October 3, 2023

Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

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

To Whom It May Concern,

On behalf of Runke Bioengineering (Fujian) Co., Ltd. (Runke Bioengineering), we are submitting a GRAS notification for arachidonic acid (ARA)-rich oil as a food ingredient for use in infant formula. 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,

October 3, 2023

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 AS AN INGREDIENT FOR USE IN INFANT FORMULA

Prepared for Runke Bioengineering (Fujian) Co., Ltd.
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Prepared by:
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GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ARACHIDONIC ACID (ARA)-RICH OIL AS AN INGREDIENT FOR USE IN INFANT FORMULA

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List of Abbreviations

2-MCPD = 2-monochloropropanediol-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
AV = acid value
BAM = Bacteriological Analytical Manual
bw = body weight
CA =corrected age
CAERS = CFSAN Adverse Event Reporting system
CAS = Chemical Abstract Service
cfu = colony forming units
CHO = Chinese hamster ovary
COA = Certificate of Analysis
cGMP = current Good Manufacturing Practice
CGMCC = China General Microbiological Culture Collection Center
cGMP = current Good Manufacturing Practice
DHA = docosahexaenoic acid
DIAMOND = DHA Intake and Measurement of Neural Development
DINO = DHA for the Improvement of Neurodevelopmental Outcome in Preterm Infants
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
GGT = gamma-glutamyl transferase
GMO = genetically modified organism
GRAS = Generally Recognized as Safe
h = hour

HACCP = Hazard Analysis and Critical Control Point
ICU = intensive care unit
IGF-1 = insulin growth factor-1
IMCAS = Institute of Microbiology Chinese Academy of Sciences
IQ = intelligence quotient
ISO = International Standardization Organization
KCl = potassium chloride
LCPUFA = long-chain polyunsaturated fatty acid
LD50 = lethal dose (50%)
LDH = lactate dehydrogenase
MCHC = mean corpuscular hemoglobin concentration
MCPD= monochloropropane-1,2-diol
MCV = mean corpuscular volume
MNPCE = micronucleated polychromatic erythrocyte
MPV = mean platelet volume
NA = not available
NOAEL = No Observed Adverse Effect Level
OECD = Organization of Economic Cooperation and Development
PCE = polychromatic erythrocytes
PMA = post-menstrual age
PT = prothrombin time
PUFA = polyunsaturated fatty acid
PV = peroxide value
QC = quality control
RAO = refined arachidonic acid-rich oil
RBC = red blood cell
ROP = retinopathy prematurity
SCORAD = SCORing Atopic Dermatitis
SD = standard deviation
SDH = sorbitol dehydrogenase
TG = triglycerides
TK = thymidine kinase
USDA = United States Department of Agriculture
VLBW = very low birth weight
WBC = white blood cell
yr = year

PART 1. SIGNED STATEMENTS AND A CERTIFICATION

1.A. Submission of GRAS Notice

Pursuant to 21 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, ARA, ARA-rich oil, ARA-rich oil derived from *Mortierella alpina* FJRK-MA01.

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

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

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

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 preterm 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 preterm infant formula. 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. Purpose for Which the Substance will be Used

Runke Bioengineering intends to market the ARA-rich oil as an ingredient in exempt (preterm 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.4. Description of the Population Expected to Consume the Substance

The population expected to consume the substance consists of preterm 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: September 3, 2023
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. 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, ARA-rich oil, arachidonic acid, ARA-rich oil from *Mortierella alpina* (*M. alpina*), 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)

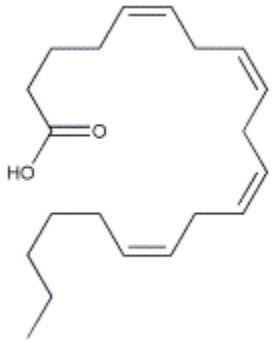
2.A.1.3. Chemical Abstract Service (CAS) Registry Number
ARA: 506-32-1

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 preterm 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 preterm 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 (FSANZ, 2003; Hadley et al., 2016).

2.A.2. Potential Toxicants in the 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-monochloropropanediol-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 MCPD 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, MCPD, or glycidyl esters in Runke Bioengineering's ARA-rich oil.

Table 1. Residual Solvents Tested for 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

	Limit of Quantitation	11004332	11008334	11012336	Methods of Analysis
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; 2-MCPD = 2-monochloropropanediol-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 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 inoculum 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 the stability. The product is packaged in airtight containers.

The ARA-rich oil is manufactured in adherence with current Good Manufacturing Practice (cGMP) to meet 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. 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	Regulatory status
Fermentation medium		
Glucose [dextrose and glucose]	50-99-7	21CFR 168.120 [21CFR 184.1866]
Yeast extract	8013-01-2	21 CFR 184.1983
Sunflower seed oil	8001-21-6	GRAS per 21 CFR 170.30
Magnesium sulfate (heptahydrate)	10034-99-8	21 CFR 184.1443
Potassium dihydrogen phosphate	7778-77-0	No 21 CFR status
Potassium chloride	7447-40-7	21 CFR 184.1622
Sodium hydroxide	1310-73-2	21 CFR 184.1763
Processing aids		
Ascorbyl palmitate	137-66-6	21CFR 182.3149
Tocopherols	10191-41-0; 1406-18-4	21CFR 182.3890
Citric acid monohydrate	5959-29-1	21CFR 184.1033
Sodium hydroxide	1310-73-2	21CFR 184.1763
Sodium sulfate	7757-82-6	21CFR 186.1797
Potassium hydroxide	1310-58-3	21CFR 184.1631
Activated clay (bentonite)	1302-78-9; 68333-91-5	21CFR 184.1155
Hexane	110-54-3	21CFR 173.270

Abbreviations: CAS = Chemical Abstract Service

1) *Mortierella alpina*
   ARA-rich oil is produced via a multi-step fermentation and refinement process using the non-modified, wild type soil fungus *M. alpina*. The 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).

2) Culture medium
   The fermentation medium contains the following ingredients: glucose, yeast extract paste, magnesium sulfate ($MgSO_4 \cdot 7H_2O$), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH), and sunflower seed oil.

3) Hexane
   Hexane is used as a solvent for crude oil extraction from *M. alpina* biomass and as a processing aid during the refinement of the oil. As outlined in the manufacturing process, crude ARA-rich oil is extracted from the fermentation biomass using hexane, which is subsequently removed by vacuum distillation. No traces of hexane (< 0.5 mg/kg) were detected in 3 non-consecutive lots of ARA-rich oil using gas chromatography headspace analysis (AOCS Cg 4-94).

4) Degumming acids
   An aqueous solution of sodium hydroxide or sodium sulfate, meeting appropriate food-grade specifications, is used as a degumming agent in the manufacturing process of ARA-rich oil.

5) Neutralizing agent
   Dilute aqueous solutions of sodium hydroxide or sodium sulfate, meeting appropriate food-grade specifications, are used to remove any free FAs in the manufacturing process of ARA-rich oil.

6) Bleaching agent
   Bleaching clay and activated carbon, of appropriate food-grade specifications, are used as bleaching agents during the refinement of crude ARA-rich oil.

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

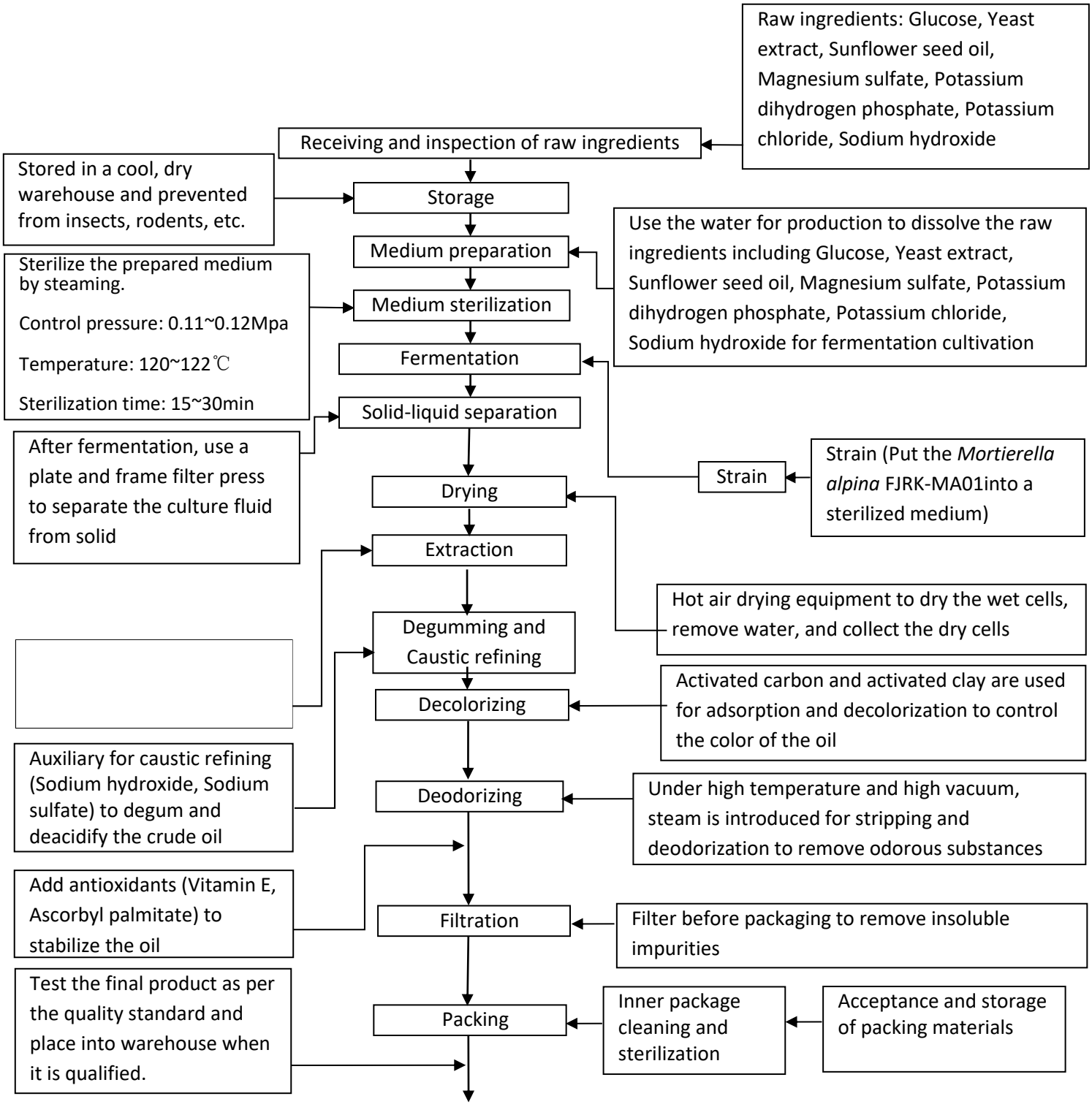


Figure 2. Manufacturing Flow Diagram of ARA-rich Oil

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 (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. 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 (AV), free FAs, unsaponifiables, anisidine value, peroxide value (PV), 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 (≥38% for Runke Bioengineering's; ≥40% in GRN 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 and 000041
ARA, C 20:4n6, relative %	≥38	≥40	≥40	38-44*
Acid value, mg KOH/g	≤0.5	≤1.0	NA	NA
Free fatty acids, %	≤0.4	≤0.2	≤0.2	<0.4
Unsaponifiable matter, %	≤3.0	≤3.0	<1.0	<3.5
Anisidine value	≤20	≤20	NA	NA
Peroxide value, meq/kg	<5.0	≤2.0	<5.0	<5.0
Residual hexane, mg/kg	≤1.0	≤1.0	NA	NA
Mercury (Hg), mg/kg	≤0.05	≤0.05	<0.5	<0.2
Lead (Pb), mg/kg	≤0.1	NA	<0.1	<0.2
Arsenic (As), mg/kg	≤0.1		<0.2	<0.5
Cadmium (Cd), mg/kg	≤0.1		NA	NA
Moisture and volatile matter content, g/100 g	≤0.1	≤0.1	NA	NA
Coliforms, cfu/g	≤1	≤3	NA	NA
Molds, cfu/g	≤10	≤10	NA	NA
Yeast, cfu/g	≤10	≤10	NA	NA
Salmonella, /25 g	Not Detected	NA	NA	NA

*Specifications for other fatty acids are included.
Abbreviations: cfu = colony forming units

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.

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

Parameters	Batch Number			Mean	Method of analysis
	11004332	11008334	11012336		
ARA, C20:4n6, relative %	41.01	42.20	41.70	41.64	AOAC 996.06 mod
Acid value, mg KOH/g	0.29	0.28	0.29	0.29	AOCS Cd 3d-63
Free fatty acids, %	0.14	0.13	0.13	0.13	AOCS Ca 5a-40; AOAC 940.28
Free fatty acids (as oleic acid), %	0.15	0.14	0.15	0.15	AOCS Cd 3d-63
Unsaponifiable matter, %	1.56	1.56	1.51	1.54	AOCS Ca 6a-40
p-Anisidine value	5.7	5.1	4.9	5.2	AOCS Cd 18-90
Peroxide value, meq/kg	0.61	0.47	0.60	0.56	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 Chapter 3, 2001
Molds, cfu/g	<10	<10	<10	<10	US FDA BAM Chapter 18, 2001
Yeast, cfu/g	<10	<10	<10	<10	
Salmonella, /25 g	ND	ND	ND	ND	US FDA BAM Chapter 5, 2021
Enterobacteriaceae, cfu/g	<10	<10	<10	<10	ISO 21528-2-2017
Cronobacter spp, /10 g	ND	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; 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 (TG; 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

	Batch Number			
Parameters, %	11004332	11008334	11012336	Mean
C16:4 Hexadecatetraenoic acid	<0.02	<0.02	<0.02	<0.02
C10:0 Capric acid	<0.02	<0.02	<0.02	<0.02
C11:0 Undecanoic acid	<0.02	<0.02	<0.02	<0.02
C12:0 Lauric acid	<0.02	<0.02	<0.02	<0.02
C14:0 Myristic acid	0.29	0.31	0.30	0.30
C14:1 Myristoleic acid	<0.02	<0.02	<0.02	<0.02
C15:0 Pentadecanoic acid	0.10	0.09	0.10	0.10
C15:1 Pentadecenoic acid	<0.02	<0.02	<0.02	<0.02
C16:0 Palmitic acid	7.10	7.21	7.06	7.12
C16:1 Omega 7	0.17	0.18	0.17	0.17
C16:1 Total (Palmitoleic acid + isomers)	0.23	0.23	0.22	0.23
C16:2 Hexadecadienoic acid	<0.02	<0.02	<0.02	<0.02
C16:3 Hexadecatrienoic acid	<0.02	<0.02	<0.02	<0.02
C17:0 Margaric acid	0.25	0.26	0.26	0.26
C17:1 Heptadecenoic acid	0.03	0.03	0.03	0.03
C18:0 Stearic acid	7.26	7.73	7.43	7.47
C18:1 Vaccenic acid	0.35	0.37	0.35	0.36
C18:1 Omega 9 (oleic acid)	8.78	9.36	8.67	8.94
C18:1 Total (oleic acid + isomers)	9.24	9.87	9.14	9.42
C18:2 Omega 6 (linoleic acid)	12.18	13.34	11.91	12.48
C18:2 Total (linoleic acid + isomers)	12.54	13.79	12.26	12.86
C18:3 Omega 3 (alpha linolenic acid)	0.05	0.05	0.05	0.05
C18:3 Omega 6 (gamma linolenic acid)	2.25	2.18	2.18	2.20
C18:3 Total (linolenic acid + isomers)	2.29	2.24	2.23	2.25

C18:4 Omega 3 (octadecatetraenoic acid)	<0.02	<0.02	<0.02	<0.02
C18:4 Total (octadecatetraenoic acid)	<0.02	<0.02	<0.02	<0.02
C20:0 Arachidic acid	0.72	0.75	0.74	0.74
C20:1 Omega 9 (gondoic acid)	0.36	0.36	0.35	0.36
C20:1 Total (gondoic acid + isomers)	0.39	0.39	0.40	0.39
C20:2 Omega 6	0.50	0.52	0.49	0.50
C20:2 Total (eicosadienoic acid)	0.50	0.52	0.49	0.50
C20:3 Omega 3	0.14	0.15	0.12	0.14
C20:3 Omega 6	1.92	1.90	1.87	1.90
C20:3 Total (eicosatrienoic acid)	2.07	2.04	1.99	2.03
C20:4 Omega 3	<0.02	<0.02	<0.02	<0.02
C20:4 Omega 6 (arachidonic acid)	41.01	42.20	41.70	41.64
C20:4 Total (eicosatetraenoic acid)	41.03	42.20	41.71	41.65
C20:5 Omega 3 (eicosapentaenoic acid)	0.06	0.06	0.06	0.06
C21:5 Omega 3 (heneicosapentaenoic acid)	<0.02	<0.02	<0.02	<0.02
C22:0 Behenic acid	0.06	1.49	0.06	0.54
C22:1 Omega 9 (erucic acid)	<0.02	<0.02	<0.02	<0.02
C22:1 Total (erucic acid + isomers)	<0.02	<0.02	<0.02	<0.02
C22:2 Docosadienoic omega 6	0.03	0.04	0.03	0.03
C22:3 Docosatrienoic, omega 3	0.02	0.02	0.03	0.02
C22:4 Docosatetraenoic omega 6	0.20	0.22	0.21	0.21
C22:5 Docosapentaenoic omega 3	<0.02	<0.02	<0.02	<0.02
C22:5 Docosapentaenoic omega 6	0.10	0.06	0.08	0.08
C22:5 Total (docosapentaenoic acid)	0.10	0.06	0.08	0.08
C22:6 Docosahexaenoic omega 3	0.32	0.20	0.25	0.26
C24:0 Lignoceric acid	1.16	1.22	1.19	1.19
C24:1 Omega 9 (nervonic acid)	0.19	0.20	0.19	0.19
C24:1 Total (nervonic acid + isomers)	0.19	0.20	0.25	0.21
C4:0 Butyric acid	<0.02	<0.02	<0.02	<0.02
C6:0 Caproic acid	<0.02	<0.02	<0.02	<0.02
C8:0 Caprylic acid	<0.02	<0.02	<0.02	<0.02
Total fat as triglycerides	89.95	95.15	90.29	91.80
Total fatty acids	86.20	91.20	86.54	87.98
Total monounsaturated fatty acids	9.97	10.60	9.93	10.17
Total omega 3 isomers	0.60	0.49	0.52	0.54
Total omega 6 isomers	58.20	60.46	58.47	59.04
Total polyunsaturated fatty acids	59.09	61.34	59.29	59.91
Total saturated fatty acids	16.96	19.07	17.14	17.72
Total trans fatty acids	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	ND		
C 15:0 (Pentadecanoic acid)	0.10	0.09	0.17		
C 15:1 (Pentadecenoic acid)	<0.02		ND		
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		ND		
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	ND		
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	ND		
C 22-5n3 (Docosapentaenoic acid)	<0.02	ND	ND		
C 22-5n6 (Docosapentaenoic acid)	0.08	ND	ND	<0.01	
C 23:0 (Tricosanoic acid)			ND		

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	
C26:0		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 (page 26-27, stamped page 38-39).
GRN 000326, RAO, available from Cargill; Table 18 (pages 40-42, stamped pages 50-52).

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). Total sterols were calculated to be 2.26 g/100 g oil, which is slightly higher than those reported in GRN 00041/000080 and GRN 000963. Variations in samples and analytical methods may contribute to the differences. For example, desmosterol values were presented in the Shenzhen University report while reports by Eurofins did not report such values. On the other hand, 24-methyl cholest-5,22-dien-3β-ol (brassicasterol) values were included in the report by Eurofins, but not in the Shenzhen University report. 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 from current notice) and vice versa.

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).

Sterols are components of many oil containing foods, and sterols in ARA-rich oil are not expected to pose any safety concerns.

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

	Batch Number (Appendix B; Eurofins)			Batch Number (Appendix C; Sterol report)			
Parameters, g/100 g	11004 332	11008 334	11012 336	11004 332	11008 334	11012 336	Mean
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 GRNs 000041 and 000080 (pages 21-22, stamped pages 27-28), 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 GRN 000041 and 000080 (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 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, 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; the 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

	Average sterol content (g/100 g oil)				
Compound	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 batches)	0.79 (n=1)	0.21 (n=3)	0.98 (n=5)	1.57 (n-=6)

*Source: GRN00041 (ARASCO®), Table 8: N=2 for individual sterols, but N=1 for total sterols. 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 has been evaluated at -10°C and ≤25°C (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 to 36 months is expected under refrigerated and frozen conditions, respectively. The oil should be stored (also 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 Dietary 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 preterm 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 preterm infant formulas, respectively. The intended use of ARA-rich oil to deliver these concentrations of ARA, corresponds to 1.973% of total fat in non-exempt term infant formula and 1.32% of total fat in exempt preterm infant formula. 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) preterm and term infants consume 120 kcal/kg 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 000080 (term infants), GRN 000094 (preterm infants), and GRN 000326 (term and preterm infants, page 60, FDA, 2010), with updated recommendations to provide 0.5% ARA by weight of FAs for preterm infants. 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 preterm 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 (preterm 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 (preterm infants) of fat/kg body weight/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 =41.7 mg ARA/kg bw/day). 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 preterm 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 preterm 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 preterm low-, very low-, and extremely low- birth weight infants, respectively (Table 12). For example, daily ARA

intake/person/day in preterm low-birth weight infants would be 33.4 mg ARA/kg bw/day x 2.5 kg bw/person = 83.5 mg ARA/person/day).

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 preterm 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 preterm 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

l**nfants**	mg ARA/kg bw/day	mg ARA-rich oil/kg bw/day	mg ARA/ infant/day
Term infants	42	110	
Preterm 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.

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. 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.

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 DHA present in Runke Bioengineering's ARA-rich oil.

To calculate EDIs of sterols/person/day, 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 preterm infants using the following formulas: 1) Total sterols and ARA content present in 1 gram of Runke Bioengineering's ARA-rich oil is 22.6 mg and 380 mg, respectively, thus, the ratio of total sterols to ARA is approximately 1:16.8; and 2) ARA of 42 mg and 33.4 mg/kg bw/day for term infants and preterm infants, respectively (please see details in Part 3.A). Thus, to calculate the EDIs of sterols for term infants, EDIs of ARA (33.4 to 42 mg/kg bw/day) were divided by 16.8 to get the EDIs of sterols. 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 through the proposed uses of ARA-rich oil would not have an impact on the relative amount of sterols already consumed via infant formulas.

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 approved for term and preterm infants. The ARA concentrations in infant formula supplementation ranged from 0.4 to 0.75% of total FAs.

Table 13. Maximum ARA Use Concentrations in Infant Formulas

	ARA source	Infants	% of total fat as ARA	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
		Preterm, hospitalized	0.40	32.4
		Preterm, post-discharge	0.40	27.7
GRN 000326 (US FDA, 2010)	*M. alpina*	Preterm	0.40	27
		Term	0.75	42
GRN 000730 (US FDA, 2018)	*M. alpina*	Preterm	0.40	27
		Term	0.75	42
GRN 000963 (US FDA, 2021)	*M. alpina*	Preterm	0.40	27
		Term	0.75	42
Current notice	*M. alpina*	Preterm	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, and 000963) 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 ARA in 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 (GRN 000963, pages 25-33; GRN 000730, pages 29-44; GRN 000326, pages 61-153; GRN 000094, pages 78 - 318; GRN 000080, stamped pages 16-23 and 48-55; GRN 000041, stamped pages 108-118 and 175-418) and will not discuss previously reviewed references in detail. Additionally, this notice discusses additional animal and human studies that have been published since the FDA's last review in 2020-2021 (or in the period of July 2020 and May 2023). The subject of the present GRAS assessment is 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 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 PUFA present in the 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 preterm 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, LCPUFA 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 preterm 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 preterm infants.

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

Studies of Runke Bioengineering's ARA-Rich Oil

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).

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 *S. 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 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 hours 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 either 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. All doses were well tolerated, and no clinical signs were observed. 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. Summary of Studies Showing No Mutagenicity and Genotoxicity of Runke Bioengineering's ARA-rich Oil

Test	Test system	Concentration/dose of ARA-rich oil
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
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
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	0, 1,000, 2,500, and 5,000 mg/kg bw/day;

	collected at 24 h following the final dosing	

Reference, Lewis et al. (2016).
Abbreviation: ARA = arachidonic acid; CPA = cyclophosphamide; PCE = polychromatic erythrocytes.

The Studies Reviewed in Previous GRAS Notices

Due to an abundance of literature, studies published since 2000 are summarized in this review. 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.

Bacterial Reverse Mutation Assay

In the study of ARA-rich oil (48.5% ARA; source, ARASCO®, Martek/DSM) by Arterburn et al. (2000a), 5 strains of *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 were tested for mutagenicity. Test concentrations were 0, 100, 333, 1,000, 3,300, and 5,000 µg/plate of ARA-rich oil in the absence and presence of S9. ARA-rich oil did not cause an increase in the number of histidine revertants, either with or without metabolic activation in any of the tester stains. Thus, ARA-rich oil was considered non-mutagenic under the conditions of this assay.

In the study of ARA-rich oil (source, refined arachidonic acid-rich oil [RAO] manufactured by Cargill and Ankang Bioengineering) by Casterton et al. (2009), a standard plate incorporation method with *S. typhimurium* TA98, TA100, TA1535, and TA1537 and *E. coli* tester strain WP2 uvrA was used to evaluate the mutagenicity of ARA-rich oil (OECD 471). The test concentrations were 0, 62, 185, 556, 1,667 and 5,000 µg/plate in the absence and presence of S9. ARA-rich oil did not cause an increase in the number of revertants, either with or without metabolic activation, in any of the tester stains. Thus, ARA-rich oil was considered non-mutagenic under the conditions of this reverse mutation assay.

Mouse Lymphoma Forward Mutation Assay (Gene Mutation in the TK-locus)

Arterburn et al. (2000a) evaluated the ability of ARA-rich oil (ARASCO®) to induce gene mutations at the thymidine kinase (TK) locus in L5178 TK+/- mouse lymphoma cells. This assay detects a broader range of mutations (base-pair as well as frameshift mutations and small deletions) in a complex eukaryotic system. Mouse lymphoma L5178Y TK+/- cells were exposed to ARA-rich oil concentrations of 0, 748, 1,000, 2,000, 2,990, 3,990, and 4,990 µg/mL in the absence and presence of S9. The mutant frequency was calculated as the ratio of the total number of mutant colonies found in each of three mutant selection dishes to the total number of cells seeded, adjusted by the absolute cloning efficiency. The test substance would have been considered

mutagenic in the gene mutation test at the TK locus if a concentration-related increase in mutant frequency was noted at least twice the mean vehicle. Background mutant frequencies were within the historical control range, and positive controls exhibited large dose-dependent increases in mutant frequencies, meeting assay acceptance criteria. No dose-related increases in mutant frequencies were observed in the absence and presence of S9. The gene mutation assay demonstrated that ARA-rich oil was not mutagenic.

In a gene mutation assay with mouse lymphoma L5178Y cells at the TK locus conducted by Casterton et al. (2009) (OECD 476), L5178Y mouse lymphoma cells were treated in duplicate for 4 and 24 h, at ARA-rich oil concentrations of 0, 429, 858, 1,715, 3,500, or 5,000 µg/mL in the absence and presence of S9. The gene mutation assay demonstrated that ARA-rich oil was not mutagenic under the test conditions.

In-vitro Chromosomal Aberration Test

In a study by Arterburn et al. (2000a), Chinese hamster ovary (CHO) cells were exposed to ARA-rich oil at concentrations of 0, 1,260, 2,510, 3,760, or 5,010 µg/mL in the absence and presence of S9 (10 h harvest time). In this assay, chromosomal breaks, deletions, rearrangements, and translocations were scored. A test substance is considered to be negative in the chromosomal aberration test if it produces neither a dose-related increase in the number of structural chromosomal aberrations nor a reproducible positive response at any of the test points. The positive control resulted in a significant increase in chromosomal aberrations, indicating a valid assay. There were no significant increases in the actual number or percent of cells with aberrations, nor in the number of cells with more than one aberration at any of the doses of ARA-rich oil in the non-activation or activation cultures. Thus, ARA-rich oil was not clastogenic under the conditions of this assay.

In a study with a chromosome aberration assay in cultured CHO cells, ARA-rich oil of doses up to 5,000 ug/mL did not induce a dose-related increase in the number of structural chromosomal aberrations or a reproducible positive response at any of the test concentrations (Casterton et al., 2009). Thus, ARA doses up to 5,000 µg/mL were not genotoxic under the test conditions.

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 20).

6.B.3.1. Oral Toxicity Study of Runke Bioengineering's ARA-rich Oil

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 was no difference 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, urine chemistry parameters, and ophthalmological parameters. The 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 additional recovery groups of animals (vehicle control [corn oil] or 5000 mg/kg bw/day; n=10/sex/group; recovery group) were observed for an additional 14 days after a 90-day treatment of ARA or corn oil treatments. 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 15). Small changes were observed in some parameters (for example, mean corpuscular hemoglobin [MCHC] concentrations, 35 g/dL in oil vehicle control vs. 36 g/dL in male low-dose rats, $P<0.05$; white blood cells (WBC), 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.

Changes in clinical chemistry parameters were comparable to the controls, biologically insignificant, and not correlated with other toxicological findings (Table 16). The small increases in cholesterol and TGs in all ARA-rich oil groups of both sexes (averages of water and vehicle controls vs. 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), aspartate amino transferase (AST; 6% increase in high-dose males only; 6-11% increase in female test groups), and alkaline phosphatase (ALP; 3% increase in high-dose males only and 3-5% increase in all female test groups) 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 urine chemistry 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 17; 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 15. 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 (maybe a typo; It should have been 16.5)	16±1

From Lewis et al., 2016. Values are mean±SD for group of 20 rats treated for 90 days prior to sacrifice.
[a]p<0.05 vs. water control; [b]p<0.05 vs. vehicle control.
aPTT data for female: mid-dose value of 165 reported in the original research article (Lewis 2016) may be a typo.
Abbreviations: MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; PT = prothrombin time; aPTT = a partial thromboplastin time; RBC = red blood cell; SD = standard deviation; WBC = white blood cell

Table 16. Clinical 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 group of 20 rats treated for 90 days prior to sacrifice.
[a]p<0.05 vs water control; [b]p<0.05 vs vehicle control.
Abbreviations: ALP = alkaline phosphatase; ALT = alanine amino transferase; AST = aspartate amino transferase; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; SD = standard deviation; SDH = sorbitol dehydrogenase.

Table 17. 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 group of 20 rats treated for 90 days prior to sacrifice.

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 18-21). 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, and detailed examination were performed. 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 for 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, or malformed head (Table 18) in the skeletons among the groups (Table 19).

Table 18. 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/d			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
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	2 ± 2.1	1 ± 0.9	6 ± 5.4	4 ± 4
Dilated and fragile ventricles brain	3 ± 2.9	-	-	-	1 ± 1
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	1 ± 1.1	1 ± 0.9	2 ± 1.8	1 ± 1
Subcutaneous hemorrhage	1 ± 1	2 ± 2.1	-	-	1 ± 1
Yellowish perivascular areas liver	-	-	1 ± 0.9	-	-
Small or absent renal papillae	4 ± 4	4 ± 4.3	4 ± 3.6	7 ± 6.3	5 ± 5
Brownish discoloration lung	4 ± 3.9	2 ± 2.1	2 ± 1.8	4 ± 3.6	2 ± 3
Common Variants					
Dilated renal pelvis	2 ± 2	6 ± 6.4	5 ± 4.5	3 ± 2.7	1 ± 1

From Falk et al., 2017.

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

Table 19. Summary of Major Malformations and Minor Skeletal Variations in the Prenatal Developmental Toxicity Study

Item	Untreated	Corn Oil	ARA LD	ARA MD	ARA HD
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). Mean±(SD.

Abbreviations: 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 and 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 cohabitated 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 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 20. 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. Oral Toxicity Studies of Other Sources of ARA-rich Oil

Acute Toxicity Studies

Gao (2017) evaluated acute toxicity of ARA-rich oil (42.1% ARA) in rats. ARA-rich oil was administered to 10 young rats (5 males and 5 females) by oral gavage at the dosage of 15.2 g/kg bw (GRN 000730, page 31). Water control and vehicle control (sunflower oil) groups were included. Animals were observed for 14 days to monitor changes in body weight, clinical signs, and food consumption. At the end of the study, all surviving animals were sacrificed, and major organs were examined. No animal died during the 14-day observation period and no clinical signs of abnormality were observed at the dose of 15.2 g/kg bw (Table 21). Furthermore, no significant differences in mean body weight, food consumption, or organ weights were found among the test and control groups. No treatment-related abnormalities were observed upon macroscopic examinations of the organs. The author found that the mean lethal dose (LD_{50}) of ARA-rich oil was far above 15.2 g/kg bw.

Similarly, Hempenius et al. (1997) reported that LD50 of ARA-rich oil was above 18.2 g/kg bw in Wistar rats. The data suggest that ARA-rich oil is 'relatively harmless' (Altug, 2003).

Subchronic Toxicity Studies with an In-utero Exposure

Four 90-day subchronic toxicity studies with an in-utero exposure of ARA-rich oil (Casterton et al., 2009; Gao et al., 2014; Hempenius et al., 2000; Lina et al., 2006) are summarized below (Table 21).

<u>The Study by Hempenius et al. (2000)</u>

Hempenius et al. (2000) evaluated the safety of ARA-rich oil (38.6% ARA) derived from *M. alpina* (source, NA) in a subchronic oral toxicity study in rats that was preceded by an in-utero exposure phase. During the in-utero phase, Wistar (Crl:(WI)WU BR) rats aged 10-11 weeks old (14 males and 28 females per group except 7 males and 14 females in the low-fat control group) received a standard diet (carrier control), a high-fat diet with corn oil, diets with 0.3, 1.5, or 7.5% ARA-rich oil, or a diet with 7.5% ARA-rich oil plus 5.5% fish oil containing DHA during the premating, mating, gestation, and lactation periods. In addition, the control group received 13% corn oil and the second control group was fed an unsupplemented rodent diet. Total levels of fats in each diet were kept constant by adding the appropriate amount of corn oil. The test substances were administered from 4 weeks prior to the mating, throughout mating, gestation, and lactation periods of parental (F_0) animals. The same diets were administered to the F_1 pups for 13 weeks after weaning.

The fertility and reproductive performance, general condition of pups, viability, sex ratio, and number of pups were not affected by ARA-rich oil with or without DHA-oil supplementation. Pups in the ARA/DHA-oil group exhibited significantly lower weight gain (by 10%) than the corn oil control from day 14 of lactation.

In the subsequent 13-week subchronic toxicity study of the F_1 groups, 20 males and 20 females of each group were randomly selected from the litters in such a way that no more than one animal/sex/litter was included in any group. At the start of the subchronic study the rats were 20-31 days old. In a 13-week subchronic study, the selected F_1 animals received the same test substances or control diets for a 13-week period.

The high-dose (7.5%) ARA-rich oil group and/or the ARA/DHA-oil group had significant decreases in ALP activity, cholesterol, TGs, and phospholipid concentrations and significant increases in creatinine and urea concentrations, which were considered related to a high-fat diet and non-adverse. Similar results have been reported in other studies of high lipid ingestion in rats. These high-dose groups had increased relative weights of the adrenals, spleen, and liver (high-dose ARA vs. ARA/DHA vs. corn oil control: female adrenals: 0.244 vs 0.268* vs. 0.221; female liver, 31.7** vs. 34.5** vs. 29.0; female spleen, 2.03* vs. 2.11** vs. 1.82; male spleen 1.68 vs. 1.77** vs. 1.59; where *<0.05, **<0.01). However, the increase in spleen weight was not associated with morphological changes; thus, it was considered a result of a physiological adaptation rather than a toxic effect.

In females, a dose-dependent increase in hepatocellular vacuolation in the liver was observed, with statistical significance noted for the high-dose ARA group and the ARA/DHA group (corn oil control vs. low-dose vs. mid-dose vs. high-dose ARA vs. ARA/DHA: 2 vs. 3. Vs. 7 vs. 10* vs 11**; where *<0.05, **<0.01). However, in males, vacuolation was present in the liver of about one-third of all ARA groups and in the corn oil control group, but absent in the ARA/DHA group.

None of these findings were observed in the mid-dose (1.5%) ARA-rich oil fed rats. In the 0.3% and 1.5% ARA-rich oil diet groups, no treatment-related effects on clinical examinations, growth, food and water intake, hematology, clinical chemistry, urinalysis, organ weights, or microscopic examination parameters were observed.

The NOAEL of ARA-rich 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) (Table 21).

The Study by Lina et al. (2006)

Lina et al. (2006) evaluated the safety of an ARA-rich oil (41.5% ARA; SUNTGA40S; Suntory Limited; Japan) derived from *M. alpina* in a subchronic study in F1 Wistar rats with an in-utero exposure. The experiment was comprised of two phases: (1) an in-utero exposure phase, in which F_0 Wistar rats aged 9-10 weeks old (12 males and 24 females per group) were administered one of 6 diets starting 4 weeks prior to mating, and throughout mating, gestation, and lactation periods; and (2) a 13-week subchronic study, in which the selected F_1 animals received the test substances or control diets for a 13-week period. The diets administered were 2 control diets (high-fat diet control containing corn oil or low-fat diet control), 0.5%, 1.5%, or 5% ARA oil, or 3.65% ARA-rich oil plus 2.11% high-DHA tuna oil.

In F_0 rats, ARA-rich oil with or without DHA oil did not affect the health, growth, fertility, or reproductive performance. In addition, it did not exhibit any treatment-related abnormalities in pup characteristics (condition, weight gain, viability, number per litter, and sex ratio).

For the subsequent subchronic toxicity study, the F_1 rats at day 21 post-partum (10 males and 10 females per group) received the same diets as the in-utero exposure phase for 13 weeks. No treatment-related abnormalities were observed in neurobehavioral observations, ophthalmoscopy, growth, hematology, clinical chemistry, urinalysis, and macroscopic and microscopic findings, although ARA-rich oil at high doses was associated with a few differences in hematology, clinical chemistry, and spleen weight.

Thus, the authors concluded that ARA-rich oil was safe at doses up to 5% in the diet which may correspond to an overall intake of approximately 3,000 mg/kg bw/day in F_0 and F1 rats, except during lactation when the intake in dams doubled.

The Study by Gao et al. (2014)

Gao et al. (2014) evaluated the potential toxicity of ARA-rich oil (48.3% ARA; Xiamen Kingdomway Group Company, China) derived from *M. alpina* strain XM027 by performing a 90-day subchronic study in F_1 Sprague Dawley rats with in-utero exposure. The experiment was comprised of two phases: (1) an in-utero exposure phase, in which F_0 sexually mature animals were fed one of 5 diets (diets containing 0.5%, 1.5%, or 5.0% ARA-rich oil, a standard rodent diet, or a high-fat diet) starting 4 weeks prior to mating, and throughout mating, gestation, and lactation periods; and (2) a 13-week subchronic study, in which the selected F_1 animals received the same diets as in the in-utero phase for 13 weeks starting weaning (day 21).

ARA-rich oil, at concentrations of 0.5%, 1.5%, and 5.0% of the diet, did not affect reproductive performance of the parental rats, or any characteristics of the pups. In the subsequent subchronic study with the offspring (F_1) rats, no treatment-related abnormalities were observed in clinical examinations, growth, food and water intake, hematology, clinical chemistry, urinalysis, organ weights, and microscopic examination parameters in test groups. Thus, the NOAEL was placed at 5% ARA-rich oil, the highest level tested. This level corresponds to approximately 3,750 mg/kg in F_0 females, 2,850 mg/kg in F_0 males, 4,850 mg/kg in F_1 females, and 4,480 mg/kg in F_1 males.

The Study by Casterton et al. (2009)

In an in-utero phase of the 90-day study in Wistar Outbred (Crl:WIWU) rats, rats received one of 5 diets (0.5%, 1.5%, and 5% ARA-rich oil [43.3% ARA; source, RAO from Cargill, the subject of GRN 000326] and two controls diets (a standard low-fat diet and a high-fat diet supplemented with 5% corn oil). The study protocol was similar to those described in Hempenius et al. (2000) and Gao et al. (2014). Briefly, F_0 rats (16 females and 8 males/group) were exposed to one of these test and control diets from 4 weeks prior to mating, throughout mating, gestation, and lactation to offspring (F_1) weaning (F_1, day 21). In a subsequent 90-day feeding study, selected F_1 animals received the same test or control diets for 13-weeks starting weaning (day 21 of life).

No treatment-related abnormalities were observed in clinical signs, food intakes, body weights, or body weight gain during the premating, gestation, and lactation periods. There were no treatment-related abnormalities in fertility and reproductive performance including indices for mating, female fecundity, female fertility, male fertility, gestation, birth, and viability, as well as precoital and gestation times.

In the subsequent subchronic toxicity study, the F_1 rats at day 21 post-partum received the same diets as the in-utero exposure phase for 13 weeks. No treatment-related abnormalities were observed for histopathology, hematology, clinical chemistry, and organ weights. Although statistical significance was shown for several parameters, none were considered treatment-related because the values were within historical control ranges, and significant differences were seen with comparison to only one control, and/or were seen in only one gender with no dose–response relationship. The only exception that could not be explained by such reasoning was the observed increase in monocytes in both high-dose males and females. However, in the absence of confirmatory histopathology and/or other changes in clinical chemistry variables in the high-dose group, it was not considered of toxicological concern.

Based on these findings, no adverse treatment-related effects for ARA-rich oil were seen at up to 5% in the diet or 3,170 mg/kg bw/day.

Subchronic Oral Toxicity Study of *M. alpina* Biomass

Nisha et al. (2009) examined the safety of an ARA-rich *M. alpina* strain CBS 528.72 biomass (13.1% ARA; manufacturer, NA). Wistar rats aged 3 weeks old (6 males and 6 females per group) were assigned to one of the following 6 diets for 13 weeks: the diet containing 0, 0.25, 0.5, 1, 2, or 3% *M. alpina* biomass.

No treatment-related abnormalities were observed in survival rate, food consumption, body weight gain, serum biochemical and hematological indices, organ weights, and histopathological examination parameters (Table 21). The authors concluded that *M. alpina* biomass was nontoxic and well tolerated by rats.

Teratogenicity Study of ARA-Rich Oil

The potential teratogenicity of ARA-rich oil (brand name, ARASCO®; 51.4% total FAs; available from Martek/DSM) and DHA-rich oil (a brand name, DHASCO®, available from Martek, DSM) was evaluated in a developmental toxicity study (Arterburn et al., 2000b) (Table 21). The female rats were approximately 11 weeks old when paired for mating. Males from the same strain and source as the females were used for mating. Pregnant female Crl:CD1 SD BR VAF/ Plus1 rats were subjected to one of 5 treatments (control, or 1,000 or 2,500 mg/kg bw/day ARA-rich oil, or 500 or 1,250 mg/kg bw/day DHA-rich oil) once daily on days 6 through 15 of gestation. Cesarean sections and necropsies were performed on day 20 of gestation. Maternal evaluations included the number of corpora lutea in ovaries, weight of uteri with visible implantation, number and placement of implantation sites, live and dead fetuses, early and late resorptions, abnormalities, and maternal necropsy. Fetal evaluations, such as soft tissue development and skeletal abnormalities, were also examined.

Both oil treatments did not cause adverse maternal toxicity or significant adverse developmental effects including fetal malformations, changes in pre- or post-implantation losses, resorptions, live births, or sex ratios. The results of the study demonstrated that ARA-rich oil of doses up to 2,500 mg/kg bw/day was not teratogenic. The NOAEL of ARA-rich oil was determined to be 2,500 mg/kg bw/day for F_0 and F_1 rats.

Bioequivalency Study

The 2011 study of Tyburczy et al. compared the bioequivalency of three different sources of ARA-rich oils as measured by tissue (brain, retina, and heart) and red blood cell (RBC) ARA levels (Table 22). All three ARA-rich oil ingredients were manufactured using *M. alpina* by three different

companies: ARASCO® was manufactured by Martek/DSM, RAO was from Cargill and Ankang, Wuhan, China, and SUNTGA40S was provided by Nippon Suisan Kaisha, Ltd. (previously Suntory, Ltd., Japan). ARASCO® served as a reference ARA-rich oil. All three ARA-rich oil ingredients have established FDA GRAS status: ARASCO®, SUNTGA40S, and RAO were the subjects of GRNs 000041/000080, 00094, and 000326, respectively.

It was hypothesized that the three sources of ARA-rich oil ingredients would be nutritionally bioequivalent and equally safe in rapidly growing neonatal pigs. Piglets were fed one of three ready-to-use formulas that provided ARA at approximately 0.64% total FAs and DHA at 0.34% total FAs from day 3 to 22 of life. 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.

For both RAO and SUNTGA40S diets, the 90% confidence intervals fell within the 80 – 125% limits for all tissue samples (including liver histology) as well as clinical chemistry and hematological parameters. The data suggested that the three sources of ARA-rich oil ingredients were bioequivalent sources of ARA with respect to tissue and RBC ARA accretion.

The three ARA formulas equally supported growth in the neonatal pigs as shown by similar body weights at every time point and no differences in organ weights. The three ARA-rich oils equally supported ARA accretion in the brain, retina, and heart. Mean ARA levels in the brain, retina, and heart were 10.97%, 10.50%, and 20.38% of total FAs, respectively, and were similar for all three dietary treatment groups. However, for hepatic ARA levels, the ARASCO® group had significantly higher ARA levels than the other 2 groups (ARASCO® vs. SUNTGA40S vs. RAO: 17.66 vs. 17.33* vs. 17.38% of FAs; *P<0.01 between ARASCO® and RAO groups). This 0.33% difference in liver ARA levels may be due to variations in ARA content in diets as the RAO diet provided 8% less ARA (0.62% vs. 0.67% total FAs) than the ARASCO® and SUNTGA40S diets. However, the magnitudes of the differences observed were too small; thus, it was considered that all three sources were

similar. Over the full study period, mean RBC ARA levels across the three dietary treatment groups decreased from 6.98 ± 0.60% of total FAs on day 3 of age to 4.62 ± 0.32% of total FAs on day 21 of age. Mean ARA levels in the RBC fraction were similar among all dietary treatment groups at every time point examined (day 3, 6.84-7.06; day 7, 5.76-5.94; day 14, 4.89-5.32; day 21, 4.55-4.71% of total FAs; NS at any time points).

In addition, no significant differences were observed in hematology and clinical chemistry parameters among the groups.

Based on growth and RBC concentrations in brain, heart, liver, and blood, hematology, clinical chemistry, and liver histology parameters, the authors concluded that all three ARA-rich oils were bioequivalent.

Neonatal Piglet Studies

The Study by Merritt et al. (2003)

Merritt et al. (2003) evaluated the safety of ARA-rich oil (40% FAs as ARA) derived from *M. alpina* (SUNTGA40S; Suntory Ltd, Japan) for use in infant formulas in a neonatal piglet model (Table 21). Forty-eight 3-day-old piglets were assigned to one of 4 bottle-fed treatments (6 males and 6 females/group) until 19 days of age: 1) a control formula (no added DHA or ARA), 2) an ARA formula providing 96 mg/100 g, 3) a DHA formula providing 55 mg DHA/100 g, and 4) a DHA+ARA blend formula providing 34 mg DHA and 62 mg ARA/100 g. All formulas were equal in fat and calorie content (approximately 1,000 kcal/L). Actual mean intakes of these groups were 250 mg ARA/kg bw/day for the ARA only group, 136 mg DHA/kg bw/day for the DHA group, and 153 mg ARA plus 84 mg DHA/kg bw/day for the blend of ARA and DHA group. The highest dose of ARA was approximately 6 and 7.5 times the maximum ARA intake levels in term and preterm infant formulas under the intended use. There were no treatment-related abnormalities in clinical signs, body weights, food intake, hematology, clinical chemistry, organ weights, and necropsy findings. The authors concluded that administration of ARA, DHA, or ARA+DHA to neonatal piglets did not result in adverse health effects at the highest doses tested.

The Study by Tyburczy et al. (2012)

Tyburczy et al. (2012) evaluated the effect of high dietary ARA-rich oil derived from *M. alpina* (ARASCO®, Martek Biosciences) on growth, clinical chemistry, hematology, and immune function in newborn piglets (Table 21). Three-day old piglets were administered one of seven diets for 25 days: one of 6 diets with varying ratios of ARA:DHA as follows (g/100 g FAs): 0.1/1.0; 0.53/1.02; 0.69/1.01; 1.06/1.04; 0.67/0.62; and 0.66/0.33; a seventh group was maternal-reared and

remained with the dam during the study. Piglets were vaccinated against *Mycoplasma hyopneumoniae* on day 7 of age and were sacrificed on day 28 of age. Serum samples collected on days 21 and 28 of age were analyzed for antibodies to *M. hyopneumoniae* while blood and serum samples collected on day 28 of age were analyzed for hematology and clinical chemistry parameters. No treatment-related abnormalities or significant differences were observed for clinical observations, feed intake, growth, hematology, clinical chemistry, organ weights, or immune status (as measured by *M. hyopneumoniae* titers and serum concentrations of immunoglobulin [Ig]A, IgM, and IgG, and high-sensitivity C-reactive protein). The data suggest that a dietary ARA concentration up to 1.06% total FAs, in combination with DHA (1.04% FAs), produced no adverse effects on measurement outcomes including the clinical chemistry, hematology, or immune function parameters. It was concluded that the dietary ARA level, when DHA is constant at 1.0% total FAs, did not influence the measured outcomes in the neonatal period.

Conclusion:

The safety of ARA-rich oil (40.3% ARA of total FA) produced by Runke Bioengineering 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) (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 they 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) established the bioequivalence of three sources of ARA-rich oils. These studies were also discussed in GRN 000963 (pages 30-32), GRN 000730 (pages 31–35), and GRN 000326 (pages 149 -153).

Table 21. Summary of Animal Toxicity Studies of ARA-rich Oils Derived from *M. alpina*

Species	Test substance	Dose	Duration	NOAEL	Reference
Studies of Runke Bioengineering's ARA					
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	ARA-rich oil-5,000 mg/kg bw/day	Lewis et al., 2016
Rat, Wistar			13 wk	ARA-rich oil-5,000 mg/kg bw/day	Lewis et al., 2016
Rat, Wistar			Developmental toxicity, GD days 6-20	Both developmental and reproductive toxicity, ARA-rich oil-5,000 mg/kg bw/day	Falk et al., 2017
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
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
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
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
Rat, Wistar	ARA-rich oil from *M. alpina* (43.3% ARA; RAO from Cargill)	0, 1, 1.5, or 5% of diet	90-day subchronic with in-utero exposure	5% of diet or 3,170 mg/kg bw/day	Casterton et al., 2009

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/d) in diet; 3.65% ARA +2.11%DHA	13 weeks 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 and ARA+DHA in F_1 were not considered adverse.	Lina et al., 2006
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
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
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
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 (D3 to D22)	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
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
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 (from day 3 to 19)	248 mg ARA/kg bw/day (or ~620 mg ARA-rich oil/kg bw/day)	Merritt et al., 2003

Abbreviations: ARA = arachidonic acid; bw = body weight; DHA = docosahexaenoic acid; wk = week.

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

Our review has focused on the papers published since the FDA's last review of 2020-2021 or the papers published through May 2023.

6.B.4.1. Preterm Infants

Recent Studies

Hellström et al. (2021) evaluated whether the enteral supplementation of FAs reduces retinopathy prematurity (ROP) in preterm infants. In this randomized clinical trial, a total of 207 infants born at less than 28 weeks' gestational age (mean age of 25.5 weeks) received an emulsion supplement containing 100 mg/kg bw/day ARA plus 50 mg/kg bw/day DHA (ARA and DHA 2:1 supplement; DSM Nutritional Products; purity, NA) or no supplementation within 3 days after birth until 40 weeks post-menstrual age (PMA) (Table 22). The primary outcome was the incidence of severe ROP. Secondary outcomes include serum levels of ARA and DHA, morbidities (other complications such as bronchopulmonary dysplasia, intraventricular hemorrhage, patent ductus arteriosus, necrotizing enterocolitis), safety and tolerance, death, and growth. There were no adverse effects of enteral supplementation on the primary and secondary outcomes including necrotizing enterocolitis and postnatal growth. The fraction of serum ARA was significantly higher in the ARA:DHA group than in the control group. There were 29 of 207 deaths (14.0%) in the entire study population, with no significant between-group differences before 40 weeks' PMA. Most infants experienced at least one adverse event (AE): 99 (98.0%) in the ARA:DHA group and 105 (99.1%) in the control group. Incidence rates of serious AEs were similar between the groups: 26 patients (25.7%) in the ARA:DHA group and 26 (24.8%) in the control group. The authors concluded that supplementing the diets with an enteral lipid solution with ARA to DHA ratio of 2:1 had no significant adverse effects on measured outcomes in preterm infants.

Frost et al. (2021) evaluated if emulsified ARA and DHA supplementation could support higher blood ARA and DHA concentrations at 2 and 8 weeks in 30 very low birth weight (VLBW) infants (Table 22). One hundred ninety-two VLBW infants with a mean birth weight of 1,040 g (mean gestational age of 28 weeks) in neonatal intensive care units received one of the following 3 treatments for 8 weeks or until discharged, whichever came first: 1) a placebo control supplement containing sunflower oil, 2) supplements containing 80 mg/kg bw/day ARA (source and manufacturer, not specified) and 40 mg/kg bw/day DHA, or 3) supplements providing 240 mg/kg bw/day ARA and 120 mg/kg bw/day DHA. The supplement was given via orally or nasogastric tube. The primary outcome was blood LCPUFA levels at 2 weeks. Secondary outcomes included blood LCPUFA levels at 8 weeks, days to reach full enteral feeds (defined as 120 kcal/kg bw/day), and the incidence rates of necrotizing enterocolitis and bronchopulmonary

dysplasia. No adverse effects were reported on the measured outcomes. The data suggest that the emulsified ARA and DHA supplement was generally well tolerated by all infants.

Gastrointestinal Tolerance and Allergenic Potential of ARA in Preterm Infants

The following 3 studies addressed the effects of ARA on gastrointestinal tolerance and evaluated the allergenic potential of ARA in preterm infants. Although the source of ARA was not identified in these studies, it is assumed that these studies employed ARA-rich oil derived from *M. alpina* because the only available ARA-rich oil ingredients for the use in infant formulas in the marketplace are derived from *M. alpina* (Table 22).

Study of Manley et al. (2011)

In a study by Manley et al. (2011), ARA supplementation at 0.6% of FAs in combination with DHA did not result in adverse effects on long-term atopic and respiratory outcomes in 657 preterm infants of <33 weeks gestational age (<1,250 g at birth). They consumed expressed breast milk from mothers taking either tuna oil with high-DHA (tuna oil) or standard-DHA (soy oil) capsules. Lactating women and their infants were randomly assigned to the high-DHA group (3 g tuna oil per day) or the standard-DHA group. If supplementary formula was required, infants were given a high-DHA preterm formula (DHA, 1% of FAs; ARA, 0.6% of FAs) or a standard preterm infant formula (DHA, 0.35% of FAs; ARA, 0.6% of FAs). The intervention in both groups continued until infants reached their expected date of delivery. Measurement endpoints included neurodevelopment, important allergic parameters (risk of asthma, eczema, or the requirement of a special diet due to food allergy), and respiratory parameters (incidence of bronchopulmonary dysplasia) over the first 18 months of life. No adverse effects of ARA/DHA supplementation were noted on the measured outcomes including the requirement for a special diet due to food allergy in preterm infants of <33 weeks of gestation. Supplementation of ARA/DHA did not result in adverse effects on important allergic parameters (risk of asthma, eczema, or the requirement for a special diet for food allergy) and respiratory parameters (incidence of bronchopulmonary dysplasia) over the first 18 months of life in 657 preterm infants of <33 weeks of gestation (Manley et al., 2011). The data indicate that supplementation of ARA at 0.6% of total FAs with varying ratios of DHA (0.35 or 1.0% of FAs) did not result in adverse effects on measured outcomes in preterm infants.

Study by Gunaratne et al. (2019)

From the Docosahexaenoic Acid for the Improvement of Neurodevelopmental Outcomes (DINO) study in which infants were given high- or low-DHA (~1% or 0.3% total FAs) and a fixed amount of ARA (0.6% of FAs) via enteral feeds from 2–4 days of postnatal age until 40 weeks PMA, Gunaratne et al. (2019) investigated allergic respiratory symptoms (wheeze or rhinitis) at 7 years

of corrected age (CA). Data were available for 569 of 657 children originally randomized. Primary outcomes were parent-reported incidence of respiratory allergic disease symptoms including wheeze and rhinitis at 7 years CA. Secondary outcomes included the incidence of eczema, wheeze, rhinitis, and rhinoconjunctivitis, and severity of any symptoms from birth to 7 years CA. Results showed that parent-reported symptoms of wheeze, rhinitis, rhinoconjunctivitis, or eczema from birth to 7 years CA did not differ between the groups. Overall, ARA-DHA supplementation did not have adverse effects on allergic parameters in preterm infants.

Study by Clandinin et al. (2005)

In a study by Clandinin et al. (2005), ARA supplementation at 0.6% of FAs in combination with two different sources of DHA did not result in adverse effects on measured outcomes including gastrointestinal tolerance in 361 preterm infants of < 35 weeks PMA. They were randomly assigned to 3 study formula groups: 1) control, formula with no added DHA or ARA; (2) an algal-DHA, formula with 17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal from fungal oil (Martek Biosciences, algal type was not specified); or (3) fish-DHA, formula with 17 mg DHA/100 kcal from tuna fish and 34 mg ARA/100 kcal from fungal oil. These levels of DHA and ARA are similar to those present in a typical mature human milk (approximately 0.3% of FAs as DHA and 0.6% as ARA). The study formulas were the sole source of nutrition for the preterm 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 breast-fed for 4 months or longer were the reference group. All infants were assessed at birth and at 40, 44, 48, 53, 57, 66, 79, 92, and 118 weeks PMA. Measurement endpoints included growth, tolerance, AEs, and Bayley development scores.

There were no differences in caloric intake from formula and tolerance parameters such as daily gastric residuals, stool frequency, stool consistency, and abdominal distention among the preterm groups during hospitalization (data not shown). Additionally, ARA/DHA supplementation did not increase the incidence of morbidity commonly associated with prematurity, including intraventricular hemorrhage, necrotizing enterocolitis (using modified Bell staging criteria), sepsis or suspected sepsis, bronchopulmonary dysplasia (defined as requiring oxygen at 36 weeks PMA with severe or chronic changes to the lungs as seen on chest radiographs), and ROP. The authors concluded that supplementation of ARA at 0.6% of total FAs, in combination with DHA (either algal oil or fish oil source), did not result in adverse effects on the measured outcomes in preterm infants.

In addition, Hellström et al. (2021) and Frost et al. (2021) reported that supplements providing 80 - 240 mg/kg bw/day ARA did not result in adverse effects on the incidence of ROP, necrotizing enterocolitis, gastrointestinal tolerance, death, or growth in preterm infants (Table 22).

Studies Justifying the ARA Use Level of 0.5% of Total FAs in Preterm Infants

Runke is intended to use 0.5% of total fat as ARA for preterm 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).

As described above, the studies by Manley et al. (2011), Gunaratne et al. (2019), and Clandinin et al. (2005) reported that ARA supplementation at 0.6% of FAs, in combination with DHA, did not result in adverse effects including gastrointestinal tolerance in preterm infants.

Previous GRAS notices provided information and/or clinical study data that supported the safety of ARA ingredients for use in infant formula. Almaas et al. (2015, 2016), Westerberg et al. (2011), and Henriksen et al. (2008, 2016) reported that human milk supplemented with 31 mg ARA (0.91% of total FAs) and 32 mg DHA (0.86% of total FAs) per 100 mL, providing 47 and 59 mg/kg bw/day of ARA and DHA (total FAs from supplements and human milk), respectively, was safe in preterm infants.

Recently, Frost et al. (2021) found that daily doses up to 240 mg/kg bw/day ARA (which may correspond to up to approximately 4% FAs as ARA) for 8 weeks were safe in preterm infants. In addition, emulsion supplementation (ARA and DHA 2:1; Formulaid™, DSM Nutritional Products) providing 100 mg/kg bw/day ARA (derived from *M. alpina*; which may correspond to up to approximately 1.7% of FAs as ARA) plus 50 mg/kg bw/day algal DHA (derived from *C. cohnii*) for up to 12 weeks, respectively, was well tolerated in preterm infants (Hellström et al., 2021; Pivodic et al., 2022. Sojobom et al., 2023; Wendel et al., 2023).

It is concluded that no studies found adverse effects of ARA supplementation at 0.5% of total FAs in preterm infants (Tables 22).

In addition, an intended use level of up to 0.5% FAs as ARA in preterm 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 preterm infants (Koletzko et al., 2014b).

Table 22. Preterm Infant Studies Reporting No Adverse Effects of ARA

Objective	Subjects	Test materials	Duration	Measurements	Reference
To determine if the enteral supplementation of ARA and DHA reduces ROP in extremely preterm infants	A substudy of the Mega Donna Mega trial; 207 infants born less than 28 wk gestation age (mean age of 25.5 wk)	Open label: Test group, Emulsion supplements providing 100 mg/kg bw/d fungal ARA and 50 mg/kg bw/day algal DHA (~1.7% of FAs as ARA; ARA and DHA 2:1 supplement; Formulaid™, DSM Nutritional Products, purity, NA; ARA *from M. alpina* and DHA from *C. cohnii*); Control group received standard care	From <3 days after birth until 40 wk PMA	ROP Activity Scales (original: ROP-ActS and modified: mROP-ActS)	Pivodic et al., 2022
				Incidence of severe ROP; morbidities; adverse events, tolerance, and growth	Hellström et al., 2021
To determine the effects of ARA and DHA on serum fatty acid profiles in extremely preterm infants	Mega Donna Mega trial; N=204			Serum fatty acid profile	Sojobom et al., 2023
To determine the effects ARA and DHA on short-term respiratory outcomes and neonatal morbidities	120 infants born less than 29 wk gestation age (mean age of 26.4 wk)	100 mg/kg bw/day fungal ARA and 50 mg/kg bw/day algal DHA (ARA and DHA 2:1 supplement; Formulaid, DSM Nutritional Products) or medium chain TG oil (control)	From second day of life to 36 wk PMA	Duration of respiratory support, incidence of BPD and other major morbidities associated with preterm birth	Wendel et al., 2023
To evaluate if emulsified ARA and DHA supplement could maintain and support higher blood ARA and DHA concentrations at 2 and 8 weeks in very	30 VLBW infants (mean age, 28 wk; mean birth weight, 1,040 g)	Placebo (sunflower oil); 80 mg/kg bw/d ARA+ 40 mg/kg bw/d DHA; 240 mg/kg bw/d ARA+ 120 mg/kg bw/d DHA (source, NA)	8 wk or until discharge	Whole blood LCPUFA; blood DHA and ARA levels; days to reach full enteral feeds; incidence of NEC and bronchopulmonary dysplasia	Frost et al., 2021

low birth weight infants					
To determine the effect of meeting the estimated ARA/DHA requirement of preterm infants on allergic and/or respiratory parameters	DINO trial, 657 preterm infants of <33 wk of gestation	High-DHA preterm formula (1% FAs as DHA from fish oil) or a standard preterm infant formula (0.35% FAs as DHA) with a fixed amount of ARA (0.6% of FAs)	Until infants reached their expected date of delivery; FU at 12 and 18 mo	Allergic (hay fever, eczema, asthma, or food allergy) and respiratory parameters (including the incidence of bronchopulmonary dysplasia)	Manley et al., 2011
			Until infants reached their expected date of delivery; FU at 7 yr CA	Incidence of eczema symptoms, severity of any symptoms, and the incidence of wheeze, rhinitis, rhinoconjunc-tivitis, and eczema	Gunaratne et al., 2019
To evaluate safety and benefits of feeding preterm infants formulas containing ARA and DHA until 92 wk PMA, with follow-up to 118 wk PMA	361 preterm infants of < 35 wk PMA	Control formula with no added ARA/DHA; (2) algal-DHA formula with 17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal, or (3) fish-DHA formula with 17 mg DHA/100 kcal from tuna fish and 34 mg ARA/100 kcal. (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; FU 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	Clandinin et al., 2005

To assess the effect of ARA and DHA supplementation on cognitive development in human milk-fed preterm infants at 6 mo of age	141 VLBW infants with birth weights of <1,500 g	100 mL human milk with control oil (a blend of soy oil and medium chain TG oil); or ARA- and DHA-rich oils providing 31 mg ARA (0.91% of total FAs/100 mL human milk) and 32 mg DHA (0.86% of total FAs/100 mL human milk); source-Martek Biosciences Total intakes*: Control group: 32 mg DHA/kg/d and 22 mg ARA/kg/d; Test group: 59 mg DHA/kg/d and 47 mg ARA/kg/d	From 1 wk after birth and lasting until discharge from the hospital (on average, 9 wk); follow-up at 6 mo	Cognitive development at 6 mo of age; growth; adverse events	Henriksen et al., 2008
To investigate the effect of ARA and DHA in early neonatal life on cognitive functions among human milk fed very low birth weight infants (<1,500 g) at 20 mo of chronological age			From 1 wk after birth until discharge from hospital (9 wk on average); follow up at 20 mo	Cognitive function tests were performed at 20 mo (Free-play sessions, Bayley Scales of Infant Development -the Ages and Stages Questionnaire); and plasma DHA and ARA concentrations	Wester-berg et al., 2011
To test the effects of ARA/DHA supplementation on cerebral white matter and improve behavioral outcome in VLBW infants at 8 years of age	129 VLBW infants with birth weights of <1500 g		From 1 wk after birth and lasting until discharge from the hospital (on average, 9	Cerebral white matter; behavioral outcome measured by Strengths and Difficulties questionnaire and selected scales from the Child Behavior Checklist.	Almaas et al., 2016

To test if the effects of ARA/DHA supplementation on cognition in VLBW infants fed human milk			wk); follow-up at 8 yr (n=98)	A battery of cognitive measures; magnetic resonance imaging data on segmental brain volumes and cerebral cortex volume, area, and thickness at 8 yr follow up	Almaas et al., 2015
To test if ARA/DHA supplementation would affect growth, metabolic markers, and cognitive function, and to describe predictors of metabolic markers and cognitive status at follow-up				Growth; IQ; metabolic profile in blood (cholesterol, fatty acids, IGF-1, adiponectin, leptin, glycated hemoglobin, carotenoids); body mass index at follow up at 8 y	Henriksen et al., 2016
To test the hypothesis that once daily DHA+ARA drops applied to buccal mucosa will increase blood levels of these fatty acids	30 extremely VLBW infants with birth weights <1,000 g and <34 wk of gestational age (median, 26 wk; 806 g at birth)	Placebo (sunflower oil); 40 mg/kg bw/day ARA + 20 mg/kg bw/day DHA; or 120 mg/kg bw/day ARA + 60 mg/kg bw/day DHA (source and purity NA)	From <72 h of age for 8 wk	RBC levels of DHA and ARA and other long chain fatty acids	Robinson et al., 2016

DHA and ARA= Percentages given as % of total FAs unless noted otherwise. Abbreviations: BDP = bronchopulmonary dysplasia; bw = body weight; CA = corrected age; d = days; EPA = eicosapentaenoic acid; h = hour; IGF-1 = insulin growth factor-1; ICU = intensive care unit; IQ = intelligence quotient; LCPUFA = long-chain polyunsaturated fatty acid; *M. alpina = Mortierella alpina*; mo = month; NA = not available; NEC = necrotizing enterocolitis; PMA = post-menstrual age; RBC = red blood cell; TG = triglyceride; VLBW = very low birth weight; wk = week; wt = weight; yr = years.

*Intake data from Westerberg et al., 2011.

6.B.4.2. Term Infants

Since the FDA's review in 2020-2021, 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. Thus, this review focuses on the allergenic potential and gastrointestinal tolerance of ARA in term infants.

Allergenic Potential and Gastrointestinal Tolerance of ARA in Term Infants

Term infants receiving different dosages of ARA (0.64–0.72% FAs as ARA) and DHA (0.32–0.36% FAs as DHA) from 1–9 days of life until up to 12 months of age did not have adverse effects on the risk of lower respiratory tract infections, wheezing/asthma, or other allergic diseases when compared to controls. Studies of term infants have not reported adverse effects on allergic or gastrointestinal symptoms associated with ARA/DHA-supplemented infant formula (Burks et al., 2008; Birch et al., 2010; Hoffman et al., 2008). The results of each study are summarized below (Table 23).

Study by Hoffman et al. (2008)

In a study by Hoffman et al. (2008), 244 healthy term infants received either a soy formula containing ARA (34 mg ARA/100 kcal) and algal DHA (17 mg DHA/100 kcal) (test group) or a control formula with no supplementation (control group). These levels correspond to approximately 0.6% of total FAs as ARA and 0.3% of total FAs as DHA. Of the 244 infants enrolled, 182 infants completed the study. Infants received study formula from 14 to 120 days of age. Body weight and other anthropometric measurements, atopic dermatitis, tolerance, and AEs were monitored.

The incidence of AEs, formula intake, stool frequency and characteristics, and parental assessment of fussiness, diarrhea, and constipation were comparable between the groups although gastrointestinal reflux was higher in the control than in the test group (control vs. test: 12 vs. 3 infants, P = 0.009). In addition, no statistically significant difference was noted in the atopic dermatitis scores, as assessed by mean SCORing Atopic Dermatitis (SCORAD) indices at 120 days of age between the two groups (control, 2.9 ± 0.76; test, 2.3 ± 0.72). The only differences noted were higher gastrointestinal reflux (control vs. test: 12 vs. 3 infants, P = 0.009) and the incidence of excessive gas (15% vs. 5%, P = 0.026) which were noted more in the control group than in the test group at 60 days of age. In the subset 47 infants who underwent blood sample analysis, no statistically significant differences were noted in blood chemistry profiles (total RBC lipids and plasma phospholipids, glucose, and kidney, liver, and pancreas function markers)

between the two groups at 14 or 120 days of age (data not shown). The authors concluded that both formulas were well tolerated and supported normal growth.

Study by Burks et al. (2008)

The study by Burks et al. (2008) evaluated the effects of DHA and ARA supplementation to an amino acid-based formula on overall growth, tolerance, and safety in 164 healthy term infants. Study 1 compared the effect on growth, tolerance, and safety in healthy infants of an amino acid-based formula (Nutramigen, Mead Johnson) to a control extensively hydrolyzed formula (casein based). Both formulas were supplemented with ARA (0.64% of total FAs; 34 mg ARA/100 kcal) and DHA (0.32% of total FAs; 17 mg/100 kcal, source was not specified). These levels are similar to those in human milk worldwide. The formulas were given from 14 ± 2 through 120 ± 4 days of age. Overall growth, formula acceptance, tolerance, and AEs were similar between the two groups. No differences between groups were detected in the number of infants who experienced at least one AE or the incidence of serious AEs. The exceptions were parent-reported fussiness, which was lower in the control group ($P<0.039$) at 90 days of age and the incidence of diarrhea, which was significantly higher in the control group (control vs. test groups, 9 vs. 0, $P<0.001$).

In Study 2, the hypoallergenicity of the new amino acid-based formula was evaluated in 32 infants and children (aged 8 months to 10 years) with a confirmed cow's milk allergy. All 29 children completed both the double-blind, placebo-controlled food challenge, with formula fed in randomized order after a pre-challenge elimination period. The new amino acid-based formula containing 34 mg ARA/100 kcal (0.64% of total FAs) and 17 mg DHA/100 kcal (0.32% of total FAs) (source, purity, manufacturer, NA) or a placebo formula, another commercially available amino acid-based formula (Nutricia; USA) were tested in a double-blind, placebo-controlled food challenge, an open challenge, and an extended 7-day feeding period. Twenty-four of the 29 children who completed both challenges (83%) had ongoing allergic manifestations at study entry, including atopic dermatitis, asthma, allergic rhinitis, allergic conjunctivitis, or gastrointestinal manifestations. Allergic symptoms (extent and severity of rash, pruritus, urticaria/angioedema, upper or lower respiratory symptoms, or gastrointestinal symptoms) and AEs were monitored. All 29 children who completed both the double-blind, placebo-controlled food challenge and open challenge had negative responses to both tests. As determined by daily parental record, acceptance, and tolerance of the amino acid-based formula were generally good. No serious AEs occurred during the double-blind, placebo-controlled food challenge, open challenge, or extended 7-day feeding period. The authors concluded that the amino acid-based formula with DHA and ARA at levels similar to those in human milk worldwide was safe in healthy term infants. The results of the same study were briefly reported in Vanderhoof (2008).

Study by Birch et al. (2010)

From the DHA Intake and Measurement of Neural Development (DIAMOND) study, Birch et al. (2010) investigated the incidence of allergic and respiratory diseases through 3 years of age in children fed DHA- and ARA-supplemented formula during infancy. In this study, 343 healthy term infants were randomized to one of four infant formulas: control (0% DHA), 0.32% DHA (17 mg/100 Kcal), 0.64% DHA, or 0.96% DHA (source -algal DHA oil derived from *Crypthecodinium cohnii*); all DHA-supplemented formulas also provided a fixed amount of ARA at 0.64% of total FAs (34 mg/100Kcal). Assigned formulas were fed from the time of enrollment (1 to 9 days of life) through 52 weeks of age. The study formulas were fed for the first 12 months of life and were to be the sole source of nutrition until 4 months of age, when additional foods could be introduced as directed by the infants' physicians. Measurements included visual acuity, RBC FAs, anthropometric measurements, formula consumption, tolerance, and AEs. No statistical differences were reported in consistency or color of bowel movements, frequency of diarrhea or constipation, or frequency of unusual gas or fussiness between formula groups at any time (data not shown). The authors stated that infants well tolerated all formulas containing ARA at 0.64% of total FAs and had normal growth throughout the first 12 months of life.

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). Enriched formula consumption was not associated with infection or allergy, except for an association between consumption of DHA/ARA/EPA-enriched formula and a reduced use of asthma medications.

Overall, human clinical studies and meta-analyses consistently report no adverse effects of ARA/DHA supplementation on allergy and gastrointestinal tolerance in term infants.

Studies Evaluating Other Measurement Endpoints

GRNs 000041, 000080, 000094, 000326, 000730, and 000963 presented comprehensive summaries of clinical study literature regarding supplementation of ARA from *M. alpina* to infant formula (FDA, 2001a, 2001b, 2006, 2010, 2018, 2021). These GRAS notices concluded that

supplementation of ARA, in combination with a safe and suitable source of DHA (from fish and algal sources), to infant formula was safe in both preterm and term infants.

GRN 000933 also summarized the recently published DIAMOND study outcomes (Colombo et al., 2017; Lepping et al., 2019). These studies did not report adverse effects of formulas containing ARA (0.64% of FAs) in combination with algal DHA, up to 0.96% of total FAs (or up to 51-61 mg DHA/kg bw/day) as the formulas were well tolerated with no side effects in term infants. In the study by Hoffman et al. (2019), 34 mg ARA/100 kcal (which may correspond to 34 mg/kg bw/day or 0.62% FAs as ARA) and a prebiotic blend were well tolerated with no side effects in healthy term infants.

The studies by Birch et al., (2005, 2007) and Drover et al., (2009) reported no adverse effects of ARA at 0.72% FAs on measured outcomes such as cognition and gastrointestinal tolerance (Table 24). Overall, the studies using 0.64-0.72% of total FAs as ARA (0.72%, Birch et al., 2005, 2007; 0.64%, Birch et al., 2010; Colombo et al., 2011; Drover et al., 2011, 2012) demonstrated the safety of ARA-rich oil derived from *M. alpina* in term infants. Table 24 summarizes the studies reporting no adverse effects of ARA at 0.72% of FAs.

Numerous systematic reviews and recommendations of ARA used in clinical trials conducted in infants have been published in the peer-reviewed literature (Jasani and Simmer, 2017; Koletzko et al., 2014a, 2014b, 2020). 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 23. Term Infant Studies Reporting No Adverse Effects of ARA on Gastrointestinal Tolerance or Allergy

Objective	Subjects	Test Material and Dose	Type and Duration of the Study	Measurements	Reference
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 + DHA 17 mg; or 3) control, non-supplemented formula ARA Source: *M. alpina*	From 12-16 days to 120 days of age	Growth rates; 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	Hoffman et al., 2008
Study 2: to evaluate the hypoallergenicity of this new amino acid 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	The new amino acid-based formula containing 17 mg DHA /100 kcal (0.32% FAs as DHA) and 34 mg ARA/100 kcal (0.64% FAs as ARA)	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	Burks et al., 2008
To determine the effect of ARA/DHA supplementation on the visual acuity of formula-fed infants from the DHA Intake and Measurement of Neural	343 healthy term infants	ARA, 0.64% of FAs (34 mg/100 kcal, from *M. alpina*) for all 3 DHA levels; DHA (from *C. cohnii* oil), 0.32%, 0.64%, or 0.96% of FAs	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 fatty acids	Birch et al., 2010

Development (DIAMOND) study		Control-unsupplemented			

DHA and ARA= percentages in diet given as % of total FA unless noted otherwise.
Abbreviations: ARA = arachidonic acid; DHA = docosahexaenoic acid; PUFA = polyunsaturated fatty acids; *M. alpina = Mortierella alpina*; mo = month; RBC = red blood cell; RCT = randomized controlled trial; TNF = tumor necrosis factor; VEP = visual evoked potentials.

Table 24. Term Infant Studies Reporting No Adverse Effects of ARA at 0.72% of Fatty Acids

Objective	Subjects	Test material and concentration in infant formula	Type and duration of the study	Measurements	Reference
To evaluate DHA and ARA-supplementation of infant formula on visual and cognitive outcomes at 4 yr of age	79 healthy term infants	4 groups: 2 Tests- test 1) 0.72% FAs as ARA (~40.3 mg ARA/kg bw/d) + 0.36% FAs as DHA (algal); test 2) only with 0.35% FAs as DHA; and unsupplemented formula control; human milk reference. Source: *M. alpina*	Intervention from birth to 17 wk of life; follow up at age of 4 yr	Cognition and visual acuity	Birch et al., 2007
To evaluate the effects of ARA/DHA supplementation in amounts typical for human milk on sweep visual evoked potential acuity as the functional outcome	103 term infants	Test-0.72% ARA + 0.36% DHA (algal oil); or control-unsupplemented. Source: *M. alpina*	Intervention from day 5 to 52 wk	Sweep VEP acuity; Red blood cell DHA concentrations; visual function and total red blood cell lipid composition; growth; gastrointestinal tolerance	Birch et al., 2005
To examine whether feeding infant formula supplemented with ARA/DHA improves cognitive function of 9-mo-olds	229 term infants	2 groups: 1) formula with 0.72% ARA + 0.36% DHA; or 2) control- unsupplemented formula Source: *M. alpina*	12-mo feeding; from 6-wk weaning or 4-6 mo weaning to 12 mo of age	Cognitive outcome measures at 9 mo of age	Drover et al., 2009

Birch et al. (2007) states that all formulas contribute 5.6 g fat per 100 kcal. Abbreviations: ARA = arachidonic acid; bw = body weight; DHA = docosahexaenoic acid; mo = month; wk = week; yr = year.

Summary of Previous GRAS Notices

For supporting the safety conclusion, the following summarizes information pertaining to safety of the ARA-rich oil produced by *M. alpina* for each of six GRAS notices (000041, 000080, 000094, 000326, 000730, and 000963).

GRN 000041, filed by Martek Biosciences Corporation

GRN 000080, filed by Mead Johnson Nutritionals

Both GRAS notices were related to ARASCO® (a brand name of ARA-rich oil marketed by Martek).

They were the first GRAS notices related to ARA-rich oil (~40% ARA) produced by *M. alpina*. ARA-rich oil was intended to be used in term infant formula at maximum use levels of 1.25-1.88% of total fat (or 0.5-0.75% of total fat as ARA) in combination with DHA at a maximum level of 1.25% of total fat (or 0.5% total fat as DHA) at a ratio ranging from 1:1 to 1:2 (DHA to ARA).

The safety of its ARA-rich oil (38-44% ARA) was summarized as follows:

1) The NOAEL of ARA-rich oil (ARASCO®) was determined to be 2,500 mg/kg bw/day in subchronic (28- and 90-day; Boswell et al. 1996; Koskelo et al. 1997) and developmental toxicity studies (Arterbum et al. 2000) in rats.
2) When administered as a mixture of DHA (DHASCO®) and ARA (1:2) in the diet, the maximum level of about 9 g/kg bw/day (about 6 g/kg bw/day ARA and 3 g/kg bw/day DHA) was found to be the NOAEL in both 28-day (Wibert et al. 1997) and a 90-day (Bums et al. 1999) feeding studies in rats. In the toxicity studies, the two oil ingredients were tested at dietary ratios of 1:1.5 to 1:2 (DHA:ARA).
3) In a piglet study, daily doses of up to 420 mg ARA and 210 mg DHA/kg bw/day for 28 days did not result in any adverse effects on measured outcomes such as body weights, organ weights, serum chemistry values, or hematocrit.
4) In term infants, formulas containing up to 0.72% of total fat as ARA and 0.36% total fat as DHA did not result in any adverse effects. The Expert Panel stated, “In trials where supplemental ARA has been used, the concentration ranged from 0.1% to 1.1% of total fat in preterm formulas and from 0.2% to 0.72% for term formulas. These values clearly fall well within the normal range of mother’s milk and the Expert recommendations.”

GRN 000094, filed by Ross Products Division of Abbott Laboratories.

This GRAS notice first described the use of ARA-rich oil (≥40% ARA; brand name, SUNTGA40S) in both term and preterm infants. Intended use levels were 0.4% in term infants, 0.4% of total FAs

as ARA, in combination with DHA at 0.25% of total FAs, in hospitalized preterm infants, and 0.4% of total FAs as ARA, in combination with DHA at 0.15% of total FAs, in post-discharge preterm infants and term infants.

The safety of ARA-rich oil was summarized as follows:

1) ARA-rich oil was not mutagenic or genotoxic.
2) The LD_{50} of ARA-rich oil was determined to be > 2 g/kg bw, the highest level tested.
3) In a 90-day subchronic toxicity study, the administration of ARA-rich oil as 1-2% of the diet in combination with 1% DHA-rich oil in the diet did not produce treatment-related abnormalities in rats.
4) In a study of neonatal piglets, groups were fed formulas containing 0.8% of total FAs as ARA-rich oil (25% ARA) or 0.3% total FAs as DHA. After 18 days, no significant differences were seen in absolute or relative liver weights in the formula-fed group compared to the control group. A statistically significant reduction in relative liver weight was seen in the group fed DHA-rich oil supplemented formula compared to the control group.
5) Results of animal toxicity studies of ARA-rich oil (25% or 40% ARA) are consistent with those of other sources of ARA-rich oil ingredients.
6) To evaluate the effects of ARA supplementation in preterm infants (born < 33 weeks gestational age; weighing 750 to 1800 g at birth), the first phase of the study covered the time of the first enteral feeding until the post-discharge visit corresponding to term CA, and the second phase ran from term CA until 12 months CA. Preterm infants were randomized to formulas supplemented with ARA-rich oil and DHA-rich oil. In the first phase of the study, supplementation with ARA (0.43% total fat as ARA, either fungal or egg-derived TG source) in combination with DHA (0.27% total fat) did not result in adverse effects on measured outcomes, such as growth, development (visual, general, language development, information processing, and temperament), blood biochemistry, plasma antioxidants, plasma and RBC FAs, tolerance, stool characteristics, morbidity, and the incidence of AEs during the initial in-hospital course. In the second phase of the study, supplementation with ARA (0.43% total fat, either fungal or egg-derived triglyceride source) in combination with DHA (0.16% total fat) did not result in adverse effects on measured outcomes described above for a duration of time after hospital discharge.

7) Several studies of other sources of ARA-rich oil have shown no short-term (21 days - 4 months post-conceptual age [PCA] or long-term (up to 92 weeks PCA, following feeding through 48 weeks PCA) adverse effects of ARA and DHA supplementation in preterm infants (Carlson et al., 1998; Clandinin et al., 1997, 1999; Faldella et al., 1996; Foreman-Van Drogelen et al., 1996; Koletzko et al., 1989, 1995a; Vanderhoof et al., 1999,2000).

8) Several studies of other sources of ARA-rich oil have shown no adverse effects of ARA and DHA supplementation on growth and developmental outcomes in term infants (Auestad et al., 1997; Birch et al., 1998,2000; Carlson et al., 1996; Hoffman et al., 2000; Kohn et al., 1994; Lucas et al., 1999; Makrides et al., 1999,2000; Scott et al., 1998; Willatts et al., 1996, 1998a, 1998b).

Overall, pre-clinical animal toxicity studies and clinical studies of ARA-rich oil support the safety of ARA supplementation of infant formulas in both preterm and term infants.

GRN 000326, filed by Cargill: Cargill's refined ARA-rich oil (RAO; ≥40% ARA) produced by *M. alpina* strain 149-N48 (Pages 61-153)

Intended use levels included 0.4% of total FAs as ARA in preterm infants and 0.75% total FAs as ARA in term infants.

This notice described that the subject of the GRAS determination of Cargill's ARA-rich oil is chemically similar or essentially equivalent to other commercially available ARA-rich oil ingredients. Cargill's ARA-rich oil's safety profile matches that of other ARA-rich oil products reported in the scientific literature. The following are the key points of safety discussion.

1) ARA-rich oil was not mutagenic or genotoxic.
2) From a subchronic toxicity study with an in-utero exposure, the NOAEL of ARA-rich oil was determined to be approximately 3,000 mg/kg bw/day. Results for ARA-rich oil are consistent with those from toxicology studies on other ARA-rich oil products.
3) Several human clinical studies provided information on selected safety endpoints after infant exposure to ARA-rich oil. Those studies included Fang et al. (2005), Groh-Wargo et al. (2005), Clandinin et al. (2005), Birch et al. (2005), Hoffman et al. (2006), Pastor et al. (2006), Siahanidou et al. (2007), Burks et al. (2008), Field et al. (2008), Henriksen et al. (2008), Hoffman et al. (2008), Makrides et al. (2005), Simmer et al. (2008a, 2008b), and Rosenfeld et al. (2009).

GRN 000730, filed by LiNyi Youkang Biology Co., Ltd.: ARA-rich oil (≥40% ARA) produced by *M. alpina* strain LU166 (Pages 28-44).

Intended use levels included 0.4% of total FAs as ARA in preterm infants and 0.75% total FAs as ARA in term infants.

This notice incorporated, by reference, the safety and metabolism studies discussed in the previous four GRAS notices (GRN 000326, pages 61-153; GRN 000094, pages 78 - 318; GRN 000080, stamped pages 16-23 and 48-55; and GRN 000041, stamped pages 108-118 and 175-418).

1) Mean lethal dose (LD_{50}) of ARA-rich oil (purity, 42.1%) was far above 15.2 g/kg bw.
2) Based on a 90-day oral toxicity study and reproductive/developmental toxicity studies of ARA-rich oil (40.3% ARA; Falk et al., 2017; Lewis et al., 2016), a NOAEL of ARA-rich oil was determined to be 5,000 mg/kg bw/day which may correspond to 2,000 mg ARA/kg bw/day. However, this notice summarized a 90-day oral toxicity study with an in-utero exposure (F_1) (Hempenius et al., 2000), which reported a NOAEL of 970 mg ARA-rich oil/kg bw/day or 374 mg ARA/kg bw/day.
3) The studies published since 2010 reported no adverse effects of ARA-rich oil in preterm infants (Almaas et al., 2015, 2016; Alshweki et al., 2015; Kitamura et al., 2016; van de Lagemaat et al., 2011; Westerberg et al., 2011). These studies reported that ARA supplementation was safe up to 0.91% total FAs. Measurements included adverse effects and safety, growth, and anthropometric parameters.
4) The studies using 0.64-0.72% of total FAs as ARA (0.72%, Birch et al., 2005, 2007; 0.64%, Birch et al., 2010; Colombo et al., 2011; Drover et al., 2011, 2012) demonstrated the safety of ARA-rich oil derived from *M. alpina* in term infants.

Overall, this notice concluded that the publicly available scientific literature on the consumption and safety of ARA-rich oils in infant clinical studies was extensive and sufficient to support the safety and GRAS status of the ARA-rich oil.

GRN 000963, filed by BASF Corporation: ARA-rich oil (≥40% ARA) produced by *M. alpina* (Pages 25-33).
Intended use levels included 0.4% of total FAs as ARA in preterm infants and 0.75% total FAs as ARA in term infants.
Publicly available preclinical toxicology studies have been summarized in the previously cited GRNs of ARA and include absorption, distribution, metabolism, and excretion (ADME), acute and subchronic toxicity, reproductive and developmental toxicity, and mutagenicity/genotoxicity studies (Bums et al., 1999; Falk et al., 2017; Gao et al., 2014; Hempenius et al., 1997, 2000; Lewis et al., 2016; Merritt et al., 2003; Nisha et al., 2009; Tyburczy et al., 2012; Wibert et al., 1997). The studies were conducted in rats and piglets. This notice cited the reviews of the studies that were included in GRNs 000326 and 000730, and brief summaries of selected studies were provided. Numerous studies have been conducted and published in support of the safety evaluation of ARA and ARA-rich oil, including in-vitro studies, in-vivo animal studies, and clinical studies in humans, including infants. The available published scientific data on the safety of ARA sourced from *M. alpina* are extensive. The compositional profile of the ARA-rich oil presents no obvious safety concerns. The totality of published study data, as presented in previous GRNs reviewed by the FDA, supports the safe use of BASF's ARA-rich oil from *M. alpina* in infant

formulas up to 0.75% of total FAs as ARA, which may correspond to 41-50 mg ARA/kg bw/day. These EDIs of ARA are in agreement with current recommendations for ARA consumption by preterm and term infants of 35-45 mg/kg bw/day (Koletzko et al., 2014a, 2014b).

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 AEs and the use of DHA and ARA oils in infant formulas (FDA, 2011b; 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 (2011b) 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 preterm and term infants who would be consuming exempt and non-exempt infant formula, respectively.

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 meet 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 0000326, 000080, and 000041. For preterm 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 preterm infants. In addition, an intended use level of up to 0.5% FAs as ARA in preterm 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 preterm 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 preterm 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 preterm 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.

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). 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 though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. The specifications and 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 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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7.B. Reference That Is Not Generally Available

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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:

Approved by: Yu-Guang ZHOU | Signature:

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

Conclusion of Identification:

According to the results of the morphological, physiological properties, sequence of 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

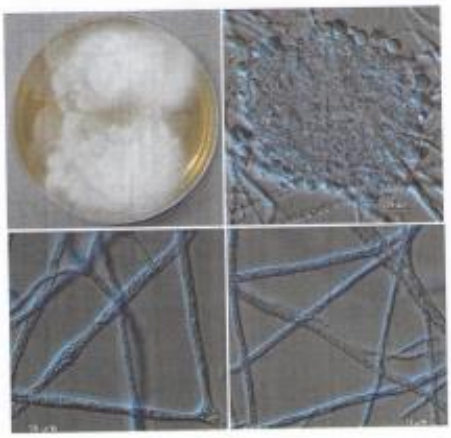
Applicant: Fujian Runke Bioengineering Corp., Ltd.

(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.

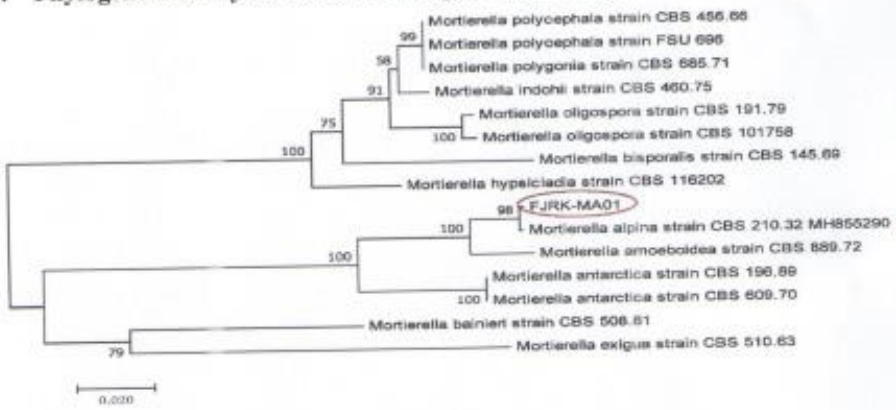


2. Partial sequence of rRNA gene

(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

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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.
This analytical report shall not be excerpted or modified without prior written approval from Eurofins. The report shall be utilized in full.
The result(s) is(are) only for internal use by the client and not for publicly available as evidence.Without the written permission of Eurofins, any party is prohibited from using the test results and the report for publicity or promotions or marketing.
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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

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.
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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

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
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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	502-2021-00126364	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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		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 identfitications 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	502-2021-00126365	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 idenfitications 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

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.
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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 (℃)	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 candles 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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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
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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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DAkkS Deutsche Akkreditierungsstelle D-PL-14292-01-00

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
△ CNAS # DAkkS □CMA
☆ means the test is subcontracted within Eurofins group
● means the test is subcontracted outside Eurofins group

Sum compounds results are calculated from the results of each quantified compound as set by regulation
The uncertainty has not been taken into account for standards that already include measurement uncertainty or on explicit request of client.
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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
^# **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	502-2022-00037070	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.
JinDu Industrial Park Zhao-an County
Zhangzhou City Fujian Province
Fax 0596-3552000

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 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		

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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

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
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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	

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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	

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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

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< LOQ: Below Limit of Quantification
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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		

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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		

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Appendix C. Sterols of ARA-rich Oil

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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.

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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 shows as average value ± standard deviation (g/100g oil)			
[a]4α-Methyl zymosterol	-[e]	-[e]	-[e]
[a]24-Methyl cholesta-5,24(25 or 28)-dien-3β-ol C28:2	-[e]	-[e]	-[e]
[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	-[e]	-[e]	-[e]
[a]24-methyl cholesta-5(25)27-dien-3β-ol	-[e]	-[e]	-[e]
[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	-[e]	-[e]	-[e]
[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; "-[e]"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, and 000963) 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. Exempt infant formula refers to formulas for preterm infants only and does not include use in other exempt formulas (e.g., hypoallergenic formulas, formulas for inborn errors of metabolism).

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.

Runke Bioengineering intends to market the ARA-rich oil as an ingredient in exempt (preterm 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. The proposed use of ARA-rich oil is to provide 0.75% and 0.50% ARA by weight of FAs in term and preterm infant formulas, respectively, in combination with a safe and suitable source of DHA. The intended use of ARA-rich oil to deliver this concentration of ARA corresponds to 1.974% of total fat in non-exempt term infant formula and 1.316% of total fat in exempt preterm infant formula. 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. Exempt infant formula use includes preterm infants as well as use in hypoallergenic infant formulas for term infants (from birth to 12 months). The intended use levels are similar to all other approved uses for incorporation of ARA-rich oil in infant formula (GRNs 000041, 000080, 000094, 000326, 000730, and 000963). 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; GRN 000730, pages 29-44; GRN 000326, pages 61-153; GRN 000094, pages 78 - 318; GRN 000080, stamped pages 16-23 and 48-55; GRN 000041, stamped pages 108-118 and 175-418.

The major sterols associated with *M. alpina* oil include desmosterol and 24-methyl sterols. In 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 preterm 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 preterm 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 long-chain PUFA 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, preterm infants should have higher postnatal long-chain PUFA requirements than full-term infants, although ARA supplementation can benefit both term and preterm infants.

Mutagenicity and Genotoxicity Studies of Runke Bioengineering's ARA-Rich Oil

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.

Animal Toxicity Studies of Runke Bioengineering's ARA-Rich Oil

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.

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, SUNTAGA40S 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

Our review has focused on the papers published since the FDA's last review of 2020-2021 or the papers published between July 2020 and May 2023.

Preterm Infants

Previous GRAS notices provided information and/or clinical study data that supported the safety of ARA ingredients for use in infant formula. Almaas et al. (2015, 2016), Westerberg et al. (2011), and Henriksen et al. (2008, 2016) reported that human milk supplemented with 31 mg ARA (0.91% of total FAs) and 32 mg DHA (0.86% of total FAs) per 100 mL, providing 47 and 59 mg/kg bw/day of ARA and DHA, respectively, was safe in preterm infants when consumed from 1 week after birth up until discharge from hospital (9 weeks on average).

The studies by Manley et al. (2011), Gunaratne et al. (2019), and Clandinin et al. (2005) reported that ARA supplementation at 0.6% of FAs in combination with DHA did not result in adverse effects on measured outcomes including gastrointestinal tolerance in preterm infants. Recently, Frost et al. (2021) found that daily doses up to 240 mg/kg bw/day ARA (which may correspond to up to approximately 4% FAs as ARA) for 8 weeks did not result in any adverse effects in preterm infants. In addition, emulsion supplement (ARA and DHA 2:1; Formulaid™, DSM Nutritional Products) providing 100 mg/kg bw/day ARA (derived from *M. alpina*; which may correspond to up to approximately 1.7% of FAs as ARA) plus 50 mg/kg bw/day DHA (derived from *C. cohnii*) for up to 12 weeks, respectively, was well tolerated in preterm infants (Hellström et al., 2021; Pivodic et al., 2022. Sojobom et al., 2023; Wendel et al., 2023).

An intended use level of up to 0.5% FAs as ARA in preterm 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 preterm infants (Koletzko et al., 2014a).

Term Infants

Since the FDA's review in 2020-2021, 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 the risk of lower respiratory tract infections, wheezing/ asthma, or other allergic diseases when compared

to controls. Studies of term infants have not reported adverse effects on allergies or gastrointestinal symptoms associated with ARA/DHA-supplemented infant formula (Birch et al., 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). The FDA considered the USDA's reports, which indicated the time-dependent increase of market shares of infant formulas containing DHA- and ARA-rich oil products: 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 adverse events (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".

In conclusion, ARA-rich oil, combined with a safe and suitable source of DHA, is not expected to adversely impact the preterm 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 preterm 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]

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Date

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001162	Oct 4, 2023
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

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

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

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

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Sunny Tsai	International Marketing Manager

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

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

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

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

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

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

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

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

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

Telephone Number	Fax Number	E-Mail Address
3018756454	7039880103	scho@aceoners.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Arachidonic acid (ARA)-Rich Oil

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

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

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

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

☒ a) GRAS Notice No. GRN 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.

The proposed use of arachidonic acid (ARA)-rich oil derived from Mortierella alpina FJRK-MA01 is to provide 0.75% and 0.50% ARA by weight of Fatty acids in term and preterm 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 Bioenginnering
(name of notifier)

has concluded that the intended use*(s)* of Arachidonic acid (ARA)-rich oil derived 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)

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

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

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	Sunny Tsai, International Sales Manager	10/04/2023

SECTION G – LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	RunkeARA-richOilFinal9-30-23SubmittedtoFDA.pdf	Administrative
	ARAcoverletter10-3-2023.pdf	Administrative