Runke Bioengineering's ARA-rich Oil

A study by Falk et al. (2017) investigated the reproductive and developmental toxicity of dietary exposure to Runke Bioengineering's ARA-rich oil (40.3% ARA) derived from *M. alpina* (Tables 19-22). In the developmental toxicity study, healthy, pregnant Wistar rats (n=24/group) were untreated (control) or administered corn oil (vehicle control), 1,000, 2,500, or 5,000 mg/kg bw/day of ARA-rich oil via gavage from gestation days 6 through 20. Morbidity, mortality, gross pathological examination, histopathological analysis, and clinical signs and symptoms were evaluated. In addition, the number and sex of each pup, number of still births and live births, occurrence of gross observations (e.g., ear opening, eye opening, hair growth, tooth eruption, and gross anomalies of litter), physical or behavioral abnormalities, body weight, and food consumption of the dams were determined. Fetuses were weighed and examined for external malformations and abnormalities in soft tissues and the skeleton. Clinical pathology evaluation of all surviving animals from all groups was performed on day 15, day 45, and prior to necropsy. The animals were fasted overnight (approximately 16 to 18 h) prior to blood collection.

Developmental Prenatal Toxicity Study (Falk et al., 2017)

Maternal study data

No treatment-related changes in body weight were observed for any of the test groups at the conclusion of the gestation period and premating or lactation periods, although sporadic increases in food consumption were observed in females during the gestation period for all dose groups.

Gestation day 20 laparohysterectomy data

No treatment-related lesions and significant differences in the weight of the reproductive organs, implantation, cornea lutea of the right and left cornu, and pre- and post-implantation loss of fetuses were observed in all ARA-rich oil groups (data not shown).

Fetal data

No significant or dose-dependent differences were observed among test and control groups with respect to the number of fetuses, the external observations including fetal size, generalized arrested development, kinked tail, bent tail, bulged eyelid, microphthalmia, subcutaneous hemorrhage (Table 19), or malformed head in the skeletons among the groups (Table 20).

Table 19. Changes in Fetal Development in the Prenatal Developmental Toxicity Study

Item	Untreated	Corn Oil	ARA LD	ARA MD	ARA HD
ARA-rich oil, mg/kg bw/day	-	-	1,000	2,500	5,000
No. of fetuses (litters)	204 (23)	188 (24)	225 (24)	214 (24)	191 (21)
General External Observations – Number (% of total)					
Smaller in size	1 (0.5)	1 (0.5)	-	1 (0.5)	-
Larger in size	3 (1.5)	4 (2.1)	-	1 (0.5)	2 (1.0)
Generalized arrested development	1 (0.5)	-	-	-	1 (0.5)
Subcutaneous hemorrhage	-	1 (0.5)	2 (0.9)	1 (0.5)	-
Number of fetuses	102	94	112	111	100
Minor Visceral Anomalies – Number (% of total)					
Dilated lateral ventricles brain	1 (1.0)	2 (2.1)	1 (0.9)	6 (5.4)	4 (4.0)
Dilated and fragile ventricles brain	3 (2.9)	-	-	-	1 (1.0)
Dilated and fragile ventricles brain with dilated neural canal, small spinal cord	3 (2.9)	-	-	-	-
Dilated lateral ventricles brain with fragile and ruptured cerebral hemisphere	-	-	3 (2.7)	-	-
Brownish discoloration around cerebral hemisphere	-	-	1 (0.9)	-	-
Hemorrhagic foci – liver	1 (1.0)	1 (1.1)	1 (0.9)	2 (1.8)	1 (1.0)
Subcutaneous hemorrhage	1 (1.0)	2 (2.1)	-	-	1 (1.0)
Yellowish perivascular areas liver	-	-	1 (0.9)	-	-
Small or absent renal papillae	4 (4.0)	4 (4.3)	4 (3.6)	7 (6.3)	5 (5.0)
Brownish discoloration lung	4 (3.9)	2 (2.1)	2 (1.8)	4 (3.6)	2 (3.0)
Common Variants					
Dilated renal pelvis	2 (2.0)	6 (6.4)	5 (4.5)	3 (2.7)	1 (1.0)

From Falk et al., 2017.

Abbreviations: ARA = arachidonic acid; bw = body weight; HD = high-dose; LD = low-dose; MD = mid-dose.

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

Item	Untreated	Corn Oil	ARA LD	ARA MD	ARA HD
ARA-rich oil, mg/kg bw/day	-	-	1,000	2,500	5,000
Number of pups	102	94	113	112	100
Major Malformations – Number (% of total)					
Cranial skeletal	17 (27%)	12 (13%)	13 (12%)	12 (11%)	14 (14%)
Ribs	5 (5%)	7 (7%)	6 (5%)	4 (4%)	4 (4%)
Vertebral	12 (12%)	26 (28%)	24 (21%)	18 (16%)	18 (16%)
Sternebrae	9 (9%)	13 (14%)	14 (12%)	14 (13%)	10 (10%)
Limbs	7 (7%)	7 (7%)	5 (4%)	8 (7%)	4 (4%)
Malformed head	-	-	-	1 (0.5%)	-
Kinked tail	-	2 (1.1%)	-	-	2 (1%)
Bent tail	1 (0.5%)	1 (0.5%)	1 (0.4%)	-	1 (0.5%)
Bulged eyelid	2 (1%)	2 (1.1%)	-	-	1 (0.5%)
Microphthalmia	1 (0.5%)	1 (0.5%)	2 (0.9%)	1 (0.5%)	-
Minor Skeletal Anomalies - Delayed/Incomplete Ossification – Number (% of total)					
Cranial	39 (38%)	12 (13%)	27 (24%)	39 (25%)	27 (27%)
Sternebrae	3 (3%)	5 (5%)	1 (1%)	2 (2%)	4 (4%)
Ribs	1 (1%)	-	2 (2%)	2 (2%)	2 (2%)

Adopted from Falk et al. (2017).

Abbreviations: ARA = arachidonic acid; HD = high-dose; LD = low-dose; MD = mid-dose.

Reproductive Toxicity (Falk et al., 2017)

In the reproductive toxicity study, male Wistar rats aged 7-8 weeks old and female Wistar rats aged 6-7 weeks old (n=20 males/group; n=24 females/group) were administered a vehicle control (corn oil), or 1,000, 2,500, or 5,000 mg/kg bw/day of ARA-rich oil via gavage throughout the mating period, pregnancy (for 22 days), and the nursing and lactation periods which lasted for 21 days (Falk et al., 2017). To evaluate the effect on spermatogenesis, male rats were given doses during the growth period and for a minimum of one complete spermatogenic cycle (84 days). The parental female rats were dosed for two complete estrous cycles (14 days) to evaluate the effect of ARA-rich oil on the estrous cycle. One male per 2 female rats was cohabited until all females became

pregnant as evidenced by a sperm-positive 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. The following observations were made from the reproductive toxicity study:

Mortality, Clinical Signs, and Food Consumption
No treatment-related mortality was observed in the parental (F_0) or pup generation (F_1) during the course of the study. F_0 mortality was 4, 2, 4, and 6% for the corn oil control, low-dose ARA, mid-dose ARA, and high-dose ARA groups, respectively. The parental (F_0) and pup generations (F_1) showed no treatment-related mortality and clinical signs and no significant differences in mean body weight or body weight gain. No differences in food consumption among groups were observed during the pre-mating, mating, and lactation periods in all ARA treatment groups, although the F_0 males in the low-dose group and the F_0 females in the mid-dose group had higher food consumption compared to the control group.

Reproductive performance: No significant differences were found for mean litter size, sex ratio, live birth index, weaning index, number of implantation sites, corpora lutea, pre- and post-implantation loss, female fertility index, gestation index, fecundity index, estrous cycle length, and gestation period.

F_0 generation; anatomic pathology: No animals in the F_0 generation exhibited treatment-related abnormalities in necropsy and histopathological parameters. No significant differences were observed in absolute and relative organ weights among groups (data not shown).

Developmental parameters and clinical pathology of the F_1 generation: Gross necropsy of the F_1 generation animals revealed no treatment-related external or internal abnormalities. There were no significant differences in absolute and relative organ weights.

Taken together, for the orally administered ARA-rich oil, the NOAEL for maternal toxicity and embryonic/fetal development and for paternal or maternal reproductive toxicity was found to be 5,000 mg/kg bw/day in rats.

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

Fertility Indices	Corn Oil	ARA LD	ARA MD	ARA HD
No. of females	24	24	24	24
No. of mated females	24	24	24	24
No. of females littered (pregnant)	24	24	24	24
Female fertility index, %	100	100	100	100
Gestation index, %	100	100	100	100
Pregnancy/fecundity index, %	100	100	100	100
Premating group estrus cycle*	3.56 ± 0.45	3.78 ± 0.47	3.59 ± 0.51	3.85 ± 0.62
Gestation period*	21.25 ± 0.62	21.56 ± 0.72	21.62 ± 0.69	21.25 ± 0.72
Percent males	61.2	53.9	53.1	52.4
Pups delivered	204	197	210	214
Mean male pup weight day 0, g	6.74 ± 0.66	5.69 ± 0.56	5.36 ± 0.26	5.36 ± 0.53
Mean male pup weight day 22, g	35.38 ± 4.84	33.25 ± 5.02	33.25 ± 4.25	33.52 ± 4.25
Mean female pup weight day 0, g	5.13 ± 0.56	5.57 ± 0.52	5.24 ± 0.56	5.45 ± 0.23
Mean female pup weight day 22, g	33.23 ± 5.25	33.56 ± 4.25	32.72 ± 5.56	34.21 ± 5.12

Adopted from Falk et al. (2017). *Mean days±SD.

Abbreviations: HD = high-dose; LD = low-dose; MD = mid-dose

6.B.3.2. Corroborative Oral Toxicity Studies of Other Sources of ARA-rich Oil

Table 22 summarizes corroborative animal toxicity studies of ARA-rich oils derived from *M. alpina,* which were extensively discussed in previous GRAS notices that received FDA's "no questions" letters. Each ARA-rich oil used in these corroborative studies described in Table 22 is compositionally similar to Runke Bioengineering's ARA-rich oil.

Studies reviewed in previous GRAS notices include acute toxicity studies of ARA-rich oil (Gao et al., 2017; Hempenius et al., 1997), subchronic toxicity studies with *in utero* exposure (Casterton et al., 2009; Gao et al., 2014; Hempenius et al., 1997; Lina et al., 2006), teratogenicity study (Arterburn et al., 2000b), neonatal piglet studies (Merritt et

al., 2003; Tyburczy et al., 2012), and bioequivalency test (Tyburczy et al., 2011) as well as acute and subchronic oral toxicity studies of *M. alpina* biomass (Nisha et al., 2009).

The mean lethal dose (LD_{50}) was higher than 18.2 g ARA-rich oil/kg bw (Hempenius et al., 1997). The NOAELs found from subchronic toxicity studies with *in utero* exposure range from 970 mg ARA-rich oil/kg bw/day (corresponding to 374 mg ARA/kg bw/day; Hempenius et al., 2000) to 4,850 mg/kg bw/day for F_1 females (Gao et al., 2014).

Because these studies were extensively reviewed in previous GRAS notices, we incorporate by reference the toxicity studies described in GRN 000326 (pages 135-153), GRN 000730 (pages 31-33), GRN 000963 (pages 30-31), and GRN 001115 (pages 39, 40, 42-43). Page numbers of specific studies summarized in those GRAS notices are presented in Table 22.

Bioequivalency Study

Tyburczy et al. (2011) demonstrated that the three ARA-rich oils, including Martek's (now DSM's) ARASCO®, Cargills' refined ARA-rich oil (RAO, or ARA-rich oil produced by its supplier, Cabio [formerly Alking Bioengineering], in Wuhan, China), and SUNTGA40S® (Nissui, Nippon Suisan Kaisha, Ltd., Tokyo, Japan), equally supported tissue and red blood cell (RBC) ARA accretion establishing bioequivalence (Table 22). ARASCO® served as a reference ARA-rich oil. All three ARA-rich oil ingredients have established U.S. FDA GRAS status: ARASCO®, SUNTGA40S®, and RAO were the subjects of GRNs 000041/000080, 000094, and 000326, respectively. It was hypothesized that the three ARA-rich oils would be nutritionally bioequivalent and equally safe in rapidly growing neonatal pigs. All of the three oils are single-cell TG oils derived from the fungus *M. alpina* and contain ARA at approximately 40% of the total FA content.

Piglets were fed one of three ready-to-use formulas that provided ARA at approximately 0.64% and DHA at 0.34% of total FAs from day 3 to 22 of life upon which tissues were harvested and analyzed for ARA and DHA accretion. Total formula intakes over the full study period averaged 29.6 ± 1.7 L (or a mean daily intake of 1.5 L or 1,500 kcal) with no significant differences among the three dietary treatment groups. Mean total intake of ARA was 10.60 ± 0.59 g, while the mean total intake of DHA was 5.30 ± 0.30 g. At day 22 of life, tissues and blood samples were harvested and analyzed for ARA and DHA accretion. Bioequivalence was assessed by 90% confidence intervals on the least squares geometric mean ratio of tissue ARA from the experimental groups (RAO and SUNTGA40S®) compared

with the reference ARA-rich oil (ARASCO®). If the confidence intervals, expressed as percentages with 100% equaling unity (i.e., 1:1 ratio), fell within the limits of 80 – 125%, the values were considered meeting the bioequivalence criteria. Selected FAs of the brain (cerebral cortex), retina, liver, and heart were harvested from pigs on day 22 of age. Bioavailability was more broadly assessed through secondary supporting outcomes, including accretion of ARA and DHA in the retina, heart, and liver as well as circulating levels of ARA and DHA in RBCs. In addition, livers were examined for histopathological changes, and clinical chemistry and hematological parameters were measured to assess safety.

All three ARA formulas were readily consumed and equally supported growth of the neonatal pigs. Mean ARA levels in the brain, retina, and heart were 10.97 ± 0.36%, 10.50 ± 0.43%, and 20.38 ± 0.82% of total FAs, respectively, and were similar for all three dietary treatment groups. ARA levels in the liver were 2% lower in pigs fed SUNTGA40S® (17.33 ± 0.78% FA) compared with the ARASCO® (17.66 ± 0.49% FA) while the RAO pigs showed an intermediary liver ARA content (17.38 ± 0.57% FA; P = 0.009). This difference may arise from normal variability across product lots and over time during the manufacturing process. A difference in liver ARA levels is consistent with an RAO diet providing 8% less ARA (0.62% vs. 0.67% total FA) than the ARASCO® control and SUNTGA40S® diets. Study formulas equally supported DHA accretion in the primary target organ (i.e., the brain) as well as in the retina and heart. In the liver, DHA levels were 7% higher in SUNTGA40S® pigs (8.23 ± 0.38) compared with the ARASCO® (7.70 ± 0.47% FA) and RAO (7.62 ± 0.62% FA) groups (P = 0.046). No other statistically significant differences in tissue FA accretion were observed among the dietary treatments. Mean ARA and DHA levels in the RBC fraction were similar among all dietary treatment groups at every time point examined.

The authors concluded that ARA supplied by the single-cell oils (ARASCO®, RAO, and SUNTGA40S®), when added to formula to provide ~0.64% ARA and in combination with ~0.34% DHA from DHASCO®, were nutritionally equally supporting the accretion of ARA in the neonatal pig brain, retina, and heart and are safe as ingredients in infant formula. The data indicate that ARA-rich oils produced by various strains of *M. alpina* may be substantially equivalent to ARASCO®.

Table 22. Summary of Corroborative Animal Toxicity Studies of ARA-rich Oils Derived from *M. alpina*

Species	Test substance	Dose	Duration	NOAEL	Reference	Previous GRN Citations
Acute Toxicity Studies						
Rat, Wistar	ARA-rich oil (48.3% ARA)	15.2 g ARA-rich oil/kg bw	Single dose; observed 14 days	LD_{50} > 15.2 g/kg bw	Gao, 2017	GRN 730, p.31; GRN 1115, p.40
Rat, Wistar	ARA-rich oil (32.7-38.6% ARA)	18.2 g ARA-rich oil/kg bw	Single dose; observed 14 days	LD_{50} > 18.2 g ARA-rich oil/kg bw	Hempenius et al., 1997	GRN 326, p.149 GRN 730, p.33; GRN 963, p.30; GRN 1115, p.39
Rat, Wistar	ARA-rich *M. alpina* biomass	Up to 5 g/kg bw	Single dose; observed 14 days	LD_{50} > 5 g biomass/kg bw or >0.63 g ARA/kg bw	Nisha et al., 2009	GRN 326, p.150, 152; GRN 730, p.33 GRN 963, p.30
Subchronic Toxicity Studies with an In-utero Exposure						
Rat, Wistar	ARA-rich oil from *M. alpina* (48.3% ARA)	0, 1, 1.5, or 5% of diet	13 wk of F_1, after in-utero exposure of F_0	F_0 females, 3,750; F_0 males, 2,850; F_1 females, 4,850; F_1 males, 4,480 mg/kg bw/day, the highest dose tested	Gao et al., 2014	GRN 963, p.32; GRN 1115, p.43
Rat, Wistar	ARA-rich oil from *M. alpina* (43.3% ARA;	0, 1, 1.5, or 5% of diet	90 days subchronic with in-utero exposure	5% of diet or 3,170 mg/kg bw/day	Casterton et al., 2009	GRN 326, p.135-152; GRN 1115, p.43

	RAO from Cargill)					
Rat, Wistar	ARA-rich oil, (41.5% of FA as ARA; SUNTGA40S)	0, 0.5, 1.5, or 5.0% (or ~3,000 mg/kg bw/day) in diet; 3.65% ARA + 2.11% DHA	13 wk of F_1, after in-utero exposure of F_0	No effect at 5.0% in F_1; changes in spleen wt, hematology, and blood lipids at high-dose; ARA+DHA in F_1 were not considered adverse	Lina et al., 2006	GRN 326, p.152-153; GRN 1115, p.43
Rat, Wistar	ARA-rich oil (38.6% ARA)	0, 0.3, 1.5, or 7.5% ARA in diet; 7.5% ARA + 5.5% DHA	13 wk of F_1, after in-utero exposure of F_0	NOAEL, 1.5% ARA-rich oil in diet or 970 mg ARA-rich oil/kg bw/day (corresponding to 374 mg ARA/kg bw/day)	Hempenius et al., 2000	GRN 326, p.149-150; GRN 963, p.31; GRN 1115, p.42
Subchronic Oral Toxicity Study of *M. alpina* Biomass						
Rat, Wistar	ARA-rich *M. alpina* biomass (13.1% ARA)	0, 0.25, 0.5, 1.0, 2.0 and 3.0% of diet	13 wk	3.0% *M. alpina* biomass in diet	Nisha et al., 2009	GRN 326, p.150, 152; GRN 730, p.33; GRN 963, p.31
Teratogenicity Study						
Rat, Sprague Dawley	ARA-rich oil (ARASCO®)	0, 1,000, or 2,500 mg/kg bw/day	From gestation days 6 to 15	ARA-rich oil: 2,500 mg/kg bw/day for both F_0 and F_1	Arterburn et al., 2000b	GRN 326, p.149

Bioequivalency Study						
Piglet	ARASCO®, RAO, or SUNTGA40S®	Diet, formula containing 35.8 mg ARA and 17.9 mg DHA/100 kcal (0.64 and 0.32% total FAs; comparing ARASCO®, RAO, and SUNTGA40S® at the same concentrations of ARA/DHA)	19 days (day 3 to day 22)	All three sources of ARA were safe and nutritionally bioequivalent at 0.64% of total FA as ARA in combination with DHA	Tyburczy et al., 2011	GRN 730, p.33
Neonatal Piglet Studies						
Piglet	ARA-rich oil from *M. alpina* (ARASCO®)	Varying ratios of ARA to DHA; 0.1-1.06% ARA of total FAs	25 days (day 3 to day 28)	1.06% ARA of total FAs, in combination with 1% FAs as DHA	Tyburczy et al., 2012	GRN 730, p.32-33 GRN 963, p.32
Piglet	ARA-rich oil (40% ARA; SUNTGA40S®)	Per each g of formula; 0, 96 mg ARA (actual mean intake, 248 mg/kg bw/d), 55 mg DHA (mean intake 136 mg/kg bw/d), or the blend of 62 mg ARA- (153 mg/kg bw/d) and 34 mg DHA-rich oils (84 mg/kg bw/d); each formula contained 962-999 kcal/L	16 days (day 3 to day 19)	248 mg ARA/kg bw/day (or ~620 mg ARA-rich oil/kg bw/day)	Merritt et al., 2003	GRN 326, p.151; GRN 963, p.31-32; GRN 1115, p.46

Abbreviations: ARA = arachidonic acid; bw = body weight; DHA = docosahexaenoic acid; FA = fatty acid; GRN = GRAS notice; LD_{50} = mean lethal dose; NOAEL = no observed adverse effect level; wk = week, wt = weight.

Conclusion:

The safety of ARA-rich oil (40.3% ARA of total FA) produced by Runke Bioengineering, the subject of this GRAS determination, is supported by 28-day and 90-day repeat dose oral toxicity studies in rats (Lewis et al., 2016) and a reproductive and developmental toxicity study in rats (Falk et al., 2017). The NOAEL was determined to be 5,000 mg/kg bw/day, the highest level tested in rats. The NOAEL of 2,000 mg ARA/kg bw/day may represent approximately 50-60 times the infant intake of ARA in human milk. However, in a subchronic toxicity study with an in-utero exposure, the NOAEL of ARA-oil was determined to be 1.5% in the diet or approximately 970 mg ARA-rich oil/kg bw/day (374 mg ARA/kg bw/day) in rats (Hempenius et al., 2000).

In addition, ARA-rich oil ingredients used in the corroborative studies described above are compositionally similar to Runke Bioengineering's ARA-rich oil as other oils contain 34-51% of total FAs as ARA. The safety of other sources of ARA-rich oil are supported by the following studies in rats: a 90-day subchronic toxicity study performed on the biomass of *M. alpina* (Nisha et al., 2009), 90-day subchronic toxicity studies with an in-utero exposure (Casterton et al., 2009; Gao et al., 2014; Hempenius et al., 2000; Lina et al., 2006), and a neonatal piglet study (Merritt et al., 2003) as well as a neonatal piglet study of a blend of ARA- and DHA-rich oils (Tyburczy et al., 2012). In addition, a study by Tyburczy et al. (2011) indicated that ARA-rich oils produced by various strains of *M. alpina* may be substantially equivalent to ARASCO®.

6.B.4. Human Clinical Studies of ARA-rich Oil

No new original human clinical studies have been published since the FDA's last review of 2022-2023 although one meta-analysis (Adjibade et al., 2022) has been published. Our review included studies that evaluated fungal ARA as an ingredient in infant formulas with a proper control formula group and those included safety parameters. Publications that are not relevant to assessing the safety of ARA in infant formula (such as those that employed different food forms including supplements or enteral feeding), or those that did that did not use fungal-derived ARA, were not included in this review.

6.B.4.1. Pre-term Infants

Our review included studies that evaluated fungal ARA as an ingredient in infant formulas with a proper control formula group and employed 0.5-1.1% ARA in the fat component of the formula (Table 23). Runke intends to use up to 0.5% of total fat as ARA for pre-term

infants. It is assumed that supplementation of ARA up to 0.5% of FAs is safe if the studies employing higher levels do not report adverse effects of ARA supplementation to formulas. We were able to identify 3 studies to meet the criteria described above (Carnielli et al., 2007; Clandinin et al., 1997, 2005). The studies by Clandinin et al. (2005) and Carnielli et al. (2007) were reviewed in GRNs 326, 730, and 1115 and are summarized in Table 23. Therefore, this notice incorporates, by reference, the studies discussed in previous GRAS notices and will not discuss previously reviewed references in detail.

Study by Clandinin et al. (1997)

Clandinin et al. (1997) evaluated the effects of varying levels of ARA and DHA on growth and the fatty acid content of individual lipid components in pre-term infants of less than 2.3 kg at birth. Pre-term infants received one of the following 4 formulas in the first 6 wk of life: 1) control formula with no supplemented fungal ARA and algal DHA, 2) formula with 0.32% ARA and 0.24% DHA, 3) formula with 0.49% ARA and 0.35% DHA, or 4) formula with 1.1% ARA and 0.76% DHA in the fat component of the formula. An analogous group of infants fed on their mothers' breast milk and a breast milk fortifier was also studied. Measurements included growth and fatty acid composition in plasma lipoprotein lipids that were analyzed at 12 d of age and after a further 4 wk of feeding. Supplementing infant formula with increasing levels of ARA and DHA produced a clear dose response in the level of ARA found in the HDL and LDL phospholipid fraction. Although the authors concluded that a formula level of 0.49% ARA and 0.35% DHA provides sufficient levels of these fatty acids to achieve a similar fatty acid content to that of infants fed breast milk for the major lipoprotein fractions examined, no adverse effects were observed at the highest levels of ARA and DHA (i.e., 1.1% ARA and 0.76% DHA in the fat component of the formula.

In a study by Carnielli et al. (2007), 22 healthy, non-breast-fed, pre-term infants (n=22) were randomly assigned equally to control (standard formula) and test groups (standard formula supplemented with 0.84% fungal ARA (ARASCO, DSM) and 0.64% algal DHA (DHASCO, algal type was not specified, but probably derived from *C. cohnii*, 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 polyunsaturated FAs. 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 a study by Clandinin et al. (2005), 361 pre-term infants of less than or equal to 35 weeks post-menstrual age (PMA) were randomly assigned to 3 study formula groups: 1) control, formula with no added DHA or ARA; 2) algal-DHA, formula with 17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal from fungal oil (~0.64% fatty acids as ARA; Martek Biosciences, algal type was not specified); or 3) fish-DHA, formula with 17 mg DHA/100 kcal from tuna fish and 34 mg ARA/100 kcal from fungal oil. These levels of DHA and ARA were similar to those present in a typical mature human milk (approximately 0.6 wt% fatty acids as ARA and 0.3 wt% of fatty acids as DHA). The study formulas were the sole source of nutrition for the pre-term infants until 57 weeks PMA (or 4 months after term) and the primary source of nutrition until 92 weeks PMA. DHA supplementation was stopped at 92 weeks PMA, and the subjects were monitored until 118 weeks PMA (18 months after term). Term infants, breastfed for 4 months or longer, were the reference group. All infants were assessed at birth and at 40, 44, 48, 53, 57, 66, 79, 92, and 118 weeks PMA. Measurement endpoints included growth, tolerance, AEs, and Bayley development scores. There were no differences in caloric intake from the formula, daily gastric residuals, stool frequency and consistency, or abdominal distention among the pre-term groups during hospitalization (data not shown). In addition, there were no differences in parents reporting fussiness, diarrhea, or constipation (data not shown), although infants in the algal DHA and fish DHA-supplemented groups had more gas than usual at 40 and 44 weeks PMA ($P<0.05$), which reached no differences at 53 or 57 weeks. Overall, the authors concluded that ARA and DHA supplementation did not increase morbidity or AEs in pre-term infants. In addition, no adverse effects of DHA supplementation were reported on the measured outcomes.

In summary, the studies by Clandinin et al. (2005), Carnielli et al. (2007), and Clandinin et al. (1997) in pre-term infants, employing 0.64%, 0.84%, and up to 1.1% ARA of the fat component of the formulas (corresponding to 43, 56, and 74 mg ARA/kg bw/day), respectively, did not report any adverse effects of ARA supplementation to infant formulas. These studies are briefly summarized in Table 23. Runke intends to use up to 0.5% of total fat as ARA for pre-term infants. This level corresponds to an ARA intake of 33.4 mg ARA/kg bw/day (which corresponds to 87.9 mg of ARA-rich oil/kg bw/day). An

intended use level of up to 0.5% FAs as ARA (or 33-34 mg ARA/kg bw/day) in pre-term infants is within safe intake levels found from clinical studies in pre-term infants.

Thus, it is concluded that there is reasonable certainty of no harm to pre-term infants of the subject of this GRAS Notice per the intended uses and use levels. In addition, an intended use level of up to 0.5% FAs as ARA in pre-term infants is consistent with current ARA recommendations: 18-45 mg/kg bw/day, preferably high intakes of 35–45 mg ARA/kg bw/day (approximately 0.6–0.75% of the total FAs) for very low birth weight pre-term infants (Koletzko et al., 2014b).

Studies Employing Capsule Supplements or Human Milk by Enteral Feeding Were Not Included in the Safety Evaluation of This Review

The studies administering ARA via supplement capsules, enteral feeding, human milk fed by enteral feeding, or intravenous administration are not considered in this review because food forms or routes of administration may impact the safety of the test substance, although no such studies reported adverse effects of ARA.

Emulsified supplement via the nasogastric tube:

Study by Frost et al. (2021): In this study, the ARA/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 providing 80 mg/kg bw/day ARA and 40 mg/kg bw/day DHA (source, manufacturer, and country not specified), or supplements providing 240 mg/kg bw/day ARA and 120 mg/kg bw/day DHA. Whole blood LCPUFA levels were measured. No adverse effects were reported on measured outcomes.

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 31 mg ARA (0.91% of total FAs per 100 mL) and 32 mg DHA (0.86% of total fatty acids as DHA; source not specified) was fed to pre-term infants each day for 9 weeks after birth with an 8-year follow-up.

Studies evaluating efficacy only were not considered for safety evaluation in this review:

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

Clandinin et al. (1999): This study evaluated blood concentrations of lipid profile (fatty acid composition of erythrocyte) and did not include any safety parameters.

Table 23. Pre-term Infant Studies Reporting No Adverse Effects of ARA Administered via Infant Formulas

Subjects	Test materials	Duration	Measurements/ Safety-related results	Reference	Previous GRN citation
361 pre-term infants of < 35 wk PMA	(1) Control formula with no added ARA/DHA; (2) algal-DHA formula with 17 mg DHA/100 kcal and 34 mg ARA/100 kcal, or (3) fish-DHA formula with 17 mg DHA/100 kcal from tuna fish and 34 mg ARA/100 kcal. (4) Reference group: term infant breast milk fed (~0.6% of FAs as ARA and ~0.3% of FAs as DHA)	Intervention until 92 wk PMA; follow-ups until 118 wk PMA; Reference group for ≥4 mo, starting between birth and 4 wk of age	Growth, gastrointestinal tolerance, adverse events, morbidity, and Bayley development scores/ No adverse effects on measured outcomes	Clandinin et al., 2005	GRN 326, p.63, 108; GRN 730, p.39; GRN 1115, Table 14 on p.51
22 pre-term infants with gestational ages of approxi-mately 31 wk	Control: Infant formula, or Infant formula supplemented with fungal ARA (0.84% ARASCO) and algal DHA (0.64%; DHASCO; approx. 42.8 mg DHA/kg/day, assuming that infants consume 6.7 g fat/kg bw/day);	From birth to 7 mo of age	Growth; plasma phospholipid FAs; estimation of endogenous synthesis of LC PUFAs/ No adverse effects on measured outcomes	Carnielli et al., 2007	GRN 326, p.115 and Table 28 on p.82; GRN 730, p.39-40; GRN 1115, Table 14 on p.53
117 pre-term infants <2300 g	5 groups: 1) Breast milk; 2) unsupplemented formula; formulas supplemented with 3) 0.32% ARA and 0.24% DHA, 4) 0.49% AA and 0.35% DHA, or 5) 1.1% ARA and 0.76% DHA;	First 6 wk of life	Growth, blood values including hematology; blood concentrations of lipid profile (fatty acid composition of	Clandinin et al., 1997	Not applicable

	Algal DHA and fungal ARA were from Martek; 91 completed the study		erythrocyte membrane phospholipids, lymphocyte membrane phospholipids, and plasma lipoprotein) /No adverse effects on measured outcomes		

0.64%, 0.84%, and 1.1% FAs correspond to ~ 42.8, 56.2, and ~73.6 mg ARA/kg bw/day, assuming that infants consume 6.7 g fat/kg bw/day.

DHA and ARA = Percentages given as % of total FAs unless noted otherwise.

Abbreviations: ARA = arachidonic acid; bw = body weight; DHA = docosahexaenoic acid; FA = fatty acid; LCPUFA = long-chain polyunsaturated fatty acid; mo = months; PMA = post-menstrual age; wk = weeks.

6.B.4.2. Term Infants

Since the FDA's review in 2022-2023, no new intervention studies were published. However, a meta-analysis by Adjibade et al. (2022) reported no adverse association between the consumption of long-chain PUFA-enriched formula and the risk of infection and allergy.

Our review focused on the studies employing 0.64-0.72% ARA in the fat component of the formulas with measurement endpoints of allergenic potential, gastrointestinal tolerance, and safety of ARA in term infants (Birch et al., 2005, 2007, 2010; Burks et al., 2008; Hoffman et al., 2008). These studies are summarized in Table 24 and also in previous GRAS notices (GRNs 326, 730, and 1115). Because these studies were extensively reviewed in previous GRAS notices, this notice incorporates, by reference, these studies discussed in previous GRAS notices and will not discuss previously reviewed references in detail. Page numbers of previous GRAS notice citations are included in Table 24.

<u>Studies Evaluating Efficacy Only were Not Considered for Safety Evaluation</u>

The studies evaluating the efficacy of ARA 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 ARA/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 ARA and DHA supplementation on ARA/DHA concentrations in the RBC phospholipids and cognition parameters (including memory, executive function and problem solving, and verbal and composite intellectual quotient). In this DHA Intake and Measurement of Neural Development (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%. 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% 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% 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% FAs as DHA and 0.64% FAs as ARA). Thus, this study is considered as an efficacy study demonstrating health benefits of DHA supplementation instead of the study evaluating the safety.

Meta-Analysis

From the meta-analysis of 8,389 formula-fed infants from the Etude Longitudinale Française depuis l'Enfance (France) birth cohort, Adjibade et al. (2022) reported no adverse association between ARA/DHA supplementation and the risk of lower respiratory tract infections and allergies. Formula enrichment was identified and confirmed from the list of ingredients of the formula consumed at 2 months. Among formula-fed infants at 2 months, 36% consumed formula enriched with ARA and DHA, and 11% consumed formula additionally enriched with eicosapentaenoic acid (EPA).

Numerous systematic reviews and recommendations of ARA used in clinical trials conducted in infants have been published in the peer-reviewed literature (Jasani et al., 2017; Koletzko et al., 2014a, 2014b, 2020). Overall, human clinical studies and meta-analyses consistently report no adverse effects of ARA/DHA supplementation on allergy and gastrointestinal tolerance in term infants. While the results of the reviews did not always identify clear benefits associated with ARA supplementation, there was no evidence of adverse effects or safety concerns (including allergenicity) associated with ARA supplementation of infant formula.

Table 24. Term Infant Studies Reporting No Adverse Effects of ARA Administered via Infant Formulas

Objective	Subjects	Test Material and Dose	Type and Duration of the Study	Measurements/Safety-related results	Reference	Previous GRN citation
To evaluate ARA/DHA supplementation in amounts typical for human milk in a large cohort of infants by using sweep visual evoked potential (VEP)	103 term infants	Test: 0.72% fungal ARA + 0.36% DHA (algal oil); or control: unsupplemented ARA source: *M. alpina* ARASCO®	Intervention from day 5 to 52 wk	Growth; gastrointestinal tolerance; sweep VEP acuity; random dot stereoacuity; lipid profile (RBC lipids) / No adverse effects on measured outcomes	Birch et al., 2005	GRN 326, p.64, 111; GRN 730, p.43; GRN 1115, p.54
To evaluate safety, benefits, and growth when supplemented with DHA and ARA formula in infants	244 healthy term infants	3 groups: 1) 21 mg ARA + 8 mg algal DHA; 2) 34 mg ARA (~0.64% FAs) + 17 mg DHA; or 3) control, non-supplemented formula ARA Source: *M. alpina*	From 12-16 days to 120 days of age	Safety (adverse events); growth; incidence of atopic dermatitis; tolerance assessed by stool frequency and characteristics as well as amounts of gas; ARA/DHA conc. in RBC, and plasma phospholipids/ No adverse effects on measured outcomes	Hoffman et al., 2008	GRN 326, p.117; GRN 730, p.44

To determine the effects on growth, tolerance, and safety in healthy infants of an amino acid-based formula	Study 1: 164 healthy term infants	The new amino acid-based formula containing 34 mg/100 kcal (0.64% total FAs) ARA and 17 mg/100 kcal (0.32% total FAs) DHA	From 14 ± 2 through 120 ± 4 days of age	Growth, formula acceptance, tolerance, and AEs/ No adverse effects on measured outcomes	Burks et al., 2008	GRN 326, p.116-117
Study 2: to evaluate the hypoallergenicity of a new amino acid-based formula in infants and children with confirmed cow's milk allergy	Study 2: 32 infants and children aged 8 mo to 10 yr with cow's milk allergy		Study 2: double-blind, placebo-controlled food challenge, with formulas, followed by open challenge. And extended 7-day home feeding period if the open challenge response was negative	Study 2:- any indication of allergy (extent and severity of rash, pruritus, or urticaria/angioedema; upper or lower respiratory symptoms; or gastrointestinal symptoms) and adverse events/ No adverse effects on measured outcomes		
To determine the effect of ARA/DHA supplementation on the safety and the visual	343 healthy term infants	ARA, 0.64% (34 mg/100 kcal, from *M. alpina*) for all 3 DHA concentrations; DHA (from *C. cohnii* oil), DHA: 0.32%, 0.64%, or 0.96%;	First 12 mo of life (from days 1-9), sole source of nutrition until < 4 mo of age	Physical growth; tolerance, and adverse events; visual acuity maturation; RBC FAs /	Birch et al., 2010	GRN 730, p.42; GRN 1115, p.56

acuity of formula-fed infants from the DIAMOND study		Control: unsupplemented		No adverse effects on measured outcomes		

Birch et al. (2007) states that all formulas contribute 5.6 g fat per 100 kcal.
DHA and ARA = percentages in diet given as % of total FA unless noted otherwise.
Abbreviations: ARA = arachidonic acid; DHA = docosahexaenoic acid; DIAMOND study = DHA Intake and Measurement of Neural Development study; FA = fatty acid; *M. alpina = Mortierella alpina*; mo = months; RBC = red blood cell; RCT = randomized, controlled trial; VEP = visual evoked potentials; wk= weeks; yr = years.

Consumer Reports

Findings from intervention studies are further supported by the safe history of use of ARA from fungal oil in infant formula. The FDA analyzed the Center for Food Safety and Applied Nutrition (CFSAN) Adverse Event Reporting System (CAERS) data to find a correlation between the gastrointestinal adverse events (AEs) and the use of DHA and ARA oils in infant formulas (FDA, 2011; FDA Docket No. 2008-P-0074-0017). The FDA considered the USDA's reports, which indicated the time-dependent increase of market shares of infant formulas containing DHA and ARA-oils: the market share of infant formulas containing DHA and ARA oils were introduced into the U.S. market in 2002 and increased from less than 10% of the market in the third quarter of 2002 to 98% of the market in 2008. The agency did not find any time-dependent increase in the proportions of gastrointestinal AEs to total AEs reported over time while the market share of infant formulas containing DHA and ARA oils increased from 0% to 98%. The FDA (2011) stated, "We found no statistically significant increases in the proportion of gastrointestinal AEs reports in CAERS when we looked over the time interval from when infant formulae containing DHA and ARA oils were first introduced until they essentially replaced non-supplemented formula in the marketplace."

Overall Conclusion

In conclusion, ARA, combined with a safe and suitable source of DHA, is not expected to adversely impact the pre-term and term infants who would be consuming exempt and non-exempt infant formula, respectively. Therefore, adverse effects resulting from the ingestion of the subject of this GRAS Notice per the intended use level are not expected.

6.C. Potential Adverse Effects

No potential adverse effects are expected under the intended use.

6.D. Safety Determination

Numerous human and animal studies have reported benefits of ARA-rich oils with no major adverse effects. Runke Bioengineering uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning the chemistry of ARA-rich oils. This GRAS determination is based on the data

and information generally available for the safety of ARA-rich oil. The literature indicates that ARA-rich oils offer infants health benefits without adverse effects.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of ARA-rich oils as well as appropriate corroborative data.

1. Runke Bioengineering's ARA-rich oil is manufactured under cGMP using common oil industry materials and processes.
2. Analytical data from multiple lots indicate that Runke Bioengineering's ARA-rich oil complies reliably with the established food-grade product specifications and meets all applicable purity standards.
3. Studies have shown that ARA-rich oil is not mutagenic or genotoxic. In addition, a subchronic study reported that NOAELs for Runke Bioengineering's ARA-rich oil was 5,000 mg/kg bw/day (or ~2,000 mg ARA/kg bw/day) in both male and female rats, the highest level tested. The NOAEL of 2,000 mg ARA/kg bw/day may represent approximately 50-60 times the infant intake of ARA under the intended use.
4. Runke Bioengineering's ARA-rich oil will be used as food ingredients in infant formulas. For term infants, intended use and use levels will be the same as those described in GRNs 000326, 000080, and 000041. For pre-term infants, intended use levels will be slightly higher than that described in previous GRAS notices (0.5% vs. 0.4% of total FAs as ARA). This level is justified because no studies found adverse effects of ARA supplementation at or above 0.5% of total FAs in pre-term infants. In addition, an intended use level of up to 0.5% FAs as ARA in pre-term infants is consistent with current ARA recommendations: 18-45 mg/kg bw/day, preferably high intakes of 35-45 mg ARA/kg bw/day (approximately 0.6–0.75% of the total FAs as ARA) for very low birth weight pre-term infants.
5. An estimate of exposure to ARA from its addition to infant formula is based on mean target ARA concentrations of 0.75% and 0.50% of total fat for term and pre-term infants, respectively. These correspond to intakes of ARA of 42 mg and 33 mg ARA/kg bw/day (corresponding to 110 and 88 mg of ARA-rich oil/kg bw/day) for term infants and pre-term infants, respectively.

6. The EDI values are based on the assumption that Runke Bioengineering's ARA-rich oil will replace currently marketed ARA ingredients. Thus, cumulative exposures are not expected to change.

6.E. Conclusions and General Recognition of the Safety of ARA-rich Oil

Several sources of ARA-rich oil have been evaluated by the FDA and other global regulatory agencies over the past 16 years for proposed incorporation of ARA-rich oils in foods for human consumption. Relevant U.S. GRAS notifications include GRNs 000041, 000080, 000094, 000326, 000730, and 000963 (FDA, 2001a, 2001b, 2006, 2010, 2018, and 2021, respectively). All GRAS notices provided information/clinical study data that supported the safety of the proposed ARA-rich oil ingredients for use in infant formulas. In all studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues attributable to ARA-rich oil derived from *M. alpina.* Because this safety evaluation was based on generally available and widely accepted data and information, it satisfies the so-called "common knowledge" element of a GRAS determination.

In addition, the intended uses of ARA-rich oil have been determined to be safe through 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 composition of Runke Bioengineering's ARA-rich oil are almost identical to those that have received FDA no question letters. No significant amounts of toxicants (e.g., hexane and MCPD) have been detected from Runke Bioengineering's ARA-rich oil.

The ARA-rich oil that is the subject of this GRAS determination is produced by the non-toxigenic, non-pathogenic fungus, *M. alpina,* and its purity is over 38%. The ARA-rich oil is manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food-grade and/or commonly used in fermentation and food manufacturing processes. Literature searches did not identify safety/toxicity concerns related to ARA-rich oil. Toxicity studies of Runke Bioengineering's ARA-rich oils include acute, subacute, subchronic toxicity, and developmental and reproductive toxicity studies in animals as well as mutagenicity and genotoxicity studies. The publicly available scientific literature on the consumption and

safety of ARA-rich oil in infant clinical studies is extensive and sufficient to support the safety and GRAS status of the proposed ARA-rich oil.

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

The information and data provided by Runke Bioengineering in this report, supplemented by the publicly available literature/toxicity data on ARA-rich oil ingredients, provide a sufficient basis for an assessment of the safety of ARA-rich oil for the proposed use as an ingredient in infant formulas when prepared according to appropriate specifications and cGMP.

6.F. Discussion of Information Inconsistent with GRAS Determination

Runke Bioengineering is not aware of information that would be inconsistent with a finding that the proposed use of ARA-rich oil in infant formulas, meeting appropriate specifications and used according to cGMP, is GRAS.

PART 7. REFERENCES

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Appendix A. Strain Identification Report

TEST REPORT

IMCAS Report No. 2023JB158

Applicant: Fujian Runke Bioengineering Corp., Ltd.

Sample described: Microbial culture (strain FJRK-MA01)

Sample quantity: One strain Date of sampling: 2023.04

Tested by: Bing-Da SUN Signature: [redacted]

Approved by: Yu-Guang ZHOU Signature: [redacted]

(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 rRNA gene, the strain FJRK-MA01 belongs to:

Mortierella alpina

Institute of Microbiology
Chinese Academy of Sciences
June 19, 2023

TEST REPORT

IMCAS Report No. 2023JB158

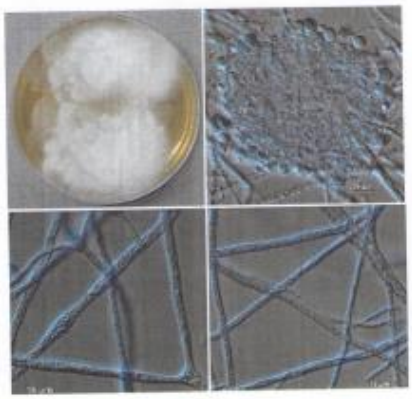
Applicant: Fujian Runke Bioengineering Corp., Ltd.

(continue)

1. Morphological properties

Fast growing on malt extract agar, colonies reaching 45~55 mm diam after five days of incubation at 25 °C, white, cottony; aerial mycelium flourish; reverse yellowish-brown, without soluble pigments.

Milky white droplets produced in mycelium, hyphae branched and without septum, 2.0~6.0 μm in width. Sporulation was rare on MEA and neither sporangiospore nor zygospore observed.

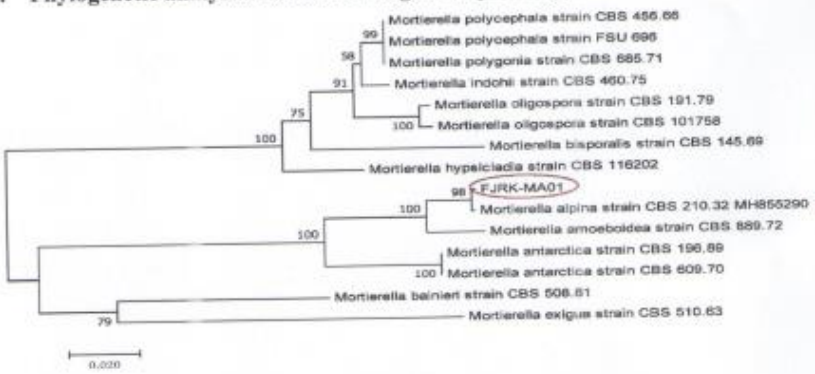


2. Partial sequence of rRNA gene

(including 18S rDNA, partial sequence; ITS1, 5.8S rRNA and ITS2, full sequence; 28S rDNA, partial sequence)

5'- CATTCATAATCAAAGTGTTTTTATGGCACTTTTAAAAAAATCCATATCCACCTTGTGTGCAATGTCATCTCACTGGAGGC
CGGCGGCTGTAAAAAGCCCGTTTGGTCACCTTTGGGATTTATATCTACTCAGAACTTTAGTGATTTTGTCTGAAAAATATTAT
GAATAACTTAATTCAAAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCATATTGCGCTCTCTGGTATTCCGGAGAGCATGCTTGTT
TGAGTATCAGTAAACACCTCAACTCCCTTTTTCTTTTTTGAAATGAGGGAGCTGGACTTGAGTGATCCCAACACTTTTCTCAC
TGAAAAGTGGCGGGTCACTTGAAATGCAGGTGCAGCTGGACTTTTCTCTGAGCTATAAGCATATCTATTTAGTCTGCCTAAAA
AACAGATTATTACCTTTGCTGCAGCTAACATAAAGGAGATGAGTTCTTGTGCTGACTGATGCAGGATTCACAGAGACAGCTTC
GCGGCTGACTTTGTAAACTCGATCTCAAATCAAGTAAGACTACCCGCTGAACTTAAGCATATCAA -3'

3. Phylogenetic analysis base on rRNA gene sequencing data



Appendix B. Certificates of Analysis

eurofins

Analytical Report

Sample Code	502-2022-00002955	Report date	09-Feb-2022
Certificate No.	AR-22-SU-007861-04		

This report is translated from report AR-22-SU-007861-03

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

Our reference:	502-2022-00002955/ AR-22-SU-007861-04
Client Sample Code:	批号：11004332 生产日期：2021.10.04
Sample described as:	Arachidonic acid oil / Arachidonic acid oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	10-Jan-2022
Analysis Starting Date:	10-Jan-2022
Analysis Ending Date:	26-Jan-2022
Arrival Temperature (°C)	14.0
Sample Weight	140g*2

		Results	Unit	LOQ	LOD
☆ QA04G	Monochloropropanediols (sum of free and esters) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Total 2-MCPD (free and bound)	<0.10	mg/kg	0.1	
	Total 3-MCPD (free and bound)	0.30	mg/kg	0.1	
☆ QA0N0	Glycidyl esters (GC-MSMS) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Glycidol (calculated)	<0.10	mg/kg	0.1	

Revision Notes
Modifies client sample description

SIGNATURE

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

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Analytical Report

Sample Code	502-2022-00002956	Report date	09-Feb-2022
Certificate No.	AR-22-SU-007862-04		

This report is translated from report AR-22-SU-007862-03

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

Our reference:	502-2022-00002956/ AR-22-SU-007862-04
Client Sample Code:	批号：11008334 生产日期：2021.10.08
Sample described as:	Arachidonic acid oil / Arachidonic acid oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	10-Jan-2022
Analysis Starting Date:	10-Jan-2022
Analysis Ending Date:	26-Jan-2022
Arrival Temperature (°C)	14.0
Sample Weight	140g*2

		Results	Unit	LOQ	LOD
☆ QA04G	Monochloropropanediols (sum of free and esters) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Total 2-MCPD (free and bound)	<0.10	mg/kg	0.1	
	Total 3-MCPD (free and bound)	0.25	mg/kg	0.1	
☆ QA0N0	Glycidyl esters (GC-MSMS) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Glycidol (calculated)	<0.10	mg/kg	0.1	

Revision Notes
Modifies client sample description

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Analytical Report

Sample Code	502-2022-00002957	Report date	09-Feb-2022
Certificate No.	AR-22-SU-007863-04		

This report is translated from report AR-22-SU-007863-03

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

Our reference:	502-2022-00002957/ AR-22-SU-007863-04
Client Sample Code:	批号：11012336 生产日期：2021.10.12
Sample described as:	Arachidonic acid oil / Arachidonic acid oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	10-Jan-2022
Analysis Starting Date:	10-Jan-2022
Analysis Ending Date:	26-Jan-2022
Arrival Temperature (°C)	14.0
Sample Weight	140g*2

		Results	Unit	LOQ	LOD
☆ QA04G	Monochloropropanediols (sum of free and esters) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Total 2-MCPD (free and bound)	**<0.10**	mg/kg	0.1	
	Total 3-MCPD (free and bound)	**0.27**	mg/kg	0.1	
☆ QA0N0	Glycidyl esters (GC-MSMS) Method: AOCS Cd 29b-13 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	Glycidol (calculated)	**<0.10**	mg/kg	0.1	

Revision Notes
Modifies client sample description

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Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2021-00126364	Report date	30-Dec-2021
Certificate No.	AR-21-SU-116947-01-EN		

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

Our reference:	502-2021-00126364/ AR-21-SU-116947-01-EN
Client Sample Code:	样品批号：11004332 生产日期：2021.10.04
Sample described as:	Arachidonic acid oil /Arachidonic acid oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	29-Nov-2021
Analysis Starting Date:	29-Nov-2021
Analysis Ending Date:	29-Dec-2021
Arrival Temperature (°C)	21.8
Sample Weight	140g*12

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

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

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

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

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		Results	Unit	LOQ	LOD
	Peroxide value	0.61	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	1218	mg/100 g	1	
	Cholesterol	8	mg/100 g	1	
	Campesterol	81	mg/100 g	1	
	Campestanol	3	mg/100 g	1	
	Stigmasterol	11	mg/100 g	1	
	Unidentified sterols	146	mg/100 g	1	
	Sitosterol	62	mg/100 g	1	
	Sitostanol+ delta-5-avenasterol	18	mg/100 g	1	
	Delta-5,24-stigmastadienol	3	mg/100 g	1	
	Delta-7-stigmastenol	10	mg/100 g	1	
	delta-7-Avenasterol	2	mg/100 g	1	
	Cycloartenol	4	mg/100 g	1	
	24-Methylenecycloartanol	3	mg/100 g	1	
	Citrostadienol	6	mg/100 g	1	
	Total plant sterols + plant stanols	1556	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)	0.29	mg KOH/g	0.05	
	Free fatty acids (as oleic acid)	0.15	%	0.01	
☆ QA01L	p-Anisidine Value Method: AOCS Cd 18-90 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	p-Anisidine Value	5.7		1	
☆ QA04E	Residual Solvents (GC-MS) Method: AOCS Cg 4-94				
	1,1,1-Trichloroethane	<0.2	mg/kg	0.2	
	1,1,2-Trichloroethane	<0.2	mg/kg	0.2	
	1,2-Dichloroethane	<0.5	mg/kg	0.5	
	1,2-Dimethoxyethane	<1.0	mg/kg	1	
	1-Butanol	<1.0	mg/kg	1	
	2-Hexanone	<1.0	mg/kg	1	
	Acetone	<1.0	mg/kg	1	
	Benzene	<0.10	mg/kg	0.1	
	Butyl acetate	<0.50	mg/kg	0.5	
	Carbon tetrachloride	<0.50	mg/kg	0.5	
	Chlorobenzene	<0.50	mg/kg	0.5	
	Chloroform	<0.10	mg/kg	0.1	
	Cyclohexane	<0.20	mg/kg	0.2	
	Dichloromethane	<0.10	mg/kg	0.1	
	Ethanol	<1.0	mg/kg	1	
	Ethyl acetate	<1.0	mg/kg	1	
	Heptane	<0.20	mg/kg	0.2	
	Hexane (sum of n-hexane, iso and 3-methyl pentane)	<0.50	mg/kg	0.5	
	Isopropanol	<1.0	mg/kg	1	
	Methanol	<1.0	mg/kg	1	
	Methyl Ethyl Ketone (MEK)	<0.20	mg/kg	0.2	
	Methyl-tert-butylether (MTBE)	<0.20	mg/kg	0.2	
	Tetralin	<5.0	mg/kg	5	
	Toluene	<0.20	mg/kg	0.2	

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		Results	Unit	LOQ	LOD
	Trichloroethylene	<0.10	mg/kg	0.1	
	Xylenes (sum)	<0.20	mg/kg	0.2	
☆ QA307	Glyceride Profile Method: AOCS Cd 11c-93				
	Diglycerides	5.8	%	1	
	Glycerol	2.7	%	1	
	Monoglycerides	1.8	%	1	
	Triglycerides	92.7	%	1	
☆ QA383	Moisture & Volatiles (Air Oven 130C) Method: AOCS Ca 2c-25				
	Moisture & Volatiles	0.02	%	0.01	
☆ QA966	Unsaponifiable Matter Method: AOCS Ca 6a-40				
	Unsaponifiable matter	1.56	%	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)	<0.02	%	0.02	
	C14:0 (Myristic acid)	0.29	%	0.02	
	C14:1 (Myristoleic acid)	<0.02	%	0.02	
	C15:0 (Pentadecanoic acid)	0.10	%	0.02	
	C15:1 (Pentadecenoic acid)	<0.02	%	0.02	
	C16:0 (Palmitic Acid)	7.10	%	0.02	
	C16:1 Omega 7	0.17	%	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)	0.25	%	0.02	
	C17:1 (Heptadecenoic Acid)	0.03	%	0.02	
	C18:0 (Stearic Acid)	7.26	%	0.02	
	C18:1 (Vaccenic acid)	0.35	%	0.03	
	C18:1 Omega 9 (Oleic Acid)	8.78	%	0.02	
	C18:1, Total (Oleic Acid + isomers)	9.24	%	0.03	
	C18:2 Omega 6 (Linoleic Acid)	12.18	%	0.02	
	C18:2, Total (Linoleic Acid + isomers)	12.54	%	0.02	
	C18:3 Omega 3 (Alpha Linolenic Acid)	0.05	%	0.02	
	C18:3 Omega 6 (Gamma Linolenic Acid)	2.25	%	0.02	
	C18:3, Total (Linolenic Acid + isomers)	2.29	%	0.02	
	C18:4 Omega 3 (Octadecatetraenoic Acid)	<0.02	%	0.02	
	C18:4 Total (Octadecatetraenoic Acid)	<0.02	%	0.02	
	C20:0 (Arachidic Acid)	0.72	%	0.02	
	C20:1 Omega 9 (Gondoic Acid)	0.36	%	0.02	
	C20:1 Total (Gondoic Acid + isomers)	0.39	%	0.02	
	C20:2 Omega 6	0.50	%	0.02	
	C20:2 Total (Eicosadienoic Acid)	0.50	%	0.02	
	C20:3 Omega 3	0.14	%	0.02	
	C20:3 Omega 6	1.92	%	0.02	
	C20:3, Total (Eicosatrienoic Acid)	2.07	%	0.02	
	C20:4 Omega 3	<0.02	%	0.02	
	C20:4 Omega 6 (Arachidonic Acid)	41.01	%	0.02	
	C20:4, Total (Eicosatetraenoic Acid)	41.03	%	0.02	
	C20:5 Omega 3 (Eicosapentaenoic Acid)	0.06	%	0.02	

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		Results	Unit	LOQ	LOD
	C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02	%	0.02	
	C22:0 (Behenic Acid)	0.06	%	0.02	
	C22:1 Omega 9 (Erucic Acid)	<0.02	%	0.02	
	C22:1 Total (Erucic Acid + isomers)	<0.02	%	0.02	
	C22:2 Docosadienoic Omega 6	0.03	%	0.02	
	C22:3 Docosatrienoic, Omega 3	0.02	%	0.02	
	C22:4 Docosatetraenoic Omega 6	0.20	%	0.02	
	C22:5 Docosapentaenoic Omega 3	<0.02	%	0.02	
	C22:5 Docosapentaenoic Omega 6	0.10	%	0.02	
	C22:5 Total (Docosapentaenoic Acid)	0.10	%	0.02	
	C22:6 Docosahexaenoic Omega 3	0.32	%	0.02	
	C24:0 (Lignoceric Acid)	1.16	%	0.02	
	C24:1 Omega 9 (Nervonic Acid)	0.19	%	0.02	
	C24:1 Total (Nervonic Acid + isomers)	0.19	%	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	89.95	%	0.1	
	Total Fatty Acids	86.20	%	0.1	
	Total Monounsaturated Fatty Acids	9.97	%	0.05	
	Total Omega 3 Isomers	0.60	%	0.05	
	Total Omega 5 Isomers	<0.05	%	0.05	
	Total Omega 6 Isomers	58.20	%	0.05	
	Total Omega 7 Isomers	0.52	%	0.05	
	Total Omega 9 Isomers	9.47	%	0.05	
	Total Polyunsaturated Fatty Acids	59.09	%	0.05	
	Total Saturated Fatty Acids	16.96	%	0.05	
	Total Trans Fatty Acids	0.18	%	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)	0.14	%	0.01	
• R290Z	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	0.181	EU/ml		
☆ ZME3X	Enumeration (MPN) of Enterobacter sakazakii Method: FDA BAM Chapter 29 mod.				
	Enterobacter sakazakii	2.3	MPN/10 ml		

COMMENT
--------- Imported conclusion from Eurofins Central Analytical Laboratories ---------

Rev. 01: Testing added per client request.

--------- Imported conclusion from Eurofins | Scientific Finland Oy ---------

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 2089 mg/100 g

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

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SIGNATURE

Claire Wang	Jack He	Shine Xie
Authorized Signatory	Authorized Signatory	Authorized Signatory

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Analytical Report

Sample Code	502-2021-00126365	Report date	30-Dec-2021
Certificate No.	AR-21-SU-116948-01-EN		

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

Our reference:	502-2021-00126365/ AR-21-SU-116948-01-EN
Client Sample Code:	样品批号：11008334 生产日期：2021.10.08
Sample described as:	Arachidonic acid oil /Arachidonic acid oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	29-Nov-2021
Analysis Starting Date:	29-Nov-2021
Analysis Ending Date:	29-Dec-2021
Arrival Temperature (°C)	21.8
Sample Weight	140g*12

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

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

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

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		Results	Unit	LOQ	LOD
	Peroxide value	0.47	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	1196	mg/100 g	1	
	Cholesterol	5	mg/100 g	1	
	Campesterol	73	mg/100 g	1	
	Campestanol	2	mg/100 g	1	
	Stigmasterol	11	mg/100 g	1	
	Unidentified sterols	127	mg/100 g	1	
	Sitosterol	62	mg/100 g	1	
	Sitostanol+ delta-5-avenasterol	19	mg/100 g	1	
	Delta-5,24-stigmastadienol	3	mg/100 g	1	
	Delta-7-stigmastenol	11	mg/100 g	1	
	delta-7-Avenasterol	3	mg/100 g	1	
	Cycloartenol	4	mg/100 g	1	
	24-Methylenecycloartanol	3	mg/100 g	1	
	Citrostadienol	7	mg/100 g	1	
	Total plant sterols + plant stanols	1506	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)	0.28	mg KOH/g	0.05	
	Free fatty acids (as oleic acid)	0.14	%	0.01	
☆ QA01L	p-Anisidine Value Method: AOCS Cd 18-90 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	p-Anisidine Value	5.1		1	
☆ QA04E	Residual Solvents (GC-MS) Method: AOCS Cg 4-94				
	1,1,1-Trichloroethane	<0.2	mg/kg	0.2	
	1,1,2-Trichloroethane	<0.2	mg/kg	0.2	
	1,2-Dichloroethane	<0.5	mg/kg	0.5	
	1,2-Dimethoxyethane	<1.0	mg/kg	1	
	1-Butanol	<1.0	mg/kg	1	
	2-Hexanone	<1.0	mg/kg	1	
	Acetone	<1.0	mg/kg	1	
	Benzene	<0.10	mg/kg	0.1	
	Butyl acetate	<0.50	mg/kg	0.5	
	Carbon tetrachloride	<0.50	mg/kg	0.5	
	Chlorobenzene	<0.50	mg/kg	0.5	
	Chloroform	<0.10	mg/kg	0.1	
	Cyclohexane	<0.20	mg/kg	0.2	
	Dichloromethane	<0.10	mg/kg	0.1	
	Ethanol	<1.0	mg/kg	1	
	Ethyl acetate	<1.0	mg/kg	1	
	Heptane	<0.20	mg/kg	0.2	
	Hexane (sum of n-hexane, iso and 3-methyl pentane)	<0.50	mg/kg	0.5	
	Isopropanol	<1.0	mg/kg	1	
	Methanol	<1.0	mg/kg	1	
	Methyl Ethyl Ketone (MEK)	<0.20	mg/kg	0.2	
	Methyl-tert-butylether (MTBE)	<0.20	mg/kg	0.2	
	Tetralin	<5.0	mg/kg	5	
	Toluene	<0.20	mg/kg	0.2	

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		Results	Unit	LOQ	LOD
	Trichloroethylene	<0.10	mg/kg	0.1	
	Xylenes (sum)	<0.20	mg/kg	0.2	
☆ QA307	Glyceride Profile Method: AOCS Cd 11c-93				
	Diglycerides	5.3	%	1	
	Glycerol	2.9	%	1	
	Monoglycerides	1.5	%	1	
	Triglycerides	93.4	%	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	1.56	%	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)	<0.02	%	0.02	
	C14:0 (Myristic acid)	0.31	%	0.02	
	C14:1 (Myristoleic acid)	<0.02	%	0.02	
	C15:0 (Pentadecanoic acid)	0.09	%	0.02	
	C15:1 (Pentadecenoic acid)	<0.02	%	0.02	
	C16:0 (Palmitic Acid)	7.21	%	0.02	
	C16:1 Omega 7	0.18	%	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)	0.26	%	0.02	
	C17:1 (Heptadecenoic Acid)	0.03	%	0.02	
	C18:0 (Stearic Acid)	7.73	%	0.02	
	C18:1 (Vaccenic acid)	0.37	%	0.03	
	C18:1 Omega 9 (Oleic Acid)	9.36	%	0.02	
	C18:1, Total (Oleic Acid + isomers)	9.87	%	0.03	
	C18:2 Omega 6 (Linoleic Acid)	13.34	%	0.02	
	C18:2, Total (Linoleic Acid + isomers)	13.79	%	0.02	
	C18:3 Omega 3 (Alpha Linolenic Acid)	0.05	%	0.02	
	C18:3 Omega 6 (Gamma Linolenic Acid)	2.18	%	0.02	
	C18:3, Total (Linolenic Acid + isomers)	2.24	%	0.02	
	C18:4 Omega 3 (Octadecatetraenoic Acid)	<0.02	%	0.02	
	C18:4 Total (Octadecatetraenoic Acid)	<0.02	%	0.02	
	C20:0 (Arachidic Acid)	0.75	%	0.02	
	C20:1 Omega 9 (Gondoic Acid)	0.36	%	0.02	
	C20:1 Total (Gondoic Acid + isomers)	0.39	%	0.02	
	C20:2 Omega 6	0.52	%	0.02	
	C20:2 Total (Eicosadienoic Acid)	0.52	%	0.02	
	C20:3 Omega 3	0.15	%	0.02	
	C20:3 Omega 6	1.90	%	0.02	
	C20:3, Total (Eicosatrienoic Acid)	2.04	%	0.02	
	C20:4 Omega 3	<0.02	%	0.02	
	C20:4 Omega 6 (Arachidonic Acid)	42.20	%	0.02	
	C20:4, Total (Eicosatetraenoic Acid)	42.20	%	0.02	
	C20:5 Omega 3 (Eicosapentaenoic Acid)	0.06	%	0.02	

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		Results	Unit	LOQ	LOD
	C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02	%	0.02	
	C22:0 (Behenic Acid)	1.49	%	0.02	
	C22:1 Omega 9 (Erucic Acid)	<0.02	%	0.02	
	C22:1 Total (Erucic Acid + isomers)	<0.02	%	0.02	
	C22:2 Docosadienoic Omega 6	0.04	%	0.02	
	C22:3 Docosatrienoic, Omega 3	0.02	%	0.02	
	C22:4 Docosatetraenoic Omega 6	0.22	%	0.02	
	C22:5 Docosapentaenoic Omega 3	<0.02	%	0.02	
	C22:5 Docosapentaenoic Omega 6	0.06	%	0.02	
	C22:5 Total (Docosapentaenoic Acid)	0.06	%	0.02	
	C22:6 Docosahexaenoic Omega 3	0.20	%	0.02	
	C24:0 (Lignoceric Acid)	1.22	%	0.02	
	C24:1 Omega 9 (Nervonic Acid)	0.20	%	0.02	
	C24:1 Total (Nervonic Acid + isomers)	0.20	%	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	95.15	%	0.1	
	Total Fatty Acids	91.20	%	0.1	
	Total Monounsaturated Fatty Acids	10.60	%	0.05	
	Total Omega 3 Isomers	0.49	%	0.05	
	Total Omega 5 Isomers	<0.05	%	0.05	
	Total Omega 6 Isomers	60.46	%	0.05	
	Total Omega 7 Isomers	0.55	%	0.05	
	Total Omega 9 Isomers	10.06	%	0.05	
	Total Polyunsaturated Fatty Acids	61.34	%	0.05	
	Total Saturated Fatty Acids	19.07	%	0.05	
	Total Trans Fatty Acids	0.18	%	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)	0.13	%	0.01	
• R290Z	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	0.153	EU/ml		
☆ ZME3X	Enumeration (MPN) of Enterobacter sakazakii Method: FDA BAM Chapter 29 mod.				
	Enterobacter sakazakii	< 0.3	MPN/10 ml		

COMMENT

--------- Imported conclusion from Eurofins Central Analytical Laboratories ---------

Rev. 01: Testing added per client request.

--------- Imported conclusion from Eurofins | Scientific Finland Oy ---------

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 2087 mg/100 g

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

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SIGNATURE

Claire Wang	Jack He	Shine Xie
Authorized Signatory	Authorized Signatory	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.
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Analytical Report

Sample Code	502-2021-00126366	Report date	30-Dec-2021
Certificate No.	AR-21-SU-116949-01-EN		

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

Our reference:	502-2021-00126366/ AR-21-SU-116949-01-EN
Client Sample Code:	样品批号：11012336 生产日期： 2021.10.12
Sample described as:	Arachidonic acid oil /Arachidonic acid oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	29-Nov-2021
Analysis Starting Date:	29-Nov-2021
Analysis Ending Date:	29-Dec-2021
Arrival Temperature (°C)	21.8
Sample Weight	140g*12

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

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

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

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		Results	Unit	LOQ	LOD
	Peroxide value	0.60	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	1227	mg/100 g	1	
	Cholesterol	6	mg/100 g	1	
	Campesterol	79	mg/100 g	1	
	Campestanol	3	mg/100 g	1	
	Stigmasterol	11	mg/100 g	1	
	Unidentified sterols	139	mg/100 g	1	
	Sitosterol	62	mg/100 g	1	
	Sitostanol+ delta-5-avenasterol	20	mg/100 g	1	
	Delta-5,24-stigmastadienol	3	mg/100 g	1	
	Delta-7-stigmastenol	10	mg/100 g	1	
	delta-7-Avenasterol	2	mg/100 g	1	
	Cycloartenol	4	mg/100 g	1	
	24-Methylenecycloartanol	2	mg/100 g	1	
	Citrostadienol	6	mg/100 g	1	
	Total plant sterols + plant stanols	1556	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)	0.29	mg KOH/g	0.05	
	Free fatty acids (as oleic acid)	0.15	%	0.01	
☆ QA01L	p-Anisidine Value Method: AOCS Cd 18-90 Accreditation: ISO/IEC 17025:2017 A2LA 2993.01				
	p-Anisidine Value	4.9		1	
☆ QA04E	Residual Solvents (GC-MS) Method: AOCS Cg 4-94				
	1,1,1-Trichloroethane	<0.2	mg/kg	0.2	
	1,1,2-Trichloroethane	<0.2	mg/kg	0.2	
	1,2-Dichloroethane	<0.5	mg/kg	0.5	
	1,2-Dimethoxyethane	<1.0	mg/kg	1	
	1-Butanol	<1.0	mg/kg	1	
	2-Hexanone	<1.0	mg/kg	1	
	Acetone	<1.0	mg/kg	1	
	Benzene	<0.10	mg/kg	0.1	
	Butyl acetate	<0.50	mg/kg	0.5	
	Carbon tetrachloride	<0.50	mg/kg	0.5	
	Chlorobenzene	<0.50	mg/kg	0.5	
	Chloroform	<0.10	mg/kg	0.1	
	Cyclohexane	<0.20	mg/kg	0.2	
	Dichloromethane	<0.10	mg/kg	0.1	
	Ethanol	<1.0	mg/kg	1	
	Ethyl acetate	<1.0	mg/kg	1	
	Heptane	<0.20	mg/kg	0.2	
	Hexane (sum of n-hexane, iso and 3-methyl pentane)	<0.50	mg/kg	0.5	
	Isopropanol	<1.0	mg/kg	1	
	Methanol	<1.0	mg/kg	1	
	Methyl Ethyl Ketone (MEK)	<0.20	mg/kg	0.2	
	Methyl-tert-butylether (MTBE)	<0.20	mg/kg	0.2	
	Tetralin	<5.0	mg/kg	5	
	Toluene	<0.20	mg/kg	0.2	

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		Results	Unit	LOQ	LOD
	Trichloroethylene	<0.10	mg/kg	0.1	
	Xylenes (sum)	<0.20	mg/kg	0.2	
☆ QA307	Glyceride Profile Method: AOCS Cd 11c-93				
	Diglycerides	5.5	%	1	
	Glycerol	2.2	%	1	
	Monoglycerides	2.0	%	1	
	Triglycerides	92.2	%	1	
☆ QA383	Moisture & Volatiles (Air Oven 130C) Method: AOCS Ca 2c-25				
	Moisture & Volatiles	0.06	%	0.01	
☆ QA966	Unsaponifiable Matter Method: AOCS Ca 6a-40				
	Unsaponifiable matter	1.51	%	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)	<0.02	%	0.02	
	C14:0 (Myristic acid)	0.30	%	0.02	
	C14:1 (Myristoleic acid)	<0.02	%	0.02	
	C15:0 (Pentadecanoic acid)	0.10	%	0.02	
	C15:1 (Pentadecenoic acid)	<0.02	%	0.02	
	C16:0 (Palmitic Acid)	7.06	%	0.02	
	C16:1 Omega 7	0.17	%	0.04	
	C16:1 Total (Palmitoleic Acid + isomers)	0.22	%	0.04	
	C16:2 (Hexadecadienoic Acid)	<0.02	%	0.02	
	C16:3 (Hexadecatrienoic Acid)	<0.02	%	0.02	
	C17:0 (Margaric Acid)	0.26	%	0.02	
	C17:1 (Heptadecenoic Acid)	0.03	%	0.02	
	C18:0 (Stearic Acid)	7.43	%	0.02	
	C18:1 (Vaccenic acid)	0.35	%	0.03	
	C18:1 Omega 9 (Oleic Acid)	8.67	%	0.02	
	C18:1, Total (Oleic Acid + isomers)	9.14	%	0.03	
	C18:2 Omega 6 (Linoleic Acid)	11.91	%	0.02	
	C18:2, Total (Linoleic Acid + isomers)	12.26	%	0.02	
	C18:3 Omega 3 (Alpha Linolenic Acid)	0.05	%	0.02	
	C18:3 Omega 6 (Gamma Linolenic Acid)	2.18	%	0.02	
	C18:3, Total (Linolenic Acid + isomers)	2.23	%	0.02	
	C18:4 Omega 3 (Octadecatetraenoic Acid)	<0.02	%	0.02	
	C18:4 Total (Octadecatetraenoic Acid)	<0.02	%	0.02	
	C20:0 (Arachidic Acid)	0.74	%	0.02	
	C20:1 Omega 9 (Gondoic Acid)	0.35	%	0.02	
	C20:1 Total (Gondoic Acid + isomers)	0.40	%	0.02	
	C20:2 Omega 6	0.49	%	0.02	
	C20:2 Total (Eicosadienoic Acid)	0.49	%	0.02	
	C20:3 Omega 3	0.12	%	0.02	
	C20:3 Omega 6	1.87	%	0.02	
	C20:3, Total (Eicosatrienoic Acid)	1.99	%	0.02	
	C20:4 Omega 3	<0.02	%	0.02	
	C20:4 Omega 6 (Arachidonic Acid)	41.70	%	0.02	
	C20:4, Total (Eicosatetraenoic Acid)	41.71	%	0.02	
	C20:5 Omega 3 (Eicosapentaenoic Acid)	0.06	%	0.02	

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		Results	Unit	LOQ	LOD
	C21:5 Omega 3 (Heneicosapentaenoic Acid)	<0.02	%	0.02	
	C22:0 (Behenic Acid)	0.06	%	0.02	
	C22:1 Omega 9 (Erucic Acid)	<0.02	%	0.02	
	C22:1 Total (Erucic Acid + isomers)	<0.02	%	0.02	
	C22:2 Docosadienoic Omega 6	0.03	%	0.02	
	C22:3 Docosatrienoic, Omega 3	0.03	%	0.02	
	C22:4 Docosatetraenoic Omega 6	0.21	%	0.02	
	C22:5 Docosapentaenoic Omega 3	<0.02	%	0.02	
	C22:5 Docosapentaenoic Omega 6	0.08	%	0.02	
	C22:5 Total (Docosapentaenoic Acid)	0.08	%	0.02	
	C22:6 Docosahexaenoic Omega 3	0.25	%	0.02	
	C24:0 (Lignoceric Acid)	1.19	%	0.02	
	C24:1 Omega 9 (Nervonic Acid)	0.19	%	0.02	
	C24:1 Total (Nervonic Acid + isomers)	0.25	%	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	90.29	%	0.1	
	Total Fatty Acids	86.54	%	0.1	
	Total Monounsaturated Fatty Acids	9.93	%	0.05	
	Total Omega 3 Isomers	0.52	%	0.05	
	Total Omega 5 Isomers	<0.05	%	0.05	
	Total Omega 6 Isomers	58.47	%	0.05	
	Total Omega 7 Isomers	0.52	%	0.05	
	Total Omega 9 Isomers	9.36	%	0.05	
	Total Polyunsaturated Fatty Acids	59.29	%	0.05	
	Total Saturated Fatty Acids	17.14	%	0.05	
	Total Trans Fatty Acids	0.18	%	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)	0.13	%	0.01	
• R290Z	Bacterial Endotoxins Method: USP 43<85>				
	Bacterial Endotoxins	0.096	EU/ml		
☆ ZME3X	Enumeration (MPN) of Enterobacter sakazakii Method: FDA BAM Chapter 29 mod.				
	Enterobacter sakazakii	< 0.3	MPN/10 ml		

COMMENT

--------- Imported conclusion from Eurofins Central Analytical Laboratories ---------

Rev. 01: Testing added per client request.

--------- Imported conclusion from Eurofins | Scientific Finland Oy ---------

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. cycloartanol, 24-methylenecycloartanol, and citrostadienol).

Amount of total GC elutables is 2088 mg/100 g

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

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Claire Wang	Jack He	Shine Xie
Authorized Signatory	Authorized Signatory	Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2022-00037068	Report date	30-Apr-2022
Certificate No.	AR-22-SU-033316-02		

This report is translated from report AR-22-SU-033316-01

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

Our reference:	502-2022-00037068/ AR-22-SU-033316-02
Client Sample Code:	样品批号：11004332 生产日期： 2021.10.04
Sample described as:	Arachidonic acid oil /Arachidonic acid oil
Sample Packaging:	Sealed metal bottle
Sample reception date:	23-Apr-2022
Analysis Starting Date:	24-Apr-2022
Analysis Ending Date:	29-Apr-2022
Arrival Temperature (°C)	21.6
Sample Weight	280g
Sample Condition	Other

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

SIGNATURE

Tracy Li
Authorized Signatory

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Analytical Report

Sample Code	502-2022-00037069	Report date	30-Apr-2022
Certificate No.	AR-22-SU-033317-02		

This report is translated from report AR-22-SU-033317-01

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

Our reference:	502-2022-00037069/ AR-22-SU-033317-02
Client Sample Code:	样品批号：11008334 生产日期： 2021.10.08
Sample described as:	Arachidonic acid oil /Arachidonic acid 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
4# SU10Z	Cronobacter spp. in 10g Method: ISO 22964:2017				
	Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	Cronobacter spp	**Not Detected**	/10 g		
4# SU1A2	Aerobic plate count Method: US FDA BAM Chapter 3, Jan 2001				
	Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Aerobic Plate Count	**<10**	cfu/g		
4 SU1A4	Salmonella Method: US FDA BAM Chapter 5, 2021				
	Accreditation: ISO/IEC 17025:2017 CNAS L3788				
	Salmonella	**Not Detected**	/25 g		
4# SU1A7	Yeasts and moulds Method: US FDA BAM Chapter 18, Apr 2001				
	Accreditation: DAkkS: D-PL-14292-01-00 & CNAS: L3788				
	Moulds	**<10**	cfu/g		
	Yeast	**<10**	cfu/g		
4# SU1CX	E.coli Method: ISO 16649-3:2015				
	Accreditation: DAKKS:D-PL-14292-01-00&CMA:211020342268&CNAS:L3788				
	E. coli	**Not Detected**	/25 g		

SIGNATURE

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Analytical Report

Sample Code	502-2022-00037070	Report date	30-Apr-2022
Certificate No.	AR-22-SU-033318-02		

This report is translated from report AR-22-SU-033318-01

Runke Bioengineering (Fujian) Co.,Ltd.
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Zhangzhou City Fujian Province
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Our reference:	502-2022-00037070/ AR-22-SU-033318-02
Client Sample Code:	样品批号：11012336 生产日期： 2021.10.12
Sample described as:	Arachidonic acid oil /Arachidonic acid 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 Condition	Other
Sample Weight	280g

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

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Tracy Li
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Analytical Report

Certificate No.	AR-23-SU-007406-02	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-007406-01

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

Sample Code:	502-2022-00063743
Client Sample Code:	批号：11004332 生产日期：2021.10.04
Sample described as:	Arachidonic acid oil /Arachidonic acid oil
Sample Packaging:	Sealed metal bottle
Arrival Temperature (°C)	26.2
Sample Weight	100g*2
Sample Condition	Other

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

Sample Code:	502-2023-00005402
Client Sample Code:	批号：11004332 生产日期：2021.10.04
Sample described as:	Arachidonic acid oil /Arachidonic acid oil
Sample Packaging:	Sealed metal can
Arrival Temperature (°C)	18
Sample Weight	140g
Sample Condition	Other

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

SIGNATURE

Ally Dong
Authorized Signatory

Jack He
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Certificate No.	AR-23-SU-007407-02	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-007407-01

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

Sample Code:	502-2022-00063744		
Client Sample Code:	批号：11008334 生产日期：2021.10.08		
Sample described as:	Arachidonic acid oil /Arachidonic acid oil		
Sample Packaging:	Sealed metal bottle		
Arrival Temperature (°C)	26.2	Sample Weight	100g*2
Sample Condition	Other		

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

Sample Code:	502-2023-00005403		
Client Sample Code:	批号：11008334 生产日期：2021.10.08		
Sample described as:	Arachidonic acid oil /Arachidonic acid oil		
Sample Packaging:	Sealed metal can		
Arrival Temperature (°C)	18	Sample Weight	140g
Sample Condition	Other		

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

SIGNATURE

Ally Dong
Authorized Signatory

Jack He
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Certificate No.	AR-23-SU-007408-02	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-007408-01

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

Sample Code:	502-2022-00063745		
Client Sample Code:	批号：11012336 生产日期：2021.10.12		
Sample described as:	Arachidonic acid oil /Arachidonic acid oil		
Sample Packaging:	Sealed metal bottle		
Arrival Temperature (°C)	26.2	Sample Weight	100g*2
Sample Condition	Other		

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

Sample Code:	502-2023-00005404		
Client Sample Code:	批号：11012336 生产日期：2021.10.12		
Sample described as:	Arachidonic acid oil /Arachidonic acid oil		
Sample Packaging:	Sealed metal can		
Arrival Temperature (°C)	18	Sample Weight	140g
Sample Condition	Other		

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

SIGNATURE

Ally Dong
Authorized Signatory

Jack He
Authorized Signatory

EXPLANATORY NOTE

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Analytical Report

Sample Code	502-2022-00039299	Report date 03-Jul-2022
Certificate No.	AR-22-SU-056888-02	

This report is translated from report AR-22-SU-056888-01

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

Our reference:	502-2022-00039299/ AR-22-SU-056888-02
Client Sample Code:	样品批号：11004332 生产日期： 2021.10.04
Sample described as:	Arachidonic acid oil /Arachidonic acid 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
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Analytical Report

Sample Code	502-2022-00039300	Report date	03-Jul-2022
Certificate No.	AR-22-SU-056889-02		

This report is translated from report AR-22-SU-056889-01

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

Our reference:	502-2022-00039300/ AR-22-SU-056889-02
Client Sample Code:	样品批号：11008334 生产日期： 2021.10.08
Sample described as:	Arachidonic acid oil /Arachidonic acid 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
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Analytical Report

Sample Code	**502-2022-00039301**	**Report date**	**03-Jul-2022**
Certificate No.	**AR-22-SU-056890-02**		

This report is translated from report AR-22-SU-056890-01

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

Our reference:	502-2022-00039301/ AR-22-SU-056890-02
Client Sample Code:	样品批号：11012336 生产日期：2021.10.12
Sample described as:	Arachidonic acid oil /Arachidonic acid oil
Sample reception date:	28-Apr-2022
Analysis Starting Date:	28-Apr-2022
Analysis Ending Date:	01-Jul-2022

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

SIGNATURE

Lucy Liu
Authorized Signatory

EXPLANATORY NOTE

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< LOQ: Below Limit of Quantification
N/A means Not applicable
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Appendix C. Sterols of ARA-rich Oil

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深圳大學高等研究院

Detected by Miss Xia Wang
Shenzhen Key Laboratory for Marine Microbiome Engineering
Institute for Advanced Study
Shenzhen University
Nanshan District Shenzhen,Guangdong,China 518060

Testing Report

1 Chemicals and reagents

The sterols campesterol, lanosterol, sitosterol (β-sitosterol), 24-methylene cholesterol, desmosterol, and zymosterol and the internal standard 6-Ketocholestanol were purchased on the market. LC-MS grade formic acid and HPLC-grade methanol were purchased from Supelco®, Merck, German. Deionized water was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA).

2. Sterol extraction

Fifty mg ARA oil was spiked with 2000 ng 6-ketocholestanol in a 15-mL explosion proof bottle and extracted with 10 mL absolute ethanol. After shaking for 2 min, the extraction mixture was heated at 95℃ by water bath for 30 min and cooled to room temperature, then 2-mL extract solution was centrifuged at 8000 rpm for 5 min.

3. Analysis

Separation, identification and quantification of sterols were performed with a coupled liquid chromatography-tandem mass spectrometry system consisting of an Acquity Ultra-performanceTM liquid chromatography H-Class and Plus-Xevo TQ-XS tandem mass spectrometer equipped with an APCI source (Waters, USA). The chromatographic analysis was performed on a BEH C18 column (50×2.1 mm, 1.7μm). The flow rate was 0.4 mL·min-1. The gradient was a linear gradient from 10% solvent B (0.1% (v/v) aqueous formic acid) to 100% solvent A (methanol) over a 2 min period. Acquity UPLC system was coupled to a TQS mass spectrometer operated in APCI modes. Quantification was performed using the multiple reaction monitoring (MRM) mode to monitor the precursor-product ion transitions of sterols. The general mass spectrometry conditions were as follows: Corona pin voltage: 2.0 kV; desolvent gas flow: 1000 L/Hr; cone gas flow: 150 L/Hr; collision gas flow: 0.17mL/ min, MRM and SIM as two detection mode, retention time of target compounds, cone hole voltage, and collision energy are shown in Table 1.

1

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Table 1. The MS parameters of the sterols

Detection Mode	compound	Retention time (min)	Parent ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (ev)
MRM	Zymosterol	3.89	367.42	81.15	20	34
			367.42	95.14	20	30
	Lanosterol	5.15	409.47	95.14	2	28
			409.47	191.24	2	14
	β-Sitoesterol	6.00	397.47	147.14	14	24
			397.47	161.19	14	20
	Campesterol	5.50	383.46	147.19	4	22
			383.46	161.18	4	20
	24-methylene Cholesterol	4.41	381.44	95.14	4	28
			381.44	147.19	4	26
	Desmosterol	4.05	367.42	81.15	2	30
			367.42	95.14	2	28
SIM	Cholesta-5,25-3β-ol	4.20*	367.42	-	2	-
	4α-methyl Zymosterol	4.30*	381.35	-	4	-
	24-methyl cholesta-5,24(25)-dien-3β-ol	4.70*	381.35	-	4	-
	24α-methyl cholesta-5,25-dien-3β-ol	4.40*	381.35	-	4	
	24β-methyl cholesta-5,25-dien-3β-ol	4.23*	381.35	-	4	-
	24,25-methylene cholesta -5-en-3β-ol	4.92*	381.35	-	4	-
	31-Norlanosterol	4.40*	395.36	-	2	-

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4. Test results of ARA oil samples from Runke

Table 3. Types and concentrations of sterols in ARA oil

Sterols	Sample Number		
	11004332	**11008334**	**11012336**
Sterol concentration showns as average value ± standard deviation (g/100g oil)			
[a]4α-Methyl zymosterol	-[c]	-[c]	-[c]
[a]24-Methyl cholesta-5,24(25 or 28)-dien-3β-ol C28:2	-[c]	-[c]	-[c]
[a]24-methyl cholesta-5,24(25)-dien-3β-ol	0.0077 ± 0.0024	0.0078 ± 0.0023	0.0088 ± 0.0012
[a]24-methyl choesta-5,25-dien-3β-ol C28:2	-[c]	-[c]	-[c]
[a]24-methyl cholesta-5(25)27-dien-3β-ol	-[c]	-[c]	-[c]
[b]24-Methylene cholesterol	0.0044 ± 0.0005	0.0041 ± 0.0002	0.0040 ± 0.0004
[a]24,25-methylene cholesta-5-en-3β-ol	-[c]	-[c]	-[c]
[a]31-Norlanosterol	-[d]	-[d]	-[d]
[b]Campesterol	0.0071 ± 0.0003	0.0072 ± 0.0004	0.0059 ± 0.0007
[a]Cholesta-5,25-dien-3β-ol	-[e]	-[e]	-[e]
[b]Desmosterol	0.6290 ± 0.0149	0.7453 ± 0.0319	0.8282 ± 0.0105
[b]Lanosterol	0.0149 ± 0.0012	0.0141 ± 0.0017	0.0122 ± 0.0021
[b]Sitosterol	0.0279 ± 0.0022	0.0257 ± 0.0017	0.0171 ± 0.0021
[b]Zymosterol	-[f]	-[f]	-[f]
Unidentified Sterols	-	-	-
Total Sterols (g/100 g oil) (average ± standard deviation, number of batches indicated)	0.6978 ± 0.0160 (n=4)	0.8043 ± 0.0301 (n=4)	0.8763 ± 0.0127 (n=4)

"[a]"MRM; "[b]"SIM; "-[c]"analyte concentration was below the instrument detection limit of 6.25×10^{-7} g/100g, "-[d]"analyte concentration was below the instrument detection limit of 5.00×10^{-7} g/100g, "-[e]"analyte concentration was below the instrument detection limit of 1.00×10^{-6} g/100g, "-[f]"analyte concentration was below the instrument detection limit of 2.50×10^{-6} g/100g.

Appendix D. 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 arachidonic acid (ARA)-rich oil 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), Matthew L. Tripp, Ph.D. (MattTrippScience Consulting), and Susan S. Cho, Ph.D. (AceOne RS, Inc.).

The Expert Panel, independently and collectively, critically evaluated the scientific information and data compiled from the literature. The Expert Panel evaluated other information deemed appropriate or necessary.

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 ARA as an ingredient in infant formulas. The FDA has issued ‘no question’ letters on previous GRAS notices (GRNs 000041, 000080, 000094, 000326, 000730, 000963, and 001115) related to food uses of ARA-rich oil derived from *M. alpina* for infant formula applications. Based on a comparison of the specifications of these products, it is concluded that ARA-rich oil in this GRAS determination is substantially equivalent to the other ARA-rich oil ingredients described in the FDA GRAS notices; thus, it is recognized that the information and data in the other GRAS notices are pertinent to the safety of the ARA-rich oil in this GRAS determination.

The Expert Panel agrees that there are adequate data in the scientific literature to conclude that ARA is a common component of infant formulas, that various ARA-rich oil

ingredients 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

Arachidonic acid is a long chain polyunsaturated fatty acid (LCPUFA) that is a primary structural component of the human brain, retina, and other tissues. Arachidonic acid is a carboxylic acid with a 20-carbon chain and four cis-double bonds; the first double bond is located at the sixth carbon from the omega end. Thus, it is classified as an omega-6 fatty acid (FA).

Human milk provides small quantities of ARA and docosahexaenoic acid (DHA): ARA concentrations ranged from 0.30 to 1.22% of total FAs (Brenna et al., 2007). The mean ARA content of American women's milk ranged from 0.40 to 0.67% of total FAs (Brenna et al., 2007; Bopp et al., 2005; Jensen et al., 2005). Arachidonic acid content in colostrum tends to be higher (usually by 50%) than that of mature milk.

The intended use of ARA-rich oil is to provide a source of ARA in infant formula at a concentration consistent with that of human milk. The ARA content of human milk varies from 0.34-1.22% of total fatty acids (FAs) among different populations. Therefore, the proposed use of ARA-rich oil is to provide 0.75% and 0.50% ARA by weight of FAs in term and pre-term infant formulas, respectively, in combination with a safe and suitable source of docosahexaenoic acid (DHA). Intended use levels are consistent with recommendations by Koletzko et al. (2014a; 2014b; 2020).

Runke Bioengineering intends to market the ARA-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 dairy such as bovine or goat milk-based; ages from birth to 12 months) in combination with a safe and suitable source of DHA. Exempt infant formula refers to formulas for pre-term infants only and does not include use in other exempt formulas (e.g., hypoallergenic formulas, and formulas for inborn errors of metabolism). The ratios of ARA:DHA are expected to be in the range of 2:1-1:1. Runke Bioengineering's ARA-rich oil will be added to ready-to-drink or powder form of infant formulas from which reconstituted infant formulas can be prepared. For term infants, the intended use level (0.75% FAs as ARA) is similar to all other approved uses for incorporation of ARA-rich oil in infant formula (GRNs 000041, 000080,

000094, 000326, 000730, 000963, and 001115). For pre-term infants, the intended use level in this GRAS determination is up to 0.5% in exempt infant formulas. The use level in non-exempt formulas in this GRAS determination is slightly higher than that described in previous GRAS notices (0.5% vs. 0.4%). Intended use levels are consistent with recommendations by Koletzko et al. (2014a; 2014b; 2020).

Runke Bioengineering's ARA-rich oil is produced by a fermentative process using the non-toxigenic, non-pathogenic *Mortierella alpina* strain FJRK-MA01. The organisms are grown in a pure culture heterotrophic fermentation process, recovered from the fermentation broth, and dried. The resulting dried algae are extracted with hexane to produce a crude oil that is further refined, decolorized, and deodorized using processes commonly employed in the vegetable oil industry. 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 rigorously tests its final production batches to verify adherence to quality control specifications. Based on certificates of analysis (COAs) consistent with the food-grade oil industry, the Expert Panel concluded that Runke Bioengineering's ARA-rich oil meets specifications for chemical identity, FA profile, and contaminants (heavy metals and microorganisms) and is free of contaminants such as residual hexane, monochloropropanediols (MCPDs), and glycidyl esters.

Product specifications are set for ARA content, acid value, free fatty acids, anisidine value, peroxide value, moisture and volatiles, unsaponifiables, residual hexane, heavy metals, and microbiological parameters. Specifications for Runke Bioengineering's ARA-rich oil are similar to those described in the previous GRAS notices (Runke Bioengineering's, ≥38%; ≥40% in GRN 000326 and 000094; 38-44% in GRNs 000080 and 000041). In addition, the FA profile of Runke Bioengineering's ARA-rich oil is similar to those described in previous GRAS notices. The data indicate that Runke Bioengineering's ARA-rich oil is substantially equivalent to existing ARA-rich oil ingredients that have been the subject of previous GRAS determinations (GRNs 000326, 000094, 000080, and 000041). Thus, it is recognized that the information and data in the other GRAS notices are pertinent to the safety of the ARA-rich oil in this GRAS determination. The safety and metabolism studies discussed in previous GRNs are as follows: GRN 000963, pages 25-33 (FDA, 2021); GRN 000730, pages 29-44 (FDA, 2018); GRN 000326, pages 61-153 (FDA, 2010); GRN 000094, pages 78-318 (FDA, 2006); GRN 000080, stamped pages 16-23 and 48-55 (FDA, 2001b); GRN 000041, stamped pages 108-118 and 175-418 (FDA, 2001a).

The major sterols associated with *M. alpina* oil include desmosterol and 24-methyl sterols. In Runke Bioengineering's ARA-rich oil, brassicasterol (24-methyl cholest-5,22-dien-3β-ol) is the most abundant phytosterol (1.21 g/100 g oil), followed by desmosterol (0.734 g/100 g oil). Total sterols were calculated to be 2.26 g/100 g oil. The estimated daily intakes (EDIs) of sterols were calculated as 2.5 mg/kg bw/day for term infants and 2.0 mg/kg bw/day for pre-term infants. These intakes are below the amounts of sterols already consumed as natural constituents in the infant formulas as 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). Sterols are components of many oil-containing foods and sterols in ARA-rich oils are not expected to pose any safety concerns.

Studies indicate that infants may not synthesize sufficient amounts of ARA and DHA *de novo* from their precursors to cover the high demand during this period of rapid accretion for normal growth and development. It is known that pre-term birth, which curtails the maternal supply of ARA and DHA to the fetus, is associated with sub-optimal neural and visual development, which can be improved by providing exogenous ARA and DHA (Kremmyda et al., 2011). After delivery, the premature infant becomes dependent on external sources for its nutritional requirements due to the shorter period and lesser extent of intrauterine LCPUFA accumulation. In addition, the infant may have a limited ability to convert essential precursor FAs, linoleic acid (18:2n-6) to ARA and linolenic acid (18:3n-3) to DHA, due to reduced concentrations and activity of desaturase enzymes (Martin et al., 2011). Thus, pre-term infants should have higher postnatal LCPUFA requirements than full-term infants, although ARA supplementation can benefit both term and pre-term infants.

Mutagenicity and Genotoxicity Studies of Runke Bioengineering's ARA-Rich Oil, the Subject of This GRAS Determination

In a study by Lewis et al. (2016), Runke Bioengineering's ARA-rich oil from *M. alpina* was found to be non-mutagenic and non-genotoxic under the test conditions.

Pivotal Animal Toxicity Studies of Runke Bioengineering's ARA-Rich Oil, the Subject of This GRAS Determination

In both a 90-day oral toxicity study in rats (Lewis et al., 2016) and a reproductive and developmental toxicity study in rats (Falk et al., 2017), the No-Observed-Adverse-Effect-

Level (NOAEL) of Runke Bioengineering's ARA-rich oil (purity, ~40.3%) was determined to be 5,000 mg/kg bw/day, the highest dose tested in rats.

Corroborative Animal Toxicity Studies of Other Sources of ARA-Rich Oil

The NOAELs of ARA-rich oil determined from subchronic toxicity studies with an in-utero exposure ranged from 970 to 4,850 mg/kg bw/day (Casterton et al., 2009; Gao et al., 2014; Hempenius et al., 2000 Lina et al., 2006) and that determined from a teratogenicity study was 2,500 mg/kg bw/day in rats (Arterburn et al., 2000). Neonatal piglet studies showed that approximately 620 mg ARA-rich oil/kg bw/day or 1.0% of total FAs as ARA were safe (Merritt et al., 2003; Tyburczy et al., 2012). In addition, a study by Tyburczy et al. (2011) established the bioequivalence of three sources of ARA-rich oils (ARASCO® from DSM/Martek, SUNTGA40S® from Nippon Suisan Kaisha, Ltd., and RAO from Cargill). These studies were also discussed in GRN 000963 (pages 30-32), GRN 000730 (pages 31–35), and GRN 000326 (pages 149-153).

Based on the above-listed studies, for purposes of safety evaluation, a NOAEL of 5,000 mg/kg bw/day was chosen for Runke Bioengineering's ARA-rich oil and 2,000 mg/kg bw/day for ARA in rats (Falk et al., 2017; Lewis et al., 2016). The NOAEL of 2,000 mg ARA/kg bw/day may represent approximately 50-60 times the infant intake of ARA under the intended use. However, subchronic toxicity studies with in-utero exposure suggest the NOAELs of other sources of ARA-rich oil products range from 970 (Hempenius et al., 2000) to 4,850 mg/kg bw/day in rats (Gao et a., 2014).

Human Clinical Studies of ARA-Rich Oil: Pre-term Infants

The studies by Clandinin et al. (2005), Carnielli et al. (2007), and Clandinin et al. (1997), employing 0.64%, 0.84%, and up to 1.1% FAs as ARA (corresponding to 43, 56, and 74 mg ARA/kg bw/day), respectively, did not report any adverse effects of ARA supplementation to infant formulas. Runke is intended to use 0.5% of total fat as ARA (corresponding to 33.4 mg ARA/kg bw/day) for pre-term infants. No studies found adverse effects of ARA supplementation even at 0.5%-1.1% of total FAs in pre-term infants. An intended use level of up to 0.5% FAs as ARA (or 33-34 mg ARA/kg bw/day) in pre-term infants are within safe intake levels found from clinical studies in pre-term infants.

In addition, an intended use level of up to 0.5% FAs as ARA (or 33-34 mg ARA/kg bw/day) in pre-term infants is consistent with current ARA recommendations: 18-45 mg/kg

bw/day, preferably high intakes of 35–45 mg ARA/kg bw/day (approximately 0.6–0.75% of the total FA intake), for very low birth weight pre-term infants (Koletzko et al., 2014a).

Human Clinical Studies of ARA-Rich Oil: Term Infants

Since the FDA's review in 2022-2023, no new intervention studies were published. However, a meta-analysis by Adjibade et al. (2022) reported no adverse association between the consumption of LCPUFA-enriched formula and the risk of infection and allergy. Term infants receiving different dosages of ARA (0.64–0.72% of total FAs) and DHA (0.32–0.36% of total FAs) from 1–9 days of life until up to 12 months of age did not have adverse effects on allergies, gastrointestinal symptoms, or growth associated with ARA/DHA-supplemented infant formula (Birch et al., 2005, 2007, 2010; Burks et al., 2008; Hoffman et al., 2008).

Consumer Reports

Findings from intervention studies are further supported by the safe history of use of ARA from fungal oil in infant formula. The FDA analyzed the Center for Food Safety and Applied Nutrition (CFSAN)'s Adverse Event Reporting System (CAERS) data to find any correlation between the gastrointestinal AEs and the use of DHA and ARA oils in infant formulas (FDA, 2011; FDA Docket No. 2008-P-0074-0017).

In conclusion, ARA-rich oil, combined with a safe and suitable source of DHA, is not expected to adversely impact the pre-term and term infants who would be consuming exempt and non-exempt infant formula, respectively.

Conclusion

We, the undersigned members of the Expert Panel, have individually, collectively, and critically evaluated the materials summarized above on the safety of Runke Bioengineering's ARA-rich oil and other information deemed appropriate and unanimously conclude that Runke Bioengineering's ARA-rich oil, manufactured as described in the dossier and consistent with current Good Manufacturing Practice (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 at levels specified in the accompanying dossier. It is our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions.

Expert Panel Members:

[redacted]

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

3/22/24
Date

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Matthew L. Tripp, Ph.D.
MattTrippScience Consulting

3/21/2024
Date

[redacted]

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

3/22/2024
Date

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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
001186	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 S. Cho	Chief Science Officer

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

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

City	State or Province	Zip Code/Postal Code	Country
Centreville	Virginia	21021	United States of America

Telephone Number	Fax Number	E-Mail Address
3018756454		susanscho1@yahoo.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Arachidonic acid-rich oil from Mortierella alpina FJRK-MA01

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 000326

☐ 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.

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 dairy such as bovine or goat milk-based; ages from birth to 12 months) in combination with a safe and suitable source of DHA. Exempt infant formula refers to formulas for pre-term infants only and does not include use in other exempt formulas (e.g., hypoallergenic formulas, and formulas for inborn errors of metabolism). The proposed use of ARA-rich oil is to provide 0.75% and 0.50% ARA by weight of FAs in term and pre-term infant formulas, respectively, in combination with a safe and suitable source of docosahexaenoic acid (DHA).

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 Arachidonic acid-rich oil from Mortierella alpina FJRK-MA01
(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. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

AceOne RS, Suite 313, 14631 Route 29, Centreville, VA 21021
(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 09:08:28 -04'00'	Susan Cho, Chief Science Officer, Agent for Runke Bioengine	04/03/2024

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
	ARAcoverletter4-2-24.pdf	Administrative
	RunkeARAfinal4-2-24SubmittedtoFDA.pdf	Administrative