Spherix Consulting Group

June 15, 2020

Rachel Morissette, Ph.D.
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
CPK-2 Building, Room 2092
5001 Campus Drive, HFS-225
College Park, MD 20740

Dear Dr. Morissette:

It is our opinion that the enclosed GRAS Notification for the Use of ARA-rich Oil as an Ingredient in Exempt and Non-exempt Infant Formula constitutes a new notification because Hubei Fuxing Biotechnology Co., Ltd utilizes a novel strain of Mortierella alpina, M. alpina AF, in the production of ARA-rich oil.

We thank you for taking the time to review this GRAS notification. Should you have additional questions, please let us know.

Sincerely,

Claire L. Kruger, PhD, DABT, CFS
Managing Partner

Enclosure:

CD containing Form 3667, cover letter, GRAS Notification for the Use of ARA-rich Oil as an Ingredient in Exempt and Non-exempt Infant Formula, and all references

Spherix Consulting Group, Inc.
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GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION FOR THE USE OF ARA-RICH OIL AS AN INGREDIENT IN EXEMPT AND NON-EXEMPT INFANT FORMULA

Prepared for:

Hubei Fuxing Biotechnology Co., Ltd.
FL. 11, Bldg 23
Baishazhou Enterprise City
Baishazhou Avenue, Wuhan District
Wuhan 430000
China

Prepared by:

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June 15, 2020

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOCS: American Oil Chemist's Society

ARA: Arachidonic acid

CCP: Critical control point

CFR: Code of Federal Regulations

CFU: Colony forming units

DHA: Docosahexaenoic acid

EDI: Estimated daily intake

EU: European Union

EFSA: European Food Safety Authority

FCC: Food Chemicals Codex

FDA: Food and Drug Administration

FFDCA: Federal Food, Drug, and Cosmetic Act

FOIA: Freedom OF Information Act

FSANZ: Food Standards of Australia and New Zealand

GMP: Good manufacturing practice

GRAS: Generally Recognized As Safe

GRN: GRAS Notification

ISO: International Organization for Standardization

LCPUFA: Long-chain polyunsaturated fatty acids

LOQ: Limit of quantitation

meq: Milliequivalents

NMKL: Nordic Committee on Food Analysis

NOAEL: No observed adverse effect level

PAH: Polyaromatic hydrocarbons

PCBs: Polychlorinated biphenyls

PUFA: Polyunsaturated fatty acid (PUFA

PPARγ: Peroxisome proliferator-activated receptor γ

I. SIGNED STATEMENT OF THE CONCLUSION OF GENERALLY RECOGNIZED AS SAFE (GRAS) AND CERTIFICATION OF CONFORMITY TO 21 CFR §170.205-170.260

A. SUBMISSION OF GRAS NOTICE

Hubei Fuxing Biotechnology Co., Ltd. is hereby submitting a GRAS notice in accordance with subpart E of part 170 of Title 21 of the United States Code of Federal Regulations.

B. NAME AND ADDRESS OF THE SPONSOR

Hubei Fuxing Biotechnology Co., Ltd.
No. 18 Fuxing Ave,
Chenhu Town, Hanchuan City 431608, Hubei Province,
China

C. COMMON OR USUAL NAME

Arachidonic acid (ARA)-rich oil, or ARA-rich oil

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

This notification does not contain any trade secrets or confidential information.

E. INTENDED USE

ARA-rich oil is intended for use as an ingredient in exempt infant formula that will be consumed by preterm infants as well as non-exempt infant formula for term infants.

F. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of ARA-rich oil as an ingredient in infant formula is based upon scientific procedures as described under 21 CFR §170.30(b). The intake of ARA-rich oil from the intended uses specified above has been determined to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). The GRAS determination is made on the basis of generally available and accepted information evaluated by independent experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food.

Hubei Fuxing Biotechnology Co., Ltd is proposing to market ARA-rich oil, produced by Hubei Fuxing Biotechnology Co., Ltd, China, as a source of ARA-rich oil used in the

manufacture of infant formula. The end-use infant formulas are exempt pre-term infant formula and non-exempt term infant formula. Consistent with other GRAS sources of ARA-rich oil (GRN 730; 326), this ingredient is produced by the fungus *Mortierella alpina* and specifications stipulate a minimum of 40% arachidonic acid in the oil.

The following safety evaluation considers the composition, intake, nutritional, microbiological, and toxicological properties of Hubei Fuxing's ARA-rich oil based on publicly available data from essentially equivalent ARA-rich oils as determined GRAS in GRN 326. Corroborative safety data are described in GRNs 730, 41, 80, and 94, each of which received "no questions" letters from the United States Food and Drug Administration (FDA). The proposed use of Hubei Fuxing's ARA-rich oil as an ingredient in non-exempt term infant formula and exempt pre-term infant formula has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based upon the following:

- The compositional data and product specifications are demonstrative of carefully controlled production and purification processes. ARA-rich oil contains no detectable contaminants of concern for human health.

- The morphology, biochemistry, and physiology of *M. alpina* are well-documented and it is not pathogenic or toxigenic. *M. alpina* is not genetically modified.

- The FDA has issued 'no question' letters for five GRAS notices for ARA-rich oils derived from *M. alpina* for infant formula (GRNs 41, 80, 94, 326, and 730). A comparison of the specifications between the ARA-rich oil that is the subject of this notification and those in GRN 326 demonstrates that the product specifications for Hubei Fuxing's ARA-rich oil are comparable to the product specifications for the ARA-rich oil generated from *M. alpina* as described in GRN 326, with some parameters being more stringently controlled, including acid value, anisidine value, mercury, and moisture. Specifications for ARA rich oils determined GRAS in GRNs 41, 80, 94, and 730 show that they are similar in composition to the ARA rich oil produced by Hubei Fuxing and therefore relevant as corroborative data.

- The intended use for ARA-rich oil is as an ingredient in exempt and non-exempt infant formulas. The functional importance of long-chain polyunsaturated fatty acids (LCPUFA) in pregnancy, lactation, and infancy have been the subject of numerous clinical evaluations, particularly as they relate to the n-6 and n-3 LCPUFA, ARA (C20:4n-6) and docosahexaenoic acid (DHA; C22:6n-3). Studies have suggested 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. Breast milk is the preferred method of providing an exogenous supply of ARA and DHA. However, when for medical or personal reasons, infant formula is chosen as a sole source or supplementary infant food, addition of ARA and DHA to the formula may support the adequate nutritional status of pre-term and term infants. Based on current knowledge regarding the importance of LCPUFA and their presence in human milk, guidelines and recommendations established by the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation collectively recommend that the level of DHA in infant formula be 0.20 to 0.50 weight percent of total fat, with the minimum amount of ARA being equivalent to the DHA content. These recommendations lead to the use of highly refined oils as sources of ARA and DHA for addition to infant formulas.

- 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 proposed use of ARA-rich oil is intended to provide 0.75% and 0.40% ARA by weight of fatty acids in term and pre-term infant formulas, respectively; this is within the range found in human milk. The intended use of ARA-rich oil to deliver this concentration of ARA, corresponds to 1.875% of total fat for non-exempt term infant formula and 1.00% of total fat for exempt pre-term infant formula. This intended use level is consistent with the levels of use cited in GRN 326 (term and pre-term infants) and is also consistent with the same use levels of ARA-rich oils cited in GRNs 80, 94, and 730.

- An estimate of exposure to ARA from its addition to infant formula at target ARA levels of 0.75 g and 0.40 g per l00 g total fat for term and pre-term infant formulas may be calculated as follows: Assuming human infants consume 100 kcal/kg body weight/day (term infants) to 120 kcal/kg body weight/day (pre-term infants), of which fat comprises about 50% of the energy, an infant will consume 5.6 g (term infants) to 6.7 g (pre-term infants) of fat/kg body weight /day (1 g fat = 9 kcal). These correspond to intakes of ARA of 42 mg and 27 mg ARA/kg body weight /day (or 104 and 67 mg of ARA-rich oil/kg body weight/day) for term infants and pre-term infants, respectively.

- The source organism, manufacturing process, product specifications, and intended uses of Hubei Fuxing’s ARA-rich oil are essentially equivalent to ARA-rich oil cited in GRN 326; therefore, publicly available animal and human safety and tolerance

studies of this ARA-rich oil are used as the pivotal data to support the safety of Hubei Fuxing's ARA-rich oil. Data from studies of other ARA-rich oils cited in GRNs 41, 80, 94, and 730 are corroborative of the safety of the Hubei Fuxing's ARA rich oil.

  - The safety of ARA-rich oils as ingredients in infant formula has been reviewed by numerous regulatory bodies worldwide. In these jurisdictions, the conclusions reached were that ARA-rich oils derived from *M. alpina*, meeting appropriate food grade specifications, provide a safe ARA source for supplementation of infant formula. These decisions have led to its availability for this use in at least 50 countries worldwide.

- Numerous animal safety studies have been conducted over a period of more than a decade on ARA-rich oils derived from *M. alpina*. The pivotal study cited in GRN 326 is a subchronic toxicity study with an *in utero* exposure which establishes a no-observed adverse event level (NOAEL) of 5% ARA-rich oil in the diet, equivalent to an average intake of ARA-rich oil of 3170 mg/kg/day (Casterton et al., 2009).

  - The safety is supported by lack of systemic toxicity and reproductive or developmental toxicity reported in corroborative studies described in GRN 730: a 28-day toxicity study, a 90-day subchronic toxicity study with an *in utero* exposure, a 90-day subchronic toxicity study, a reproductive and developmental toxicity study, and a neonatal piglet study (additional corroboration provided from a neonatal piglet study of a blend of ARA- and DHA-rich oils). These studies corroborate the results from the pivotal 90-day toxicology study which established a NOAEL of 3170 mg/kg/day. In addition, studies showed a lack of adverse effects on developmental or reproductive parameters. Tolerance and safety in a neonatal piglet model determined that dietary ARA concentration of up to 96 mg ARA/100 kcal was safe and well tolerated.

  - A corroborative, unpublished bacterial reverse mutation assay of Hubei Fuxing's ARA-rich oil was negative.

- There were no test article-related adverse effects reported in clinical studies of infant formula containing ARA-rich oils in pre-term infants when used at levels up to 0.91% total fatty acid content using multiple sources of ARA-rich oil, including *M. alpina*.

- Clinical studies, detailed in GRN 326, using 0.64-0.72% of total fatty acids as ARA also confirmed safety of infant formula containing ARA-rich oil derived from *M. alpina* in term infants.

Taken together, the available data from studies conducted on ARA-rich oils from *M. alpina* establish a strong body of evidence for the safety of ARA-rich oil as a source of ARA for supplementation of infant formula.

The GRAS status of ARA-rich oil (compliant with the established food grade specifications), under the intended conditions of use proposed by Hubei Fuxing, has been determined through the deliberations of Roger Clemens, DrPH, CNS, CFS, FACN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, CNS, FACN, and Thomas Sox PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of ARA-rich oil and the potential human exposure to ARA-rich oil resulting from its intended use as an ingredient in infant formula, and have concluded:

> *There is no evidence in the available information on ARA-rich oil that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when ARA-rich oil is used at levels that might reasonably be expected from the proposed applications. ARA-rich oil is GRAS for use as an ingredient in the manufacture of infant formula.*

ARA-rich oil is thus safe and GRAS at the proposed levels of ingestion. It is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

G. PREMARKET APPROVAL

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the substance is GRAS under the conditions of intended use.

H. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, PhD, DABT, Managing Partner, Spherix Consulting Group, Inc., at 11821 Parklawn Drive, Suite 310, Rockville, MD 20852. Telephone: 301-775-9476; Email: ckruger@spherixgroup.com, or be sent to FDA upon request.

I. FREEDOM OF INFORMATION ACT (FOIA)

Parts 2 through 7 of this notification do not contain data or information that is exempt from disclosure under the FOIA.

J. INFORMATION INCLUDED IN THE GRAS NOTIFICATION

To the best of our knowledge, the information contained in this GRAS notification is complete, representative and balanced. It contains both favorable and unfavorable information, known to Hubei Fuxing Biotechnology Co., Ltd. and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

Signature of Authorized Representative of
Hubei Fuxing Biotechnology Co., Ltd.

2020. 6. 11

Date

II. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT OF THE NOTIFIED SUBSTANCE

A. COMMON OR USUAL NAME

Arachidonic acid (ARA)-rich oil, or ARA-rich oil

B. TRADE NAME

Arachidonic Acid Oil

C. DESCRIPTION OF ARA-RICH OIL

Arachidonic acid (ARA, 20:4n-6)-rich oil is a source of ARA in infant formula, produced by the fungus *Mortierella alpina*, and consists of at least 40% ARA in addition to other long chain saturated and unsaturated fatty acids.

1. Background on Arachidonic Acid

Arachidonic acid is an n-6 polyunsaturated fatty acid (PUFA) found in the phospholipids of the cell membrane, and is particularly abundant in the brain, muscles, and liver. Arachidonic acid is a precursor of eicosanoids, important signaling molecules that include prostaglandins, thromboxanes, and leukotrienes. Virtually all cellular ARA is esterified in membrane phospholipids where its localization is tightly regulated through multiple interconnected pathways (Hadley et al., 2016).

Arachidonic acid is not an essential fatty acid, meaning that humans can synthesize it from other PUFAs, such as linoleic acid. Long chain PUFAs are essential for the normal development of infants and children. Arachidonic acid is a key nutrient in human breast milk, as both term and pre-term infants have limited ability to convert the precursor PUFAs to ARA, due to reduced concentrations and activity of desaturase enzymes (Hadley et al., 2016; Martin et al., 2011). The supplementation of infant formula with ARA at levels consistent with those in human milk is important because the n-6 and n-3 fatty acids present in human milk have critical roles in membrane structure and as precursors of potent and highly reactive eicosanoids (Hadley et al., 2016). Arachidonic acid begins to accumulate in neuronal tissue in the developing fetus during the third trimester, and gradually increases until it plateaus around age 4. Arachidonic acid is one of the most abundant fatty acids in the brain, and is present in similar quantities to another PUFA, docosahexaenoic acid (DHA).

2. Source and Strain Identity

M. alpina is in the Zygomycetes Class in the Zygomycota Order. All Zygomycetes share two properties that readily distinguish them from the remaining classes of fungi. First, their asexual spores are endogenously formed, and second, their mycelium shows no cross walls except in regions where a specialized cell is formed from a hyphal tip (non-septate mycelium). The Zygomycetes include a group of soil inhabitants known as terrestrial Zygomycetes. The organism is easily isolated from the soil and has been identified in soils from diverse geographies (Deacon, 2006). Like many fungi, *M. alpina* is associated with common root crops and therefore, is in the direct food chain of many mammals.

Mortierella species have been widely studied in isolated laboratory culture and their morphology, biochemistry, and physiology are well documented. *M. alpina* has been described in Japanese publications and patents as a potential source of ARA and, consequently, it has been the subject of many intensive laboratory investigations (Bajpai et al., 1991; Lindberg and Molin, 1993; Shinmen et al., 1989; Totani and Oba, 1987), none of which have reported *M. alpina*-associated pathogenicity or toxigenicity to humans or animals (Domsch et al., 1980; Scholer et al., 1983).

ARA-rich oil that is the subject of this GRAS determination is manufactured from *M. alpina* strain AF, endogenously found in soil, isolated by Huazhong University of Science and Technology, and used by Hubei Fuxing. The strain was deposited on March 10th, 2003 at the Type Culture Collection Committee of the Chinese Academy of Sciences (Deposit No.: CGMCC No. 0903). *M. alpina* strain AF has been cultured at Hubei Fuxing Biotechnology Co. since 2003.

M. alpina AF was verified to be *M. alpina* through analysis of the *18S* and *26S* rRNA gene sequences. The sample submitted for analysis was from a slant culture used to maintain the strain before being used in the production process. The *18S* and *26S* genes were sequenced, then those sequences were submitted to BLAST (basic local alignment sequencing tool) analysis to verify that the genes are identical to the *18S* and *26S* sequences for *M. alpina*.

The *18S* rRNA sequence of *M. alpina* AF was 100% identical to other *M. alpina* strain *18S* rRNA sequences (Table 1). The gene hits with 100% sequence identity included three hits from the *18S* gene in *M. alpina*.

Table 1. Top BLAST Hits for *18S* rRNA Sequencing Results from Hubei Fuxing's *M. alpina* AF

NCBI Accession Number	Description	Max Score	Total Score	Query Cover	E value	Identity
KJ890360.1[1]	*Mortierellales sp. AGED 18S* ribosomal RNA gene, partial sequence	1397	1397	100%	0.0	100%
AB534492.1[2]	Uncultured fungus gene for *18S* rRNA, partial sequence, clone: I_3_76	1397	1397	100%	0.0	100%
AB521052.1[3]	*Mortierella sp. CO-21* gene for *18S* ribosomal RNA, partial sequence	1397	1397	100%	0.0	100%
AB476409.1[4]	***Mortierella alpina*** gene for *18S* rRNA, partial sequence	1397	1397	100%	0.0	100%
AJ271630.1[5]	***Mortierella alpina*** *18S* rRNA gene (partial), *5.8S* rRNA gene, *26S* rRNA gene (partial), internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), strain CBS 224.37	1397	1397	100%	0.0	100%
AJ271629.1[5]	***Mortierella alpina*** *18S* rRNA gene (partial), *5.8S* rRNA gene, *26S* rRNA gene (partial), internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), strain CBS 528.72	1397	1397	100%	0.0	100%

NCBI: National Center for Biotechnology Information
[1]Tan L. et al., 2014, unpublished, direct submission to NCBI
[2]Takada Hoshino Y. and Morimoto S. 2010
[3]Tagawa M. et al., 2010
[4]Sekiguchi H. et al., 2009, unpublished, direct submission to NCBI
[5]Mackenzie D.A. et al., 2000

The *26S* rRNA sequence from *M. alpina* AF was 99% identical to *26S* rRNA *M. alpina* genes (Table 2), as well as *M. polygonia* and *M. globalpina*, which are closely related to *M. alpina* (Wagner et al., 2013).

Table 2. Top BLAST Hits for *26S* rRNA Sequencing Results from Hubei Fuxing's *M. alpina* AF

NCBI Accession Number	Description	Max Score	Total Score	Query Cover	E value	Identity
LC125559.1[1]	***Mortierella alpina*** gene for *26S* rRNA, partial sequence, strain: AB14-3	1327	1327	100%	0.0	99%
LC125558.1[1]	***Mortierella alpina*** gene for *26S* rRNA, partial sequence, strain: AB14-1	1323	1323	100%	0.0	99%
LC125544.1[1]	***Mortierella alpina*** gene for *26S* rRNA, partial sequence, strain: AB7-2	1317	1317	100%	0.0	99%
LC125553.1[1]	***Mortierella alpina*** gene for *26S* rRNA, partial sequence, strain: AB11-6	1314	1314	100%	0.0	99%
LC125551.1[1]	***Mortierella alpina*** gene for *26S* rRNA, partial sequence, strain: AB11-3	1314	1314	100%	0.0	99%
LC125542.1[1]	***Mortierella alpina*** gene for *26S* rRNA, partial sequence, strain: AB6-11	1314	1314	100%	0.0	99%
JN940866.1[2]	***Mortierella alpina*** strain CBS 210.32 *28S* ribosomal RNA (LSU) gene, partial sequence	1284	1284	100%	0.0	99%
LC125576.1[1]	*Mortierella polygonia* gene for *26S* rRNA, partial sequence, strain: AB16-17	1280	1280	100%	0.0	99%
LC125547.1[1]	*Mortierella polygonia* gene for *26S* rRNA, partial sequence, strain: AB7-7	1280	1280	100%	0.0	99%
LC125543.1[1]	*Mortierella polygonia* gene for *26S* rRNA, partial sequence, strain: AB7-1	1280	1280	100%	0.0	99%
AB517932.1[3]	*Mortierella globalpina* gene for *28S* ribosomal RNA, partial sequence	1269	1269	100%	0.0	99%

NCBI: National Center for Biotechnology Information
[1]Tsuji M., et al., 2016. Unpublished, submitted to NCBI
[2]Schoch CL et al., 2012.
[3]Hirose D, et al., 2009. Unpublished, submitted to NCBI

Together, these sequencing results confirm that Hubei Fuxing is culturing *M. alpina* to produce ARA-rich oil.

D. PRODUCTION PROCESS

ARA-rich oil is produced at Hubei Fuxing Biotechnology Co., Ltd in Wuhan, China. Hubei Fuxing has been certified as meeting the Food Safety System Certification (FSSC) 22000 standards.

1. Production of ARA-Rich Oil

The production of ARA-rich oil occurs in the following steps: expansion of *M. alpina* AF culture, collection and extraction of the crude oil, and refining of the crude oil into the final product. One production period is considered one batch.

All culturing steps in the generation of ARA-rich oil occur under aerobic conditions in the absence of light in a closed system. Each culture step is monitored for microbial contamination by light microscopy to confirm healthy cell morphology and cell population with no evidence of contamination before advancing to the next step for producing ARA-rich oil. All media are sterilized. Prior to initiation and culturing of *M. alpina* AF, all raw materials and food contact materials are inspected and must comply with internal quality parameters as a critical control point.

a. Initiation of M. alpina AF culture

M. alpina AF is maintained as spores, stored in liquid nitrogen. The solid slant culture is maintained as a stock for future cultures, and the genotypic identity of the strain is validated every year.

To initiate a culture from spores, spores are transferred to a shake flask with sterile culture medium for inoculation. The culture is monitored for the absence of microbial contamination and the cell density is assessed as an indicator of a healthy population. The culture is considered ready for expansion to the seed tank when the biomass meets cell density quality control specifications.

b. Expansion of M. alpina AF culture

The initiation biomass is transferred and divided equally to four Level 1 seed tanks with stirring and aeration until the biomass meets the cell density quality control specification. The biomass from the Level 1 seed tank is then equally distributed into four Level 2 seed fermentation tanks and cultured until the biomass meets cell density quality control specifications. The entire culture is then transferred to eight Level 3 fermentation tanks to promote arachidonic acid production. Level 3 fermentation step is complete when the biomass, total oil and ARA content

internal control standards for the semi-finished product are met. The ARA content of the biomass in the Level 3 fermentation tank is a critical control point for the production process. The expansion process for *M. alpina* AF is shown in Figure 1.

The fermentation steps are controlled to ensure consistent pH, aeration rate, and temperature. All culture steps are monitored for evidence of contamination and for cell division as an indicator of a viable fungal population every 8 hours. If any visible contamination is detected by light microscopy, the entire biomass is discarded via sewage disposal.

c. Crude oil extraction

After the biomass in the fermentation tank has generated sufficient ARA content to meet internal quality control specifications, the biomass is filtered from the medium and the medium is discarded. The biomass is washed 3 times with sterilized water. The biomass is then dried with food grade commercial driers under controlled temperature and pressure such that the moisture of the dried biomass meets internal quality specifications. The crude oil is extracted with butane under controlled temperature and pressure conditions. After extraction, the fungal dregs of the biomass are discarded, and the butane is removed from the crude oil. The solvent leaching process is repeated multiple times to remove the butane from the crude oil. The butane is reserved, refined, and reused for the next production. The crude oil extraction is shown in Figure 1.

The crude oil has no more than 5% biomass and the following quality attributes are recorded: peroxide, taste/smell, acid value, phosphorus, unsaponifiable matter, and anisidine value. The crude oil is stored in stainless steel barrels under a layer of N_2 to minimize oxidation at no more than -5^{o}C for an average of 2 months before refining. The storage time will not exceed 2 years. The crude oil is stored temporarily in a warehouse before continuing to ARA-rich oil refining in the next step.

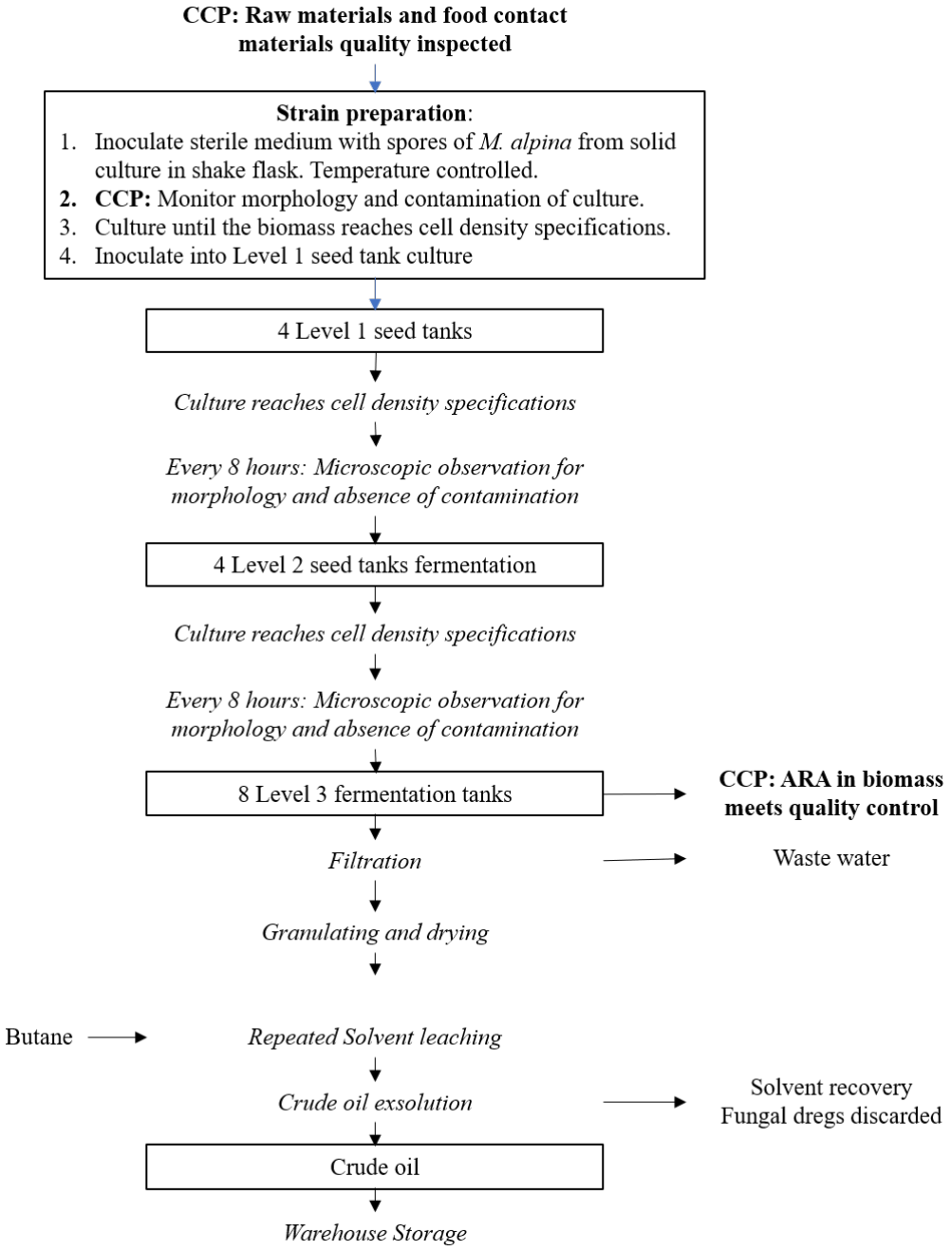

Figure 1. Production of ARA-rich Crude Oil

The *M. alpina* AF solid slant culture is used to inoculate four Level 1 seed tanks. After inoculation, *M. alpina* AF is cultured until the biomass reaches density quality control specifications. All cultures are and monitored for contamination and normal cell morphology. The biomass from the Level 1 tank is used to inoculate the four Level 2 tanks. The biomass is cultured until the reaches density quality control specifications. The biomass is then used to inoculate eight Level 3 fermentation tanks for the final culture step. After culturing in the fermentation tank, a sample is taken to measure ARA before producing the crude oil. Culturing is finished when sufficient ARA content is measured in the biomass. Then the biomass is filtered from the medium, dried, and butane is used to extract the crude oil. *CCP: critical control point*

d. ARA-rich oil refining

The oil refining process is shown in Figure 2. To continue with the production process, the phospholipids and free fatty acids in the crude ARA-rich oil are removed with citric acid and EDTA (degumming). The phospholipid content must comply with internal quality specifications by the end of this step and is controlled as a critical control point. All waste materials are discarded via sewage disposal. Next, the oil is deacidified with sodium hydroxide. The acid value must comply with internal quality specifications and is controlled as a critical control point. Next the crude oil is subjected to washing with water to remove saponins and residual soaps. Nitrogen, activated carbon, and activated clay are used to further refine the oil in the decoloration step. The oil is deodorized by steam and nitrogen, and a critical control point is in place to control the peroxide value of the oil. Vitamin E and ascorbyl palmitate are used as antioxidants in the finished ARA-rich oil. Following decoloration, the activated carbon and clay are removed by filtration. The refining process has several quality control parameters in place throughout production to ensure a product that complies with product specifications. The finished ARA-rich oil is then subjected to quality control by testing for compliance with the product specifications. The final product is reworked if it fails to meet product specifications for ARA-rich oil. Finished ARA-rich oil is stored in food contact material grade aluminum bottles at -10^{o}C for an average of 3 month until delivered to the customer. The finished ARA-rich oil is stored for no more than 18 months.

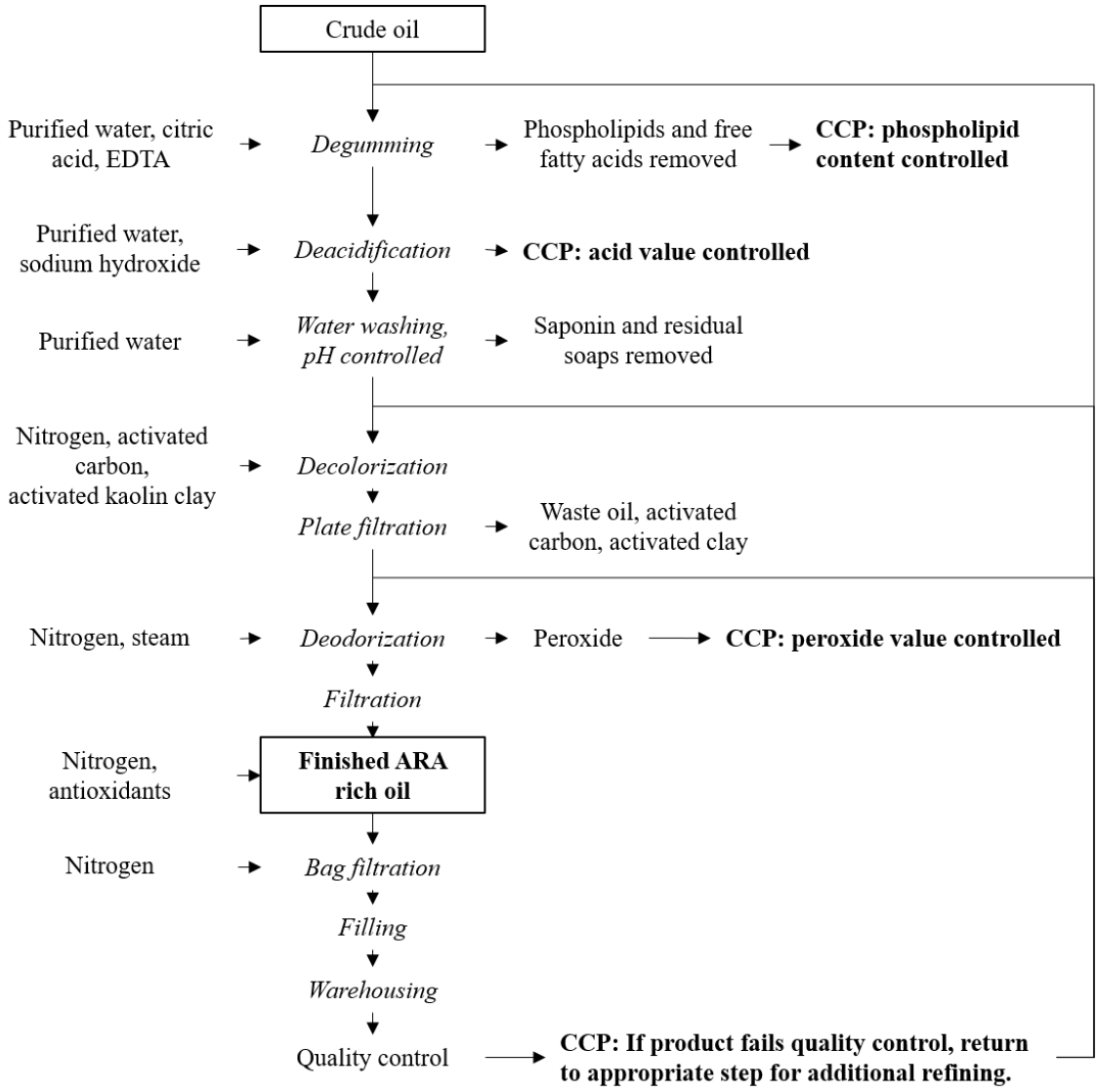

Figure 2. Refining of Crude Oil to Yield Final Product, ARA-rich Oil

The crude oil is refined via degumming, deacidification, water processing, decolorization, and deodorization to generate the final product, ARA-rich oil. *CCP: critical control point*

2. Raw Materials, Processing aids and Food Contact Substances

All processing aids used in the production of ARA-rich oil comply with Title 21 of the Code of Federal Regulations (21 CFR) and/or Food Chemicals Codex (FCC) specifications. All raw materials used in the culture of *M. alpina* AF are food grade materials that comply with 21 CFR. Similarly, all aluminum bags and drums comply with food grade acceptance criteria. These processing aids and their role in the production of ARA-rich oil are described below in Table 3.

Table 3. Processing Aids and Raw Materials Used in the Manufacture of ARA-Rich Oil

Processing Aid	Role in Production	US Regulations
Oral Glucose/Dextrose, monohydrate	Fermentation	21 CFR §168.111
Yeast powder/dry yeast	Fermentation	21 CFR §172.896
Yeast extract	Fermentation	21 CFR §184.1983
Potassium dihydrogen phosphate	Fermentation	No 21 CFR citation, complies with FCC monograph
Butane	Extraction	21 CFR §184.1165
Sodium Carbonate	Fermentation	21 CFR §184.1742
Sodium Hydroxide	Oil Refining	21 CFR §184.1763
Citric Acid, monohydrate	Oil Refining	21 CFR §184.1033
L-Ascorbyl palmitate	Anti-oxidant	21 CFR §182.3149
Activated clay, kaolin	Oil Refining	21 CFR §186.1256
Activated Carbon	Oil Refining	No 21 CFR citation, complies with FCC monograph
EDTA, disodium salt	Oil-Refining	21 CFR §172.135
Vitamin E oil (RRR-Tocopherol concentrate, mixed)	Oil Refining	21 CFR §184.1890
Aluminum bottles	Storage	Favorable FDA opinion*

CFR: Code of Federal Regulations
FCC: Food Chemicals Codex

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Product Specifications

To ensure a consistent food-grade product, Hubei Fuxing tests each batch of ARA-rich oil for compliance with a defined set of product specifications (Table 4). These parameters are assessed by the compendial Guobiao (GB) Chinese national standard methods issued by the Standardization Administration of China and are based on American Oil Chemists' Society (AOCS) methods. Data from three batches of ARA-rich oil demonstrate control of the production process and compliance with the product specifications.

Table 4. Product Specifications and Batch Data for Three Batches of Hubei Fuxing's ARA-Rich Oil

Parameter	Specification	Method	LOQ	Batch Number		
				A19050301J	A19050501J	A19050701J
Physical/Chemical Parameters						
Appearance	Pale to dark yellow oil	GB/T 5525-2008	-	Pass	Pass	Pass
Taste & Smell	Characteristic taste and smell	GB/T 5525-2008	-	Pass	Pass	Pass
25.4 mm color	Y ≤ 35, R ≤ 5	GB/T 22460-2008	-	Y=3.0, R=0.5	Y=2.5, R=0.5	Y=2.4, R=0.5
ARA (C20:4n6), %	≥ 40	GB 5009.168-2016 Third Method	0.02	45.7	45.9	44.8
Moisture, g/100g	≤ 0.05	GB 5009.236-2016	0.01	0.03	0.03	0.02
Impurities and Contaminants						
Acid value, mg KOH/g	≤ 0.5	GB 5009.229-2016	0.05	0.24	0.25	0.24
Trans fatty acid (%)	≤ 1.0	GB 5009.257-2016	0.02	0.29	0.33	0.32
Free fatty acids, % oleic acid	≤ 0.2	ISO 660:2009	0.05	0.12	0.12	0.12
Insoluble impurity (%)	≤ 0.2	GB/T 15688-2008	-	0.02	0.02	0.02
Unsaponifiable matter, %	≤ 3.0	GB/T 5535.2-2008	0.1	0.99	0.95	0.99
p-Anisidine value	≤ 10	GB/T 24304-2009	-	5.5	5.2	6.5
Peroxide value, meq/kg	≤ 2.0	GB 5009.227-2016 First Method	0.0006	N.D.	N.D.	N.D.
Residual solvent (mg/kg)	≤ 1	GB 5009.262-2016	1	N.D.	N.D.	N.D.
Metal Contaminants						
Mercury (Hg), mg/kg	≤ 0.01	GB 5009.17-2014 First Chapter second method	0.005	N.D.	N.D.	N.D.
Lead (Pb), mg/kg	≤ 0.1		0.05	N.D.	N.D.	N.D.
Arsenic (As), mg/kg	≤ 0.1		0.05	N.D.	N.D.	N.D.
Cadmium (Cd), mg/kg	≤ 0.1		0.01	N.D.	N.D.	N.D.
Copper (Cu), mg/kg	≤ 1.0		0.1	N.D.	N.D.	N.D.
Microbial and Other Contaminants						
Coliforms, cfu/mL	≤ 3	GB 4789.3-2016 Second method	-	<1.0	<1.0	<1.0
Molds, cfu/mL	≤ 10	GB 4789.15-2016 First method	-	<1.0	<1.0	<1.0
Yeast, cfu/mL	≤ 10	GB 4789.15-2016 First method	-	<1.0	<1.0	<1.0
Salmonella, /25g	Negative	GB 4789.4-2016	-	N.D.	N.D.	N.D.
Aerobic plate count, cfu/mL	≤ 1000 CFU/g	GB 4789.2-2016	-	<1.0	<1.0	<1.0
Aflatoxin B1 (µg/kg)	N.D.	DIN EN 14123, mod.	0.1	N.D.	N.D.	N.D.

GB: mandatory Chinese national standard issued by the Standardization Administration of China
GB/T: recommended Chinese national standard issued by the Standardization Administration of China
N.D.: not detected
LOQ: Limit of quantitation
meq: milliequivalents
cfu: colony forming units
DIN EN: Deutches Institut für Normang (German Institute for Standardization) European Standards

2. Other Quality Attributes

To characterize the quality of ARA-rich oil, Hubei Fuxing quantified the levels of fatty acids, trace elements, mycotoxins, plant sterols and stanols, polyaromatic hydrocarbons, polychlorinated biphenyls, and veterinary drugs and toxin residues in the finished product.

a. Percentage of fatty acids in ARA-rich oil

To evaluate the variability in the levels of individual fatty acids in ARA-rich oil, Hubei Fuxing quantified the amount of fatty acids in three batches of finished product. This testing is performed on every batch of ARA-rich oil. Arachidonic acid is the predominant fatty acid present in the ARA-rich oil (Table 5).

Table 5. Percentage Fatty Acids in ARA-Rich Oil

Fatty Acids	Batch Number		
	A19050301J	A19050501J	A19050701J
C4:0 Butyric Acid	<0.02	<0.02	<0.02
C6:0 Caproic Acid	<0.02	<0.02	<0.02
C8:0 Caprylic Acid	<0.02	<0.02	<0.02
C10:0 Capric Acid	0.03	0.02	0.03
C11:0 Hendecanoic Acid	<0.02	<0.02	<0.02
C12:0 Lauric Acid	<0.02	<0.02	<0.02
C13:0 Tridecaonic Acid	<0.02	<0.02	<0.02
C14:0 Myristic Acid	0.46	0.41	0.44
C14:1 Myristoleic Acid	<0.02	<0.02	<0.02
C15:0 Pentadecanoic Acid	0.15	0.14	0.13
C15:1 Pentadecenoic Acid	<0.02	<0.02	<0.02
C16:0 Palmitic Acid	9.61	9.63	9.85
C16:1 Palmitoleic Acid	0.3	0.22	0.26
C17:0 Margaric Acid	0.33	0.33	0.3
C17:1 Margaroleic Acid	<0.02	<0.02	<0.02
C18:0 Stearic Acid	6.27	6.32	6.53
C18:1 Oleic Acid	5.42	5.3	5.66
C18:1(n-9) Elaidic Acid	0.1	0.11	0.1
C18:2 Linoleic Acid	7.75	7.18	8.48
C18:2(n-6) Linoleaidic Acid	0.19	0.22	0.21
C18:3(n-3) Alpha-Linolenic Acid	0.52	0.44	0.59
C18:3(n-3) Trans-Linolenic Acid	<0.02	<0.02	<0.02
C18:3(n-6) Gamma-Linolenic Acid	2.81	2.81	2.78
C20:0 Arachidic Acid	<0.02	0.81	0.78
C20:1 Gondoic Acid	0.33	0.34	0.35
C20:2 Eicosadienoic Acid	0.64	0.66	0.63
C20:3(n-3) Eicosatrienoic Acid	0.12	0.11	0.11
C20:3(n-6) Eicosatrienoic Acid	5.01	5.65	5.22
C20:4 Eicosatetraenoic (Arachidonic) Acid	**45.7**	**45.9**	**44.8**
C20:5(n-3) Eicosapentaenoic Acid	0.28	0.24	0.27
C21:0 Heneicosanoic Acid	0.07	0.06	0.06
C22:0 Behenic Acid	2.91	2.92	2.9
C22:1 Erucic Acid	0.07	0.07	0.07

Table 5. Percentage Fatty Acids in ARA-Rich Oil			
Fatty Acids	**Batch Number**		
	A19050301J	**A19050501J**	**A19050701J**
C22:2(n-6) Docosadienoic Acid	0.05	0.05	0.05
C22:6(n-3) Docosahexaenoic Acid	0.3	0.04	<0.02
C23:0 Tricosanoic Acid	0.06	0.06	0.05
C24:0 Lignoceric Acid	9.38	9.66	9.13
C24:1 Nervonic Acid	0.3	0.31	0.3
Mono-unsaturated fatty acids total	6.51	6.37	6.74
Omega-3 fatty acids	1.21	0.82	0.97
Omega-6 fatty acids	62.2	62.4	62.1
Omega-9 fatty acids	6.22	6.14	6.48
Poly-unsaturated fatty acids total	63.4	63.3	63.1
Saturated fatty acids total	30.1	30.4	30.1
Total EPA + DHA Omega-3 fatty acids	0.57	0.28	0.27
Total of trans fatty acids	0.29	0.33	0.32
Total omega fatty acids	69.6	69.4	69.6
Method: GB 5009.168-2016 Third Method, performed by Eurofins Tech. Service, Suzhou 215000, Jiangsu Province, P.R. China Limit of Quantitation: 0.02%			

b. Minerals in ARA-rich oil

The presence of specific elements was assessed in three batches of ARA-rich oil. The most abundant elements are phosphorus, silicon, and sulfur. Mercury, manganese, molybdenum, chromium, cadmium, and iron were all below the level of quantitation (Table 6). This analysis is performed twice a month.

Table 6. Minerals in ARA-Rich Oil

Trace elements	LOQ	Batch Number		
		AR16041301H	AR16010401J	AR16010701J
Mercury (Hg)[a]	0.005 mg/kg	N.D.	N.D.	N.D.
Manganese (Mn)[b]	0.1 mg/kg	N.D.	N.D.	N.D.
Molybdenum (Mo)[b]	0.1 mg/kg	N.D.	N.D.	N.D.
Nickel (Ni)[b]	0.1 mg/kg	N.D.	N.D.	N.D.
Chromium (Cr)[b]	0.1 mg/kg	N.D.	N.D.	N.D.
Cadmium (Cd)[b]	0.01 mg/kg	N.D.	N.D.	N.D.
Iron (Fe)[b]	0.1 mg/kg	N.D.	N.D.	N.D.
Phosphorus (P)[b]	5 mg/kg	29.5	48.2	38.2
Silicon (Si)[c]	1 mg/kg	19	27	16
Sulfur (S)[c]	1 mg/kg	3.2	1.1	2.6

LOQ: Limit of Quantitation
N.D.: not detected
[a] Method: (AAS) BS EN 13806:2002
[b] Method: (ICP-MS) BS EN ISO 17294-2 2004
[c] Method: (ICP-AES) AOCS Ca 17-01

c. Mycotoxins in ARA-rich oil

The presence of mycotoxins, including aflatoxins, fumonisins, nivalenol, zearalenone, ochratoxin A, patulin, and sterigmatocystin was analyzed in three batches of ARA-rich oil. These mycotoxins are known to contaminate the food supply and their presence is monitored in ARA-rich oil. None of these toxins were present above the limit of quantitation (Table 7). This analysis is performed every three months.

Additionally, there have been no reports of mycotoxin production from *M. alpina* or any other of the many species of the genus *Mortierella.* Strains of *M. alpina* used by other manufacturers of ARA-rich oil also do not produce mycotoxins (FDA, 2001a; 2001b; 2006).

Table 7. Mycotoxins in ARA-Rich Oil

Mycotoxins	LOQ	Batch Number		
		AR16041301H	AR16010401J	AR16010701J
Aflatoxins				
Aflatoxin B1[a]	0.1 µg/kg	N.D.	N.D.	N.D.
Aflatoxin B2[a]	0.1 µg/kg	N.D.	N.D.	N.D.
Aflatoxin G1[a]	0.1 µg/kg	N.D.	N.D.	N.D.
Aflatoxin G2[a]	0.1 µg/kg	N.D.	N.D.	N.D.
Fumonisins				
Fumonisin (B1+B2+B3)[b]	30 µg/kg	N.D.	N.D.	N.D.
Fumonisin B1[b]	10 µg/kg	N.D.	N.D.	N.D.
Fumonisin B2[b]	10 µg/kg	N.D.	N.D.	N.D.
Fumonisin B3[b]	10 µg/kg	N.D.	N.D.	N.D.
Other Mycotoxins				
Nivalenol (NIV)[c]	20 µg/kg	N.D.	N.D.	N.D.
Zearalenone (ZON)[c]	10 µg/kg	N.D.	N.D.	N.D.
Ochratoxin A[d]	1 mg/kg	N.D.	N.D.	N.D.
Patulin[e]	3 µg/kg	N.D.	N.D.	N.D.
Sterigmatocystin[c]	10 µg/kg	N.D.	N.D.	N.D.

LOQ: limit of quantitation
N.D.: not detected
[a]Method: Internal method based on EN 14123
[b]Method: AOAC 2001.04 mod
[c]Method: Internal method, LC-MS/MS
[d]Method: GB/T 23502-2009
[e]Method: GB/T 5009.185-2003

d. PAH in ARA-rich oil

The presence of polyaromatic hydrocarbons (PAH) in ARA-rich oil was tested in three batches of ARA-rich oil. No PAH residues were detected above the limit of quantitation (Table 8). This analysis is performed every three months.

Table 8. Polyaromatic Hydrocarbons (PAH) In ARA-Rich Oil				
PAH	**LOQ**	**Batch Number**		
		AR16041301H	**AR16010401J**	**AR16010701J**
5-Methylchrysene	1 µg/kg	N.D.	N.D.	N.D.
Benzo(a)anthracene	0.5 µg/kg	N.D.	N.D.	N.D.
Benzo(a)pyrene	0.5 µg/kg	N.D.	N.D.	N.D.
Benzo(b)fluoranthene	0.5 µg/kg	N.D.	N.D.	N.D.
Benzo-(c)-fluorene	1 µg/kg	N.D.	N.D.	N.D.
Benzo(g,h,i)perylene	0.5 µg/kg	N.D.	N.D.	N.D.
Benzo-(j)-fluoranthen	0.5 µg/kg	N.D.	N.D.	N.D.
Benzo(k)fluoranthene	0.5 µg/kg	N.D.	N.D.	N.D.
Chrysene	0.5 µg/kg	N.D.	N.D.	N.D.
Cyclopenta(c,d)pyrene	1 µg/kg	N.D.	N.D.	N.D.
Dibenzo(a,e)pyrene	1 µg/kg	N.D.	N.D.	N.D.
Dibenzo(a,h)anthracene	0.5 µg/kg	N.D.	N.D.	N.D.
Dibenzo(a,h)pyrene	1 µg/kg	N.D.	N.D.	N.D.
Dibenzo(a,i)pyrene	1 µg/kg	N.D.	N.D.	N.D.
Dibenzo(a,l)pyrene	1 µg/kg	N.D.	N.D.	N.D.
Indeno(1,2,3-cd)pyrene	0.5 µg/kg	N.D.	N.D.	N.D.
LOQ: limit of quantitation N.D.: not detected Method: Internal Method: GC-MS				

e. Plant sterols and stanols in ARA-rich oil

Three batches of ARA-rich oil were assessed for the presence of plant sterols and stanols (Table 9). The quantities of plant sterols and stanols in three batches of ARA-rich oil consist of less than 1% of the finished product. This analysis is performed twice a year.

Table 9. Plant Sterols and Stanols in ARA-Rich Oil				
Plant Sterols and Stanols	**Method**	**Batch Number**		
		AR16041301H	**AR16010401J**	**AR16010701J**
Brassicasterol (g/100 g)	NMKL 198:2014	0.15	0.16	0.16
Campestanol (g/100 g)	NMKL 198:2015	N.D.	N.D.	N.D.
Campesterol (g/100 g)	NMKL 198:2016	0.04	N.D.	N.D.
Cholesterol (g/100 g)	NMKL 198:2017	N.D.	N.D.	N.D.
Delta-5,24-stigmastadienol (g/100 g)	NMKL 198:2018	N.D.	0.01	N.D.
Delta-7-Avenasterol (g/100 g)	NMKL 198:2019	N.D.	N.D.	N.D.
Delta-7-stigmastenol (g/100 g)	NMKL 198:2020	N.D.	N.D.	N.D.
Sitostanol+delta-5-avenasterol (g/100 g)	NMKL 198:2021	0.02	0.02	0.02
Sitosterol (g/100 g)	NMKL 198:2022	0.09	0.07	0.07
Stigmasterol (g/100 g)	NMKL 198:2023	N.D.	0.01	0.01
Total plant sterols + plant stanols (g/100 g)	NMKL 198:2024	0.44	0.56	0.51
Unidentified sterols (g/100 g)	NMKL 198:2025	0.13	0.28	0.24
NMKL: Nordic Committee on Food Analysis N.D.: not detected Limit of Quantification: 0.01 g/100 g				

f. Polychlorinated biphenyls (PCBs) in ARA-rich oil

No PCBs were detected above the level of quantitation in three batches of ARA-rich oil, (Table 10). This analysis is performed every three months.

Table 10. Polychlorinated Biphenyls (PCBs) in ARA-Rich Oil

	Batch Number		
PCB	**AR16041301H**	**AR16010401J**	**AR16010701J**
PCB 1 (mg/kg)	N.D.	N.D.	N.D.
PCB 101 (mg/kg)	N.D.	N.D.	N.D.
PCB 104 (mg/kg)	N.D.	N.D.	N.D.
PCB 105 (mg/kg)	N.D.	N.D.	N.D.
PCB 118 (mg/kg)	N.D.	N.D.	N.D.
PCB 126 (mg/kg)	N.D.	N.D.	N.D.
PCB 128 (mg/kg)	N.D.	N.D.	N.D.
PCB 138 (mg/kg)	N.D.	N.D.	N.D.
PCB 153 (mg/kg)	N.D.	N.D.	N.D.
PCB 170 (mg/kg)	N.D.	N.D.	N.D.
PCB 18 (mg/kg)	N.D.	N.D.	N.D.
PCB 180 (mg/kg)	N.D.	N.D.	N.D.
PCB 187 (mg/kg)	N.D.	N.D.	N.D.
PCB 188 (mg/kg)	N.D.	N.D.	N.D.
PCB 195 (mg/kg)	N.D.	N.D.	N.D.
PCB 201 (mg/kg)	N.D.	N.D.	N.D.
PCB 206 (mg/kg)	N.D.	N.D.	N.D.
PCB 209 (mg/kg)	N.D.	N.D.	N.D.
PCB 28 (mg/kg)	N.D.	N.D.	N.D.
PCB 29 (mg/kg)	N.D.	N.D.	N.D.
PCB 44 (mg/kg)	N.D.	N.D.	N.D.
PCB 50 (mg/kg)	N.D.	N.D.	N.D.
PCB 52 (mg/kg)	N.D.	N.D.	N.D.
PCB 66 (mg/kg)	N.D.	N.D.	N.D.
PCB 77 (mg/kg)	N.D.	N.D.	N.D.
PCB 8 (mg/kg)	N.D.	N.D.	N.D.
PCB 87 (mg/kg)	N.D.	N.D.	N.D.
Total PCB (mg/kg)	N.D.	N.D.	N.D.

Method: ASU L00.00-34.
Limit of Quantitation: 0.02 mg/kg
N.D.: not detected

F. STABILITY OF ARA-RICH OIL

The stability of three batches of ARA oil was investigated under ambient conditions (25^{o}C, humidity 75%) for 14 months. The ARA-rich oil was stored in aluminum bottles, the commercial packaging for the product, and flushed with nitrogen gas to minimize oxidation. Samples were tested using the same methods as described in Table 4 at 0, 1, 2, 4, 6, 8, 10, 12 and 14 months after packaging. The results are shown in Table 11, demonstrating compliance with product specifications up until 14 months and supporting a shelf life of 12 months when stored after the manufacture date under ambient conditions.

Table 11. Stability of ARA-Rich Oil

Parameter	Specification	Time (months)								
		0	1	2	4	6	8	10	12	14
Batch 17090901J										
Color (25.4 mm)	Y ≤ 35, R ≤ 5	Y2.0, R0.1	Y2.2 R0.1	Y2.4, R0.2	Y2.4, R0.3	Y2.7, R0.3	Y2.9, R0.3	Y3.1, R0.4	Y3.2, R0.5	Y3.5, R0.5
Taste & Smell	With its characteristic smell, no other peculiar smell found	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Acid Value (mg KOH/g)	≤ 0.5	0.09	0.12	0.17	0.22	0.26	0.32	0.38	0.42	0.48
Peroxide Index (meq/kg)	≤ 10	0	0.27	0.34	0.68	1.07	1.26	1.41	1.65	1.83
ARA (%)	≥ 40	41.65	41.76	41.45	41.32	41.36	41.25	41.19	41.16	41.07
p-Anisidine value	≤ 10	1.50	1.55	1.66	1.73	1.92	2.13	2.37	2.64	3.01
Coliforms (cfu/mL)	≤ 3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Molds (cfu/mL)	≤ 10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Yeast (cfu/mL)	≤10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Salmonella	Negative in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aerobic Plate Count (APC) (cfu/mL)	≤ 1000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Batch 17091801J										
Color (25.4 mm)	Y ≤ 35, R ≤ 5	Y2.0, R0.1	Y2.0, R0.1	Y2.0, R0.2	Y2.3, R0.3	Y2.7, R0.3	Y2.9, R0.3	Y3.0, R0.4	Y3.2, R0.4	Y3.4, R0.5
Taste & Smell	With its characteristic smell, no other peculiar smell found	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Acid Value (mg KOH/g)	≤ 0.5	0.11	0.13	0.19	0.27	0.28	0.34	0.46	0.48	0.51
Peroxide Index (meq/kg)	≤ 10	0	0.18	0.32	0.54	1.01	1.23	1.32	1.73	2.02
ARA (%)	≥ 40	43.56	43.44	43.48	43.22	43.07	42.95	42.84	41.66	41.33
p-Anisidine value	≤ 10	1.52	1.60	1.72	1.89	2.02	2.48	2.63	2.89	3.17
Coliforms (cfu/mL)	≤ 3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Molds (cfu/mL)	≤ 10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Yeast (cfu/mL)	≤10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Salmonella	Negative in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aerobic Plate Count (APC) (cfu/mL)	≤ 1000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Batch 17101501J										
Color (25.4 mm)	Y ≤ 35, R ≤ 5	Y2.0, R0.1	Y2.0, R0.1	Y2.4, R0.2	Y2.5, R0.3	Y2.8, R0.3	Y2.9, R0.4	Y3.1, R0.4	Y3.3, R0.4	Y3.4, R0.6
Taste & Smell	With its characteristic smell, no other peculiar smell found	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Acid Value (mg KOH/g)	≤ 0.5	0.22	0.27	0.29	0.33	0.34	0.37	0.42	0.43	0.45
Peroxide Index (meq/kg)	≤ 10	0	0.13	0.26	0.48	0.81	0.98	1.20	1.45	1.97

Table 11. Stability of ARA-Rich Oil

Parameter	Specification	Time (months)								
		0	1	2	4	6	8	10	12	14
ARA (%)	≥ 40	42.23	42.19	42.45	42.08	41.97	41.86	41.75	41.60	41.39
p-Anisidine value	≤ 10	1.03	1.30	1.34	1.59	1.77	1.98	2.13	2.29	2.41
Coliforms (cfu/mL)	≤ 3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Molds (cfu/mL)	≤ 10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Yeast (cfu/mL)	≤10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Salmonella	Negative in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aerobic Plate Count (APC) (cfu/mL)	≤ 1000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

ND: not detected
cfu: colony forming units
Storage Conditions: 25ºC, humidity 75%

III. DIETARY EXPOSURE

The ARA-rich oil produced by Hubei Fuxing is manufactured from the same species of *Mortierella* as described in GRN 326. The dietary exposure for this product will be the same as the dietary exposure description from GRN 326, as well as GRN 730, a more recent ARA-rich oil GRAS notice. We incorporate by reference the estimates of exposures from these two GRAS notices. The estimates of exposure for these two GRAS notices are summarized below for convenience.

A. INTENDED EFFECT

ARA-rich oil is intended to be used as a source of ARA, a fatty acid naturally present in human breast milk and known to play a role in infant development. GRN 326 details the overall range of ARA concentration in human milk across the global population to be 0.34-1.22%. Briefly, Brenna et al. (2007) conducted a meta-analysis of ARA concentrations in mature human milk based on published data from 65 studies spanning 1986 to 2006 and involving 2474 women worldwide. The mean and standard deviation of ARA concentration as a percentage of total fatty acids was 0.47% ± 0.13% (range: 0.24-1.0%). The authors noted that the highest concentrations of ARA in human milk were seen in coastal regions and possibly associated with marine-rich diets. This evaluation reveals a broad range of ARA levels in human milk on a worldwide basis and shows the range of possible infant exposure to ARA, which provides a guide for levels of ARA supplementation in infant formulas.

The supplementation of infant formula with ARA at levels consistent with those in human milk is important because the n-6 and n-3 fatty acids present in human milk have critical roles in membrane structure and as precursors of potent and highly reactive eicosanoids (Mandal et al., 2008). Together, ARA (n-6) and DHA (n-3) are involved in brain development and have been noted to be of particular importance for pre-term infants due to an insufficient intra-uterine supply of ARA and DHA and low fat reserves (Fleith and Clandinin, 2005; Heird and Lapillonne, 2005; Eilander et al., 2007; Gibson and Makrides, 2001; Innis, 2007; Hadders-Algra et al., 2007; Koletzko et al., 2008). Although pre-term infants are capable of endogenous synthesis of ARA from precursor fatty acids, this capacity appears to be sub-optimal to meet the demands of developing tissues. Thus, there is a particular benefit to pre-term infants who consume a diet containing pre-formed long-chain polyunsaturated fatty acids (FSANZ, 2003).

Based on scientific consensus and current knowledge regarding the importance of long chain PUFAs in the infant diet and their presence in human milk, supplementation of infant formula with ARA together with DHA has been recommended by the World Association of

Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation (Koletzko et al., 2008). For pre-term infant formula, the recommended intakes of long chain PUFAs are 20-60 mg/kg body weight/day for ARA and 20-40 mg/kg body weight/day for DHA. For term infant formulas, the recommended intakes are 20-40 mg/kg body weight/day for ARA and 40 mg/kg body weight/day for DHA. In situations where infants are not breast-fed, those organizations collectively recommend that the level of DHA in infant formula be 0.2-0.5 weight percent of total fat, with the minimum amount of ARA being equivalent to the DHA content.

B. HISTORY OF USE

The use of ARA-rich, long-chain polyunsaturated oils derived by fermentation of *M. alpina* for supplementation of infant formula has been assessed by various international bodies. Fungal oils have been used in commercially available infant formulas in at least 50 countries since the early 1990s. In the United States, ARA-rich oil generated from *M. alpina* has been the subject of multiple GRAS notifications (GRNs 730, 326, 94, 80, and 41). The information provided in GRN 94 for ARA-rich oil (SUNTGA40S) and in the published opinion on the novel food authorization for this oil (EFSA, 2008) noted that the production of long chain PUFAs by micro-organisms, including *M. alpina,* has been employed for several years. Food Standards Australia New Zealand concluded that ARA-rich oil was a safe source of ARA for use in infant formula in 2003 (FSANZ 2003). In addition, *M. alpina* is not a known human pathogen and has not been reported to produce mycotoxins (Streekstra 1997).

C. INTENDED USE

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 among different populations. Therefore, the proposed use of ARA-rich oil is to provide of 0.75% and 0.40% ARA by weight of fatty acids in term and pre-term infant formulas, respectively. The intended use of ARA-rich oil to deliver this concentration of ARA, corresponds to 1.875% of total fat in non-exempt term infant formula and 1.00% of total fat in exempt pre-term infant formula. This intended use level is consistent with the levels cited in GRN 80 (term infants), GRN 94 (pre-term infants), GRN 326 (term and pre-term infants) and GRN 730 (term and pre-term infants). The ratios of ARA:DHA are expected to be in the range of 2:1-1:1.

D. ESTIMATED DAILY INTAKE

The assumptions upon which this estimation is made are the same as those cited in GRN 326, pg 60 (FDA, 2010). An estimate of exposure to ARA from its intended use is based on target ARA concentrations of 0.75% and 0.40% of total fat in term and pre-term infant formula, respectively. Assuming human infants consume about 100 kcal/kg body weight/day (term infants) to 120 kcal/kg body weight/day (pre-term infants), of which fat comprises about 50% of those calories, an infant will consume about 5.6 g (term infants) to 6.7 g (pre-term infants) of fat/kg body weight/day (1 g fat = 9 kcal). These correspond to intakes of ARA of 42 mg and 27 mg ARA/ kg body weight/day (corresponding to 104 and 67 mg of ARA-rich oil/kg body weight/day or 420 and 270 mg ARA-rich oil powder/kg body weight/day) for term infants and pre-term infants, respectively.

IV. SELF-LIMITING LEVELS OF USE

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

V. COMMON USE IN FOOD BEFORE 1958

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

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

The FDA has issued 'no question' letters for five GRAS notices for food uses of ARA-rich oils derived from *M. alpina* for infant formula (GRNs 41, 80, 94, 326, and 730). A comparison of the specifications between the ARA-rich oil that is the subject of this notification and those in the previous GRNs shows that Hubei Fuxing's ARA-rich oil and the ARA-rich oil that is the subject of GRN 326 are similar, with some parameters in the Hubei Fuxing ARA-rich oil being more stringently controlled, including acid value, anisidine value, mercury, and moisture (Table 12). Therefore, the toxicology and corroborating safety data that support the use of the ARA-rich oil that is the subject of GRN 326 also support the GRAS status of Hubei Fuxing's ARA-rich oil. The product specifications for Hubei Fuxing's ARA-rich oil are also similar to GRNs 41, 80, 94, and 730 and data cited for these oils are highly relevant as corroborative data to support safety of Hubei Fuxing's ARA-rich oil.

Table 12. Specifications of Hubei Fuxing's ARA-Rich Oil Compared with Previous GRAS Notices for ARA-rich oil from *M. alpina*.

Parameter	**Hubei Fuxing's ARA-rich oil**	**GRN 326**	GRN 730	GRN 94	GRNs 80 and 41*
ARA, C20:4n6, relative %	**≥ 40**	**≥ 40**	≥ 40	≥ 40	38-44[a]
Acid value, mg KOH/g	**≤ 0.5**	**≤ 1.0**	≤ 0.5	N.S.	N.S.
Free fatty acids	**< 0.2**	**≤ 0.2**	< 0.1[b]	≤ 0.2	≤ 0.4
Unsaponifiable matter, %	**≤ 3.0**	**≤ 3.0**	≤ 1.5	≤ 1.0	≤ 3.5
Anisidine value	**≤ 10**	**≤ 20**	≤ 10	N.S.	N.S.
Peroxide value, meq/kg	**≤ 2.0**	**≤ 2.0**	≤ 2.5	< 5.0	< 5.0
Residual solvents (Butane or Hexane), mg/kg	**≤ 1.0**	**≤ 1.0**	N.S.	N.S.	N.S.
Mercury (Hg), mg/kg	**≤ 0.01**	**≤ 0.05**	≤ 0.05	< 0.5	< 0.2
Lead (Pb), mg/kg	**≤ 0.1**	**N.S.**	N.S.	< 0.1	< 0.2
Arsenic (As), mg/kg	**≤ 0.1**	**N.S.**	≤ 0.1	< 0.2	< 0.5
Cadmium (Cd), mg/kg	**≤ 0.1**	**N.S.**	≤ 0.1	N.S.	N.S.
Moisture, g/100g	**≤ 0.05**	**≤ 0.1**	≤ 0.1	N.S.	N.S.
Coliforms, cfu/mL	**≤ 3**	**≤ 3**	≤ 1	N.S.	N.S.
Molds, cfu/mL	**≤ 10**	**≤ 10**	≤ 1	N.S.	N.S.
Yeast, cfu/mL	**≤ 10**	**≤ 10**	≤ 1	N.S.	N.S.
Salmonella /25g	**N.D.**	**N.S.**	N.D.	N.S.	N.S.
Aerobic plate count, cfu/mL	**< 1000**	**N.S.**	< 100	N.S.	N.S.

*GRN 41 and 80 are for the same product, ARASCO (arachidonic acid-rich single-cell oil), used at different levels infant formula. GRN 80 expands the proposed use level in GRN 41.
[a]Specifications for other fatty acids are included.
[b]Free fatty acids measured as %oleic acids for GRN 730.
N.S.: not specified; N.D.: not detected; cfu: colony forming units

Table 13 is a comparison of the fatty acid profile between Hubei Fuxing's ARA-rich oil and the ARA-rich oil in GRN 326. Variations in the composition of the oil are present in myristic, palmitic, palmitoleic, margaric, alpha-linolenic acid, eicosatrienoic, dicosadienoic, and docosahexaenoic acids. These differences are not expected to pose a risk to the consumer, as these fatty acids are not expected to dramatically affect the safety of the ingredient and are naturally found in the diet. Additionally, none of the fatty acids that are different between GRN 326 and the current

notice are present as a large percentage of the oil. This demonstrates that the fatty acid profile for Hubei Fuxing's ARA-rich oil is similar to the fatty acid profile described in GRN 326, an ARA rich oil also generated from *M. alpina*.

Table 13. Fatty Acid Profiles for Hubei Fuxing's ARA-Rich Oil and GRN 326 ARA-rich Oil

Fatty Acids	Current Notice (Average ± SD, n=3 batches)	GRN 326
C4:0 Butyric Acid	ND	-
C6:0 Caproic Acid	ND	-
C8:0 Caprylic Acid	ND	ND
C10:0 Capric Acid	0.027 ± 0.0058	0.04
C11:0 Hendecanoic Acid	ND	-
C12:0 Lauric Acid	ND	0.01
C13:0 Tridecaonic Acid	ND	-
C14:0 Myristic Acid	0.44 ± 0.025	0.26
C14:1 Myristoleic Acid	ND	0.01
C15:0 Pentadecanoic Acid	0.14 ± 0.01	0.09
C15:1 Pentadecenoic Acid	ND	-
C16:0 Palmitic Acid	9.70 ± 0.13	6.02
C16:1 Palmitoleic Acid	0.26 ± 0.04	0.02
C17:0 Margaric Acid	0.32 ± 0.017	0.18
C17:1 Margaroleic Acid	ND	-
C18:0 Stearic Acid	6.37 ± 0.14	5.27
C18:1 Oleic Acid	5.46 ± 0.18	4.78
C18:1(n-9) Elaidic Acid	0.10 ± 0.01	-
C18:2 Linoleic Acid	7.80 ± 0.65	7.87
C18:2(n-6) Linoleaidic Acid	0.21 ± 0.02	-
C18:3(n-3) Alpha-Linolenic Acid	0.52 ± 0.08	0.04
C18:3(n-3) Trans-Linolenic Acid	ND	-
C18:3(n-6) Gamma-Linolenic Acid	2.80 ± 0.017	2.1
C20:0 Arachidic Acid	0.80 ± 0.021	0.75
C20:1 Gondoic Acid	0.34 ± 0.01	0.22
C20:2 Eicosadienoic Acid	0.64 ± 0.015	0.44
C20:3(n-3) Eicosatrienoic Acid	0.11 ± 0.006	0.03
C20:3(n-6) Eicosatrienoic Acid	5.29 ± 0.33	3.69
C20:4 Eicosatetraenoic (Arachidonic) Acid	45.47 ± 0.59	43.3
C20:5(n-3) Eicosapentaenoic Acid	0.26 ± 0.021	0.14
C21:0 Heneicosanoic Acid	0.063 ± 0.006	0.1
C22:0 Behenic Acid	2.91 ± 0.01	3.11
C22:1 Erucic Acid	0.07 ± 0.00	0.17
C22:2(n-6) Docosadienoic Acid	0.12 ± 0.16	0.02
C22:6(n-3) Docosahexaenoic Acid	0.17 ± 0.18	0.04
C23:0 Tricosanoic Acid	0.057 ± 0.006	-
C24:0 Lignoceric Acid	9.39 ± 0.27	10.12
C24:1 Nervonic Acid	0.303 ± 0.006	0.51
Mono-unsaturated fatty acids total	6.54 ± 0.19	-
Omega-3 fatty acids	1.00 ± 0.2	-
Omega-6 fatty acids	62.23 ± 0.15	-
Omega-9 fatty acids	6.28 ± 0.18	-
Poly-unsaturated fatty acids total	63.27 ± 0.15	-
Saturated fatty acids total	30.20 ± 0.17	27.5

SD: standard deviation; ND: not detected, limit of detection = 0.02%
-: fatty acid not assessed in GRAS notice

Based on a comparison of the manufacturing process and specifications for these products, the ARA-rich oil from Hubei Fuxing is compositionally similar to the ARA-rich oil described in GRN 326 and also similar to the ARA-rich oils described in the previously mentioned GRNs. Therefore, the information and data in GRN 326 are pivotal to the safety determination of Hubei Fuxing's ARA-rich oil and the data and information from the other cited GRAS notices are corroborative to the safety of the ARA-rich oil in this GRAS determination. The GRAS notices cited provide publicly available information that established there is reasonable certainty of no harm to target consumers from ingesting ARA-rich oil from the intended uses and use levels. ARA-rich oil is therefore GRAS as an ingredient in infant formula at the intended use levels.

This notice incorporates by reference the pivotal and corroborative safety and metabolism studies discussed in previous GRNs (GRN 730: pages 28–47, GRN 326: pages 61-153, GRN 94: pages 78-318; GRN 80: stamped pages 16-23 and 48-55, GRN 41: stamped pages 108-118 and 175-418) and will not discuss previously reviewed references in detail.

A. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

In breast milk, ARA is mainly found in the form of triglycerides, although it can also occur in phospholipids, and accounts for approximately 0.77% of fatty acids (Martin et al., 1993). In general, dietary triglycerides undergo enzymatic hydrolysis in the upper intestine to free fatty acids 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 triglycerides (Kroes et al., 2003). The new or reconstructed triglycerides enter the lymph in the form of chylomicrons for transport to the blood, which allows distribution and incorporation into plasma lipids, erythrocyte membranes, platelets, and adipose tissue. The chylomicrons are hydrolyzed by lipoprotein lipase during passage through the capillaries of adipose tissue and the liver to release free fatty acids to the tissues for metabolism or for cellular uptake, with subsequent re-esterification into triglycerides and phospholipids for storage as energy or as structural components of cell membranes. The metabolism of fatty acids occurs in the mitochondria following their transport across the mitochondrial membrane in the form of acylcarnitine. Fatty acids are metabolized predominantly via beta-oxidation, a process that involves a shortening of the fatty acid 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 fatty acids across the mitochondrial membrane is contingent upon the length of the carbon chain; fatty acids of 20 carbons or more are transported into the mitochondria to a lesser degree than shorter chain fatty acids.

Fatty acids can only be desaturated endogenously up to the n-9 position due to lack of certain enzymes in humans (Kremmyda et al., 2011). For this reason, linoleic (18:2n-6) and

linolenic (18:3n-3) acids must be obtained from the diet. Further elongation and desaturation of these fatty acids to produce long-chain PUFA is possible but is not very efficient in humans. Examples of PUFAs include ARA (20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and DHA (22:6n-3). Thus, these fatty acids may be conditionally essential depending on essential fatty acid availability.

During the last trimester of pregnancy, the placenta provides the fetus with ARA. Pre-term birth, which curtails maternal supply of ARA to the fetus, is associated with sub-optimal neural and visual development, which can be improved by providing exogenous ARA (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 fatty acids linoleic acid (18:2n-6) to ARA due to reduced concentrations and activity of desaturase enzymes (Martin et al., 2011). Supplementation of these precursor fatty acids may not provide normal concentrations of the downstream fatty acid. Thus, pre-term infants have higher post-natal long chain PUFA requirements than full-term infants, although ARA supplementation can benefit both term and pre-term infants.

B. TOXICOLOGY AND GENOTOXICITY STUDIES

1. Summary

The safety of ARA-rich oils has been assessed in multiple published reports documenting the absence of genotoxicity and the absence of toxicity in subchronic toxicity studies in rats including *in utero* exposure, and tolerance in neonatal piglets.

Pivotal genotoxicity studies and a subchronic toxicity study with an *in utero* exposure have been published by Casterton et al. (2009). The lack of genotoxic potential of Hubei Fuxing's ARA-rich oil is also supported by an unpublished bacterial reverse mutation assay. Additional genotoxicity studies corroborating the safety of the ARA-rich oil produced by Hubei Fuxing are described in Hempenius et al. (1997) and Lewis et al. (2016). A summary of the published genotoxicity studies performed using ARA-rich oils derived from *M. alpina* are described in Table 14.

Table 14. Summary of Genotoxicity Studies Performed using *M. alpina* derived ARA-rich Oil

Reference	%ARA in ARA-rich oil	Study type	Cells/Model Used	Doses used	Results
Pivotal Genotoxicity Studies					
Casterton et al., 2009	43.3%	Bacterial Reverse Mutation, OECD 471 compliant study	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2 *uvr*A	0, 62, 185, 556, 1667, and 5000 µg/plate.	No dose of ARA rich oil induced a positive response in the presence or absence of S9 activation.
		Chromosome Aberration, OECD 473 compliant study	Chinese Hamster Ovary (CHO) cells	1, 2, 3.9, 5, 7.8, 10, 15.6, 20, 31.3, 39, 62.5, 78, 125, 156, 250, 313, 500, 625, 1000, 1250, 2500, 3135, and 5000 µg/mL	No dose of ARA rich oil increased the number of structural chromosomal aberrations or induced a positive test response in the presence or absence of S9 activation.
		Gene Mutation of the TK-locus, OECD 476 compliant study	Mouse Lymphoma (L5178Y)	0, 429, 858, 1715, 3500, and 5000 µg/mL	No dose of ARA rich oil increased the mutant frequency in the presence or absence of S9 activation
Corroborative data from GRN 326 pages 121 – 122, GRN 730, pages 30 – 31					
Hempenius et al., 1997	38.6%	Bacterial Reverse Mutation, OECD 471 compliant study	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2 *uvr*A	62-5000 µg/plate	No dose of ARA rich oil induced a positive response in the presence or absence of S9 activation.
		Gene Mutation of the TK-locus, OECD 476 compliant study	Mouse Lymphoma (L5178Y)	0.078-5 mg/mL	No dose of ARA rich oil increased the mutant frequency in the presence or absence of S9 activation
		Chromosome Aberration, OECD 473 compliant study	Chinese Hamster Ovary (CHO) cells	Doses not described	ARA rich oil did not induce a biologically relevant or statistically significant increase in structural chromosome aberrations in the study
		Mouse Micronucleus Assay, OECD 474	Male and female CD-1 mice	0, 1250, 2500, and 5000 mg/kg single intraperitoneal dose	No dose of ARA-rich oil produced micronuclei in polychromatic erythrocytes in the study. No cytotoxicity was observed.
Lewis et al., 2016	40.34%	Bacterial Reverse Mutation, OECD 471 compliant study	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2 *uvr*A	0.062, 0.185, 0.556, 1.667 2.5, 3.75, and 5 mg/plate	No dose of ARA rich oil induced a positive response in the presence or absence of S9 activation.

Table 14. Summary of Genotoxicity Studies Performed using *M. alpina* derived ARA-rich Oil					
Reference	**%ARA in ARA-rich oil**	**Study type**	**Cells/Model Used**	**Doses used**	**Results**
		Chromosome Aberration, USFDA Redbook 2000 compliant study	Human peripheral blood lymphocytes	1.25, 2.5, and 5.0 mg/mL	No dose of ARA rich oil increased the number of structural chromosomal aberrations or induced a positive test response in the presence or absence of S9 activation.
		Mammalian Erythrocyte Micronucleus Assay, USFDA Redbook 2000 compliant study	Male and female Wistar rats	1000, 2500, and 5000 mg/kg body weight	No dose of ARA-rich oil produced micronuclei in polychromatic erythrocytes in the study. No cytotoxicity was observed.

The pivotal toxicity study described in GRN 326 is on an ARA-rich oil that is essentially equivalent in production process, source organism, product specifications, and ARA content. A subchronic toxicity study with an *in utero* exposure study in rats was performed. It established a NOAEL of 5% ARA-rich oil in the diet equivalent to ARA-rich oil intake of 3170 mg/kg/day. This study is discussed in detail in Casterton et al. (2009) and GRN 326, pages 128-152. These findings are further corroborated by multiple subchronic toxicity studies in rats (Hempenius et al., 2000; Lina et al., 2006; Nisha et al., 2009; Gao et al., 2014; Lewis et al., 2016; Falk et al., 2017;) and tolerance studies in neonatal piglets (Merrit et al., 2003; Tyburczy et al., 2012), summarized in Table 15 below.

Each ARA-rich oil used in the corroborative studies described below is compositionally similar to Hubei Fuxing's ARA-rich oil and also has at least 40% ARA. These studies were discussed in GRN 730, pages 31–35. The safety of the ARA-rich oil manufactured by Hubei Fuxing is supported by the following studies in rats: two 28-day repeat dose toxicity studies, a 90-day subchronic toxicity study, three 90-day subchronic toxicity studies with an *in utero* exposure, a reproductive and developmental toxicity study, and a neonatal piglet study (additional corroboration provided from a neonatal piglet study of a blend of ARA- and DHA-rich oils). All corroborative data supporting the safety of Hubei Fuxing's ARA-rich oil are summarized in Table 15. Table 15 also summarizes corroborative data discussed in GRN 326, pages 149-153.

Table 15. Summary of Animal Toxicology Studies Performed using ARA-rich Oil

Reference	Species	%ARA in ARA-rich oil	Dose	Study type	No observed adverse effect level (NOAEL)
Pivotal Toxicity Study					
Casterton et al., 2009	Rat	43.3%	0.5, 1.5, 5.0% ARA in the diet	90 day subchronic toxicity study with an *in utero* exposure	5% of the diet, equivalent to an overall average intake of 3170 mg/kg/day
Corroborative data from GRN 730, pages 31 – 35					
Tyburczy et al., 2012	Piglet	40%	0.1-1.0% ARA of total fat	19-25 days, neonatal piglet	1% ARA of total fat in the diet was safe with no adverse events
Gao et al., 2014	Rat	48.3%	0%, 0.5%, 1.5% and 5% of the diet	90-day subchronic toxicity study with *in utero* exposure	5% of the diet
Lewis et al., 2016	Rat	40.3% ARA	0, 1000, 2500, or 5000 mg/kg/day	90-day subchronic toxicity	> 5000 mg/kg/day
Falk et al., 2017	Rat	40.3% ARA	0, 1000, 2500, or 5000 mg/kg/day	Gestation days 6-20, developmental toxicity	> 5000 mg/kg/day
Corroborative data from GRN 326, pages 149 – 153					
Hempenius et al., 2000	Rat	38.6% ARA	3000, 15000, or 75000 ppm	90-day subchronic toxicity study with an *in utero* exposure	15000 ppm (approximately 970 mg/kg/day)
Merritt et al., 2003	Piglet	Arachidonate-enriched triglyceride oil, 41.5% ARA	0, 96 mg ARA/kcal, 62 mg ARA/kcal	16 days, neonatal piglets	Both levels of ARA in the diet were safe with no adverse events
Nisha et al., 2009	Rat	*M. alpina* biomass, 35.6% ARA	0, 2500, 5000, 20000, or 30000 ppm	90-day subchronic toxicity	5000 mg/kg/day
Lina et al., 2006	Rat	Arachidonate-enriched triglyceride oil, 41.5% ARA	0%, 0.5%, 1.5% or 5.0% in the diet	90-day subchronic toxicity preceded by an *in utero* exposure	5% in the diet (approximately 3000 mg/kg/day)

2. Genotoxicity Studies

a. Pivotal Genotoxicity Studies (Casterton et al., 2009)

The genotoxicity of Cargill Alking's ARA-rich oil (RAO) was assessed in a bacterial reverse mutation (Ames) assay, a chromosome aberration assay in cultured Chinese hamster ovary cells, and a gene mutation assay with mouse lymphoma L5178Y cells at the thymidine kinase locus (Casterton et al., 2009). Each study was conducted in the presence and absence of S9 metabolic activation. These studies were performed in compliance with OECD guidelines.

RAO was negative in all three genotoxicity assays, each of which was conducted in the presence and absence of S9 metabolic activation. In the Ames assay, RAO did not produce a dose-related increase in mean revertants and did not produce a positive response at any of the test concentrations. In the chromosome aberration assay, RAO induced neither a dose-related increase in the number of structural chromosomal aberrations nor a reproducible positive response at any of the test concentrations. Finally, in the mouse lymphoma assay, RAO produced no relevant increases in mutant frequency at any of the test concentrations.

b. Corroborative Genotoxicity Studies

The mutagenicity of Hubei Fuxing's ARA-rich oil was assessed in an unpublished Ames assay using the plate incorporation method. This assay was performed using the following bacterial strains of *S. typhimurium* histidine auxotroph: TA97, TA98, TA100, and TA102. The Ames test was also performed in the presence (+S9) or absence (-S9) of PCB-induced rat liver homogenate as an in vitro activation system. ARA-rich oil was used at 0.2, 0.5, 1, 2.5, and 5 mg/dish in 0.1 mL for each strain of *S. typhimurium.* The ARA-rich oil was diluted in dimethyl sulfoxide (DMSO), which was used as the vehicle control. The spontaneous revertants were included as the indicated strains of *S. typhimurium* with no treatment as the negative control. A positive control for all strains, including the S9-treated strains, was also included in the assay. The positive control for strains TA97, TA98, and TA102 in the absence of S9 was 50 µg/plate Dexon. The positive control for TA100 in the absence of S9 was 2.5 µg/plate sodium azide. In the presence of S9 enzymatic activation, TA97, TA98, and TA100 used 10 µg/plate 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (2-AF) as a positive control. The positive control for TA102 with S9 activation was 50 µg/plate 1,8-dihydroxy anthraquinone. The assay was repeated twice with the average number of colonies shown with standard deviations below in Table 16.

If the ARA-rich oil treatment groups had twice the number of colonies as the spontaneous revertant group, then ARA-rich oil would be considered genotoxic. None of the treatments, in the presence or absence of S9 activation, caused an increase in colony number over the spontaneous revertant control. No dose response relationship was observed in any of the strains of *S. typhimurium*. The positive controls for each strain had a robust increase in colony number. These results indicate that ARA-rich oil is not genotoxic under the conditions used in this assay.

The lack of genotoxic potential of Hubei Fuxing's ARA-rich oil is also supported by an Ames assay, in vitro chromosomal aberration test, and an *in vivo* mammalian erythrocyte micronucleus test performed on the ARA-rich oil in GRN 730, another ARA-rich oil generated from *M. alpina*. The ARA-rich oil described in GRN 730 yielded negative results for these genotoxicity studies (Lewis et al., 2016).

Table 16. Ames Test Results with ARA-Rich Oil

***S. typhimurium* Strain**		**TA97**		**TA98**		**TA100**		**TA102**	
	mg/plate	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
ARA-rich oil	0.2	150.5 ± 0.71	158.5 ± 0.71	42.5 ± 0.71	42.5 ± 2.12	159.5 ± 0.71	164.5 ± 0.71	247.0 ± 1.41	237.0 ± 1.41
	0.5	153.0 ± 1.41	157.0 ± 4.24	42.0 ± 1.41	42.0 ± 1.41	160.0 ± 2.83	157.5 ± 13.44	248.0 ± 4.24	239.5 ± 4.95
	1	150.5 ± 3.54	159.5 ± 2.12	43.0 ± 1.41	41.0 ± 1.41	166.0 ± 2.83	166.0 ± 2.83	246.0 ± 5.66	249.0 ± 4.24
	2.5	154.5 ± 0.71	161.0 ± 2.83	42.0 ± 1.41	44.0 ± 1.41	162.5 ± 0.71	166.0 ± 5.66	248.5 ± 10.61	258 ± 2.83
	5	158.5 ± 0.71	156.0 ± 9.90	46.5 ± 0.71	45.5 ± 0.71	161.5 ± 2.12	158.5 ± 2.12	254.5 ± 3.54	253.5 ± 9.19
DMSO		154.0 ± 4.24	153.0 ± 2.83	41.0 ± 1.41	41.5 ± 0.71	156.5 ± 3.54	156.0 ± 1.41	256.5 ± 0.71	258.5 ± 2.12
Spontaneous revertant		150.0 ± 2.83	156.0 ± 2.83	42.5 ± 0.71	42.0 ± 2.83	153.0 ± 2.83	157.5 ± 0.71	256.5 ± 7.78	261.5 ± 0.71
Positive Control Group (ug/plate)									
NaN_3	2.5	-	-	-	-	1611.5 ± 40.31	-	-	-
2-AF	10	-	1158.5 ± 58.69	-	1845.0 ± 25.46	-	1907.0 ± 0.00	-	-
Dexon	50	2428.0 ± 63.64	-	2357.0 ± 14.14	-	-	-	1195.0 ± 63.64	-
1,8-dihydroxy anthraquinone	50	-	-	-	-	-	-	-	1535.0 ± 7.07

Average of two experiments shown with standard deviation
2-AF: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide
DMSO: dimethyl sulfoxide, served as vehicle control

3. Toxicology Studies

a. Pivotal Subchronic Toxicity with an In Utero Exposure

Casterton et al. (2009) evaluated the potential toxicity of refined ARA-rich oil (43.3% ARA) derived from *M. alpina* by performing a 90-day subchronic dietary toxicity study in F_1 Sprague Dawley (SD) rats with an *in utero* exposure. This study was preceded by a 4-week pre-treatment period of parental (F_0) rats and exposure of the F_0 dams throughout mating, gestation, and lactation. Two control diets were used in the study, a standard diet (low-fat) and a high fat diet (standard diet supplemented with 5% corn oil). The results indicated that ARA-rich oil, at concentrations of 0.5%, 1.5%, and 5.0% of diet, did not affect the reproductive performance of the parental rats. No mortality or morbidity was reported for the pups at any dose tested. In the subchronic study with the offspring (F_1) rats, no treatment-related abnormalities were observed. Thus, the NOAEL was determined to be 5% ARA-rich oil, the highest level tested. This level corresponds to an overall average of male and female F_0 and F_1 rats of 3170 mg/kg/day.

b. Corroborative Studies

Lina et al. (2006) evaluated the potential toxicity of refined ARA-rich oil (41.5% ARA) derived from *M. alpine* in a 90-day subchronic study in F_1 Wistar rats with an *in utero* exposure. This study was preceded by a 4-week pre-treatment period of parental (F_0) rats and exposure of the F_0 dams throughout mating, gestation, and lactation. High-fat and low-fat controls received the basal diet with or without 5.76% corn oil. The results indicated that ARA-rich oil, at concentrations of 0.5%, 1.5%, and 5.0% of diet, did not affect the reproductive performance of the parental rats. No mortality or morbidity was reported for the pups at any dose tested. In the subchronic study with the offspring (F_1) rats, no treatment-related abnormalities were observed. The NOAEL was determined to be 5% ARA-rich oil, equivalent to approximately 3000 mg/kg/day in F_0 and F_1 animals.

A 90-day subchronic toxicity study preceded by an *in utero* exposure in Wistar rats, performed by Hempenius et al. (2000), used dose levels of 3000, 15000, and 75000 ppm ARA-rich oil. This study was preceded by a 4-week pre-treatment period of parental (F_0) rats and exposure of the F_0 dams throughout mating, gestation, and lactation. The study was controlled such that each treatment group received the same amount of fat in the diet by adding appropriate amounts of corn oil. The ARA-rich oil used in this study was 38.6% ARA and was derived from *M. alpina*.

The animals fed 75000 ppm ARA-rich oil had increased adrenal, spleen, and liver weights, and females had increased incidence of hepatocellular vacuolation. Statistically significant differences in alkaline phosphatase activity, cholesterol, triglycerides and phospholipids, creatinine and urea were considered related to a high n-3 fatty acid containing diet and non-adverse. Similar results have been reported in other studies of high lipid ingestion in

rats. None of these findings were observed in the 15000 ppm ARA-rich oil fed rats; therefore, Hempenius et al. (2000) determined a NOAEL of 15000 ppm ARA-rich oil, equivalent to approximately 970 mg/kg/day. The results of this study are supported by an earlier reported 28-day repeated oral toxicity study that described no toxicity observed at 3000 mg/kg/day in Wistar rats (Hempenius et al., 1997).

Gao et al. (2014) evaluated the potential toxicity of refined ARA-rich oil (48.3% ARA) derived from *M. alpina* by performing a 90-day subchronic study in F_1 Sprague Dawley (SD) rats with an *in utero* exposure. This study was preceded by a 4-week pre-treatment period of parental (F_0) rats and exposure of the F_0 dams throughout mating, gestation, and lactation. The results indicated that ARA-rich oil, at concentrations of 0.5%, 1.5%, and 5.0% of diet, did not affect either reproductive performance of the parental rats. No mortality or morbidity was reported for the pups at any dose tested. In the subchronic study with the offspring (F_1) rats, no treatment-related abnormalities were observed. Thus, the NOAEL was determined to be 5% ARA-rich oil, the highest level tested. This level corresponds to approximately 3750 mg/kg in the F_0 females, 2850 mg/kg in the F_0 males, 4850 mg/kg in F_1 females, and 4480 mg/kg in F_1 males.

In a study by Lewis et al. (2016), the safety of ARA-rich oil (40.3% ARA) from *M. alpina* was evaluated by conducting a 28-day toxicity study and a 90-day subchronic toxicity study in Wistar rats. The 28-day and 90-day studies used dietary exposure to 1000, 2500, and 5000 mg/kg body weight/day of ARA-rich oil and two control diets: water and corn oil (vehicle control). There were no treatment-related effects of ARA-rich oil on clinical observations, body weight, feed consumption, behavior, hematology, clinical chemistry, coagulation, urinalysis parameters, or necropsy findings. Increases in cholesterol and triglyceride levels were considered related to a high oil diet and non-adverse. The NOAEL from the subchronic toxicity study was determined to be 5000 mg/kg body weight/day, the highest dose tested. The ARA-rich oil used in the Lewis et al. (2016) study contained 40.3% ARA. The ARA-rich oil produced by Hubei Fuxing is > 40% ARA (Table 5).

A study by Falk et al. (2017) investigated the reproductive and developmental toxicity of dietary exposure to ARA-rich oil (40.3% ARA) derived from *M. alpina.* In the developmental toxicity study, pregnant Wistar rats were untreated (control) or administered corn oil (vehicle control), 1000, 2500, or 5000 mg/kg body weight/day of ARA-rich oil via gavage from gestation days 6 through 20. In the reproductive toxicity study, female Wistar rats were administered vehicle control (corn oil), or 1000, 2500, or 5000 mg/kg body weight/day of ARA-rich oil via gavage throughout the mating period, pregnancy, and the nursing and lactation periods.

Differences in the number of fetuses, fetal skeletal malformations, and external and visceral anomalies in the developmental study and mortality, clinical signs, fertility indices, physical observations, gross necropsy findings, and gestation period length in the reproductive

toxicity study were not dose-related or significantly different from control groups and were not considered treatment-related. The NOAEL for maternal toxicity and embryo/fetal development and for paternal or maternal treatment-related reproductive toxicity for the ARA-rich oil administered by oral gavage was 5000 mg/kg body weight/day in rats.

Tyburczy et al. (2012) evaluated the effect of physiologically high dietary ARA-rich oil derived from *M. alpina* on growth, clinical chemistry, hematology, and immune function in newborn piglets. Three-day old piglets were administered one of seven diets for 25 days: 6 diets with varying ratios of ARA:DHA as follows (ARA/DHA as a percentage of all fatty acids): 0.1/1.0; 0.53/1.0; 0.69/1.0; 1.1/1.0; 0.67/0.62; and 0.66/0.33. A seventh group was maternal-reared and remained with the dam during the study. No treatment-related abnormalities were observed in formula intake, growth, clinical chemistry, hematology, or immune status measurements. The authors concluded that a dietary ARA concentration up to 1% total fatty acid (or 49 mg/100 kcal of the formula) was safe and had no adverse effect on any of the safety outcomes measured.

The safety of ARA-rich oil was further corroborated by another neonatal piglet study, performed using a blend of ARA and DHA in formula (Merritt et al., 2013). The formulas used in the study were: a control formula with no added ARA or DHA, a test formula with 55 mg DHA/100 kcal, a test formula with 96 mg ARA/100 kcal, and a mix of 34 mg DHA and 62 mg ARA/100 kcal. All formulas were equal in fat content and calories. No ARA or DHA-related effects were indicative of adverse health consequence to the animals. Additionally, there were no statistically significant differences between the test formulas and control in clinical signs, body weights, food consumption, clinical chemistry, hematology, organ weights or gross or histopathology (Merritt et al., 2003).

C. CLINICAL STUDIES

The clinical studies discussed in previous GRNs are briefly summarized below and in Table 17. Studies using 0.64-0.72% of total fatty acids 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. No studies reported adverse effects of ARA or ARA-rich oil. There have been no adverse effects reported on the consumption of ARA-rich oils in pre-term infants when used at levels up to 0.91% total fatty acid content (Almaas et al., 2015, 2016; Alshweki et al., 2015; Kitamura et al., 2016; van de Lagemaat et al., 2011; Westerberg et al., 2011; Carnelli et al., 2007; Clandinin et al., 2005; Groh-Wargo et al., 2005). These studies used multiple sources of ARA-rich oil, including *M. alpina*. We incorporate by reference the clinical studies described in GRN 326, pages 61-88 and GRN 730 pages 35-44.

The latest ARA-rich oil GRAS notice that received no questions from the FDA was in 2017. Since that time, two clinical studies conducted by Hoffman et al. (2019) and Lorenzo et al. (2019) have been published on the safe use of ARA-rich oils for infants.

A multicenter, double-blind, randomized, controlled, parallel-group, prospective trial in healthy term infants was performed to assess the equivalence of DHA and different levels of ARA in combination with a prebiotic (1:1 polydextrose and galactooligosaccharides, 4 g/L) on the concentration of ARA and DHA in red blood cells (Hoffman et al., 2019). Healthy 10-18 day old infants were enrolled in the study and randomized into the following three groups: control, fed infant formula with 17 mg DHA/100 kcal and 34 mg ARA/100 kcal (n=31); test group 1: infant formula with 17 mg DHA/100 kcal, 25 g ARA/100 kcal (n=29); and test group 2: infant formula with 17 mg DHA/100 kcal, 34 mg ARA/100 kcal and the prebiotic (n=20). The results of the study describe that availability of DHA in red blood cells was not affected by the different concentrations of ARA or the presence of the prebiotic. No statistically significant group differences in weight, length or head circumference growth rates were detected for any age range or gender at any time point during the study. Parent reported study formula intake (fluid ounces/day) was significantly lower at day 60 in the ARA + prebiotic blend group vs. the control; however, no group intake differences were observed at days 30, 90, or 120. Mean reported intakes increased from day 30 to 120 for all study groups, indicating normal intake for the time period. No statistically significant group differences in gassiness, fussiness, stool frequency or consistency were reported. At day 30, there was a significant difference in stool consistency between the ARA + prebiotic blend group and the control, but this finding was consistent with previous studies of infants receiving the prebiotic blend. No statistically significant group differences were detected in overall incidence of adverse events.

An interventional, randomized and double-blinded study in healthy full-term infants was performed to understand the effect of minor alleles for the fatty acid desaturase genes on the concentrations of ARA and DHA in cheek cell samples (Lorenzo et al., 2019). This cohort was part of the COGNIS (a neurocognitive and immunological study of a new formula for healthy infants) study. Healthy term infants were randomized into two groups: group 1, infants fed with standard formula (n=85); group 2: infants fed with the experimental formula, containing a fungal oil composed of 15.8 mg/100 mL ARA and 11.2 mg/100 mL DHA(n=85). A reference group of breast-fed infants was included in the analysis (n=50). Formula-fed infants with minor alleles in the fatty acid desaturase genes were associated with declined desaturase activity and therefore lower ARA and DHA levels, regardless of ARA/DHA supplementation. No discussion of adverse events or safety parameters were reported in this publication.

Table 17. Corroborative Pre-term and Term Infant Clinical Studies			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Birch et al., 2005	Doubly masked, randomized controlled trial with 39-week duration and follow up until 52 weeks, following study initiation in term infants.	• Control group: infant formula (n=52) • Test group: infant formula supplemented with 0.72% ARA (fungal oil) and 0.36% DHA (algal oil). Percentages in diet given as % of total fatty acids. (n=51)	• Evaluation of sweep visual evoked potential (VEP) acuity in the LCPUFA supplemented group was significantly better than that in the non-supplemented control group at all time points measured ($p<0.001$ to 0.01). • Red blood cell concentration of ARA was 15-18% higher in the LCPUFA supplemented group than in the control group. Red blood cell DHA concentrations in the LCPUFA group were 215% higher than in the control group by 39 weeks. Both increases were statistically significant ($p<0.001$ to 0.01). • For both groups, all anthropometric outcome data were normally distributed. No significant effect of diet was found on growth evaluated by weight, length, or head circumference and both diets were well tolerated.
Clandinin et al., 2005	A prospective, randomized double-blind study; 92 weeks post-menstrual age (PMA) with follow up in second phase at 118 weeks PMA	• Control: infant formula (n=119) • Test group 1: Formula with 34 mg ARA + 17 mg algal DHA/100 kcal (n=112) • Test group 2: Formula with 34 mg ARA + 17 mg fish DHA/100 kcal (n=130) • Reference Group: term infants (n=105) breast-fed for ≥ 4 months	• Results showed that weight of the infant group given ARA together with DHA was significantly ($p<0.05$) greater than the control group from 66 to 118 weeks PMA but did not differ from infants in the reference group at 118 weeks PMA. • Bayley mental (MDI) and psychomotor development (PDI) scores at 118 weeks PMA (18 months after term) were higher in infants given ARA/DHA supplemented formula compared to the control group. The MDI and PDI scores for the infants in the breast-fed term reference group were near the reference norm and significantly higher than the preterm groups. • Mean weight, length and head circumference and respective growth rates did not differ among the preterm groups. • Analysis of clinical data including severity of medical conditions relating to prematurity, serum chemistry and hematology found no safety issues related to the supplemented formulas. There were no increases in morbidity or adverse events in the groups given supplemented formulas relative to the control.

Table 17. Corroborative Pre-term and Term Infant Clinical Studies			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Groh-Wargo et al., 2005	A controlled, double blind study conducted up to 40-weeks gestational corrected age of infants Sixty preterm infants with birth weights from 750 to 1800 g and gestational age at birth <33 weeks	• Control: milk formula (n=22) • Test group 1: milk formula supplemented with 0.42% ARA (egg-derived triglyceride) and 0.26% DHA (fish oil); (% based on grams/100 grams total fatty acids) (n=18) • Test group 2: milk formula supplemented with 0.42% ARA (fungal oil) and 0.26% DHA (fish oil); (% based on grams/100 grams total fatty acids) (n=20)	• No significant differences were seen among the three groups were seen at any time point in weight, length, or head circumference. • Bone mineral content and bone mineral density did not differ among groups. At 12 months, term corrected age (TCA) infants who were fed ARA/DHA-supplemented formulas had significantly greater lean body mass and significantly less fat mass than infants who were fed the non-supplemented control formula. • The ARA/DHA-supplemented formulas supported normal growth and bone mineralization in premature infants who were born at < 33-week gestation. No differences among the groups were seen in the percentage of infants with adverse clinical complications. • At 12 months TCA, preterm infants that were fed the ARA/DHA supplemented formula had increased lean body mass and significantly less fat mass by one year of age than infants fed non-supplemented formula. • The authors concluded supplementation of infant formula with ARA and DHA had a beneficial effect on growth and lean body mass.
Birch et al., 2007	Randomized trial with follow up study at 4 years of children that had been fed supplemented diets for 17 weeks during infancy	• Control group infants fed commercial un-supplemented formula (n=26) • Test group: Infants fed supplemented formula with 0.72% ARA (fungal oil) and 0.35% DHA (algal oil) (% based on total fatty acid content.) (n=26) • Reference group: 32 breast-fed infants that had been enrolled in the same 17-week randomized trial	• At 4 years of age, the group that had received the control formula as infants had significantly poorer visual activity ($p<0.03$) and verbal IQ ($p<0.003$) than the children who were breast-fed for an average of 43 weeks or those who had been fed formula containing ARA/DHA during the first 17 weeks of life. The formula supplemented group had visual acuity and verbal IQ scores that did not differ significantly from breast-fed children at 4 years of age. The result of this trial suggested that ARA/DHA supplementation of infant formula for at least the first 4 months after birth supports visual activity and IQ maturation similar to that of breast-fed infants. • No discussion of adverse events or safety parameters reported.

Table 17. Corroborative Pre-term and Term Infant Clinical Studies			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Carnelli et al., 2007	Randomized pre-term infants, from birth to 7 months	• Control, non-supplemented formula (n=11) • Fungal ARA (0.84%) 12.0 mg + DHA (fish oil), 7.1 mg per 100 mL of formula (n=11)	• Measured absolute long chain PUFA synthesis and percentage of long chain PUFA synthesis relative to dietary intake and plasma phospholipids. • All infants grew normally during the trial (7 months) and no significant difference between groups was found in weight gain.
Birch et al., 2010	Double-masked, randomized trial in healthy term infants, first 12 months of life (from days 1-9), sole source of nutrition until < 4 months of age. This report is from the DIAMOND (DHA Intake And Measurement of Neural Development) study.	• Control: non-supplemented infant formula (n=56) • Test group 1: 0.64% ARA + 0.32% DHA supplemented infant formula (n=64) • Test group 2: 0.64% ARA + 0.64% DHA supplemented infant formula (n=59) • Test group 3: 0.64% ARA + 0.96% DHA supplemented infant formula (n=65)	• Infants fed control formula had significantly poorer visual evoked potential visual acuity at 12 months of age than infants that received any of the ARA + DHA supplemented infant formula. No difference between ARA + DHA groups were observed. • No significant effects on weight, length, bowel movements, or adverse events. • Infant formulas were well-tolerated and all groups had normal growth throughout first 12 months of life.

Table 17. Corroborative Pre-term and Term Infant Clinical Studies			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Colombo et al., 2011	Double-blind, randomized, controlled, parallel-group prospective trial in 122 term infants, from birth to 12 months of age. This report is from the DIAMOND (DHA Intake And Measurement of Neural Development) study	• Control: non-supplemented infant formula • Test group 1: 0.64% ARA + 0.32% DHA supplemented infant formula • Test group 2: 0.64% ARA + 0.64% DHA supplemented infant formula • Test group 3: 0.64% ARA + 0.96% DHA supplemented infant formula	• Infants in all DHA+ARA supplemented conditions had lower heart rates than those in the non-supplemented groups, no dose response was found. • Infants supplemented at the two lower DHA doses spent proportionately more time engaged in active stimulus processing than infants fed non-supplemented formula, while infants fed the highest dose were intermediate and did not differ from any other group. • No safety parameters reported.
Drover et al., 2011	Double-masked, randomized, controlled, prospective trial. 181 term infants First 12 months of life, sole source of nutrition until <4 months of age; follow up at 18 months This report is from the DIAMOND (DHA Intake And Measurement of Neural Development) study	• Control: non-supplemented infant formula (n=28) • Test group 1: 0.64% ARA + 0.32% DHA supplemented infant formula (n=29) • Test group 2: 0.64% ARA + 0.64% DHA supplemented infant formula (n=32) • Test group 3: 0.64% ARA + 0.96% DHA supplemented infant formula (n=28)	• No diet group differences on the mental development index, the psychomotor development index, or the behavior rating scale. • DHA-supplemented subjects had higher mental development index scores than non-supplemented subjects. • Formulas were well tolerated. • No significant differences were observed in adverse events in any groups.

Table 17. Corroborative Pre-term and Term Infant Clinical Studies			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Westerberg et al., 2011	Randomized, double-blinded, placebo-controlled intervention trial in very low birth weight infants. Infants were given milk + oil for an average of 63 days from birth to discharge from the hospital	• Human milk with placebo (n=48) • Human milk with 0.5 mL oil (containing 31 mg ARA plus 32 mg DHA) per 100 mL milk (n=44)	• Cognitive function tests were performed at 20 months and found positive effects from the supplementation on functions related to attention. • Plasma DHA concentration was positively correlated with sustained attention and mental development index. • No safety parameters were reported.
van de Lagemaat et al., 2011	Randomized, controlled trial evaluating the effect of post discharge formula, term formula and human milk in 139 pre-term infants for 6 months.	• Control: human milk (n=46) • Test group 1: Post-discharge formula (0.4% ARA, 0.4% DHA) (n=52) • Test group 2: term formula (0.2% ARA, 0.2% DHA) (n=41)	• No significant differences in weight, length, or head circumference between any of the groups. • Formula fed infants had higher red blood cell DHA and DHA/ARA ratio than human milk fed infants. • Post-discharge formula fed infants had higher red blood cell DHA, EPA and DHA/ARA ratio than term formula and milk fed infants. • Post-discharge formula fed infants had higher red blood cell ARA than term formula fed infants, with similar values as those found in human milk fed infants.

Table 17. Corroborative Pre-term and Term Infant Clinical Studies			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Drover et al., 2012	Double-masked, randomized, controlled, prospective trial. 182 term infants First 12 months of life, sole source of nutrition until <4 months of age, follow up at 2, 2.5 and 3.5 years. This report is from the DIAMOND (DHA Intake And Measurement of Neural Development) study	• Control: non-supplemented infant formula (n=19) • Test group 1: 0.64% ARA + 0.32% DHA supplemented infant formula (n=23) • Test group 2: 0.64% ARA + 0.64% DHA supplemented infant formula (n=24) • Test group 3: 0.64% ARA + 0.96% DHA supplemented infant formula (n=22)	• No diet group differences on the Bracken Basic Concept Scale. • The control fed group had higher raw scores and standard scores on the Peabody Picture Vocabulary Test than the 0.32% and 0.96% DHA fed groups at 2 years of age, but these differences were not observed at 3.5 years of age. • No safety parameters were reported.
Almaas et al., 2015	Randomized, double-blinded, placebo-controlled study in 129 very low birth weight infants with birth weights < 1500 g. Consumed test formula for 9 weeks after birth. Follow up at 8 years of age.	• Control: human milk (n=40) • Test group: Human milk supplemented with 21 mg ARA (0.91% of total fatty acids) and 32 mg DHA (0.86% of total fatty acids) (n=45)	• No significant differences between the intervention group and the control group on any cognitive measures. • No safety parameters were reported.

Table 17. Corroborative Pre-term and Term Infant Clinical Studies			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Alshweki et al., 2015	Randomized trial, newborns < 1500 g and/or < 32 weeks of gestational age	• Control: breast milk (n=25) • Test group 1: formula containing 2:1 ARA: DHA (0.62-0.72% ARA and 0.31-0.36% DHA) (n=24) • Test group 2: formula containing 1:1 ARA:DHA (0.30-0.37% ARA and 0.30-0.37% DHA) (n = 21)	• ARA was significantly higher in the test group receiving 2:1 ARA:DHA than the test group receiving 1:1 ARA:DHA. • Psychomotor development scores were higher in the group receiving 2:1 ARA:DHA than the 1:1 ARA:DHA group, similar to the control. • No significant differences between to the two test groups were observed for weight, length, or head circumference.
Kitamura et al., 2016	Randomized, double-blind trial in low or very low birth weight infants with body weight of >1000 g Intervention started at after discharge from intensive care unit and lasted for 1 month	• Control: 1 mg ARA + 9.1 mg DHA/100 mL (n=16) • Test group: 4.6 mg ARA + 9.1 mg DHA/100 mL (n=19)	• No difference was found in body weight gain, height gain and head circumference gain development. • Authors reported no adverse events. • The ARA content in red blood cells was higher in the test group than the control.
Almaas et al., 2016	Randomized, double-blinded, placebo-controlled study in 129 very low birth weight infants with birth weights < 1500 g. Consumed test formula for 9 weeks after birth. Follow up at 8 years of age	• Control: human milk (n=53) • Test group: Human milk supplemented with 21 mg ARA (0.91% of total fatty acids) and 32 mg DHA (0.86% of total fatty acids) (n=45)	• No significant differences between the intervention group and the control group were found on white matter microstructure or behavioral data. • No safety parameters were reported

Table 17. Corroborative Pre-term and Term Infant Clinical Studies			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Hoffman et al., 2019	Multicenter, double-blind, randomized, controlled, parallel-group, prospective trial. Healthy 10-18 day old term infants receiving formula through 120 days of age	• Control: infant formula with 17 mg DHA/100 kcal and 34 mg ARA/100 kcal (n=31) • Test 1: infant formula with 17 mg DHA/100 kcal, 25 g ARA/100 kcal (n=29) • Test 2: infant formula with 17 mg DHA/100 kcal, 34 mg ARA/100 kcal and a prebiotic blend of 1:1 polydextrose and galacto oligosaccharides at 4 g/L (n=20)	• No statistically significant group differences from control infants were detected for any age range or gender at any time point during the study in weight, length or head circumference growth rates. • Parent reported study formula intake (fluid ounces/day) was significantly lower at day 60 in the ARA + prebiotic blend group vs. the control, however no group intake differences were observed at days 30, 90, or 120. Mean reported intakes increased from day 30 to 120 for all study groups, indicating normal intake for the time period. • No statistically significant group differences in gassiness, fussiness, stool frequency or consistency were reported. At day 30, there was a significant difference in stool consistency between the ARA + prebiotic blend group and the control, but this finding was consistent with previous studies of infants receiving the prebiotic blend. • No statistically significant group differences were detected in overall incidence of adverse events.
Lorenzo et al., 2019	Interventional, randomized and double-blinded study of 176 full term, healthy infants	• Reference group: breast fed infants (n=50) • Test group 1: infants fed with standard formula (n=85) • Test group 2: infants fed with experimental formula, with 15.8 mg/100 mL fungal oil ARA and 11.2 mg/100 mL DHA (n=85)	• No safety parameters reported • Formula fed infants with minor alleles in the fatty acid desaturase genes were associated with declined desaturase activity and lower ARA and DHA levels, regardless of ARA/DHA supplementation

D. ALLERGENICITY

M. alpina is a common soil fungus to which humans are frequently exposed (Streekstra, 1997). *M. alpina* is not reported to be allergenic, is non-pathogenic and does not form potentially allergenic spores. A search performed on April 8, 2020, on PubMed using the term "*M. alpina*" and "allergy" yielded no results. Searching for "arachidonic acid" and "allergy" found a report of decreased allergies in infants receiving infant formula supplemented with ARA (Foiles et al., 2016). Therefore, ARA-rich oil is not expected to induce an allergic response.

E. REGULATORY APPROVALS ACROSS THE WORLD

ARA-rich oils derived by fermentation of the fungus *M. alpina* have been used in commercially available infant formulas in at least 50 countries since the early 1990s. They are considered safe for use in infant formula in the United States (GRNs 41, 80, 94, 326, and 730), have been the subject of extensive safety reviews conducted by the European Food Safety Authority and the Food Standards Australia New Zealand (EFSA, 2008; FSANZ 2003), and are considered novel foods in Australia, New Zealand, and the European Union.

VII. SUPPORTING DATA AND INFORMATION

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B. EXPERT PANEL STATEMENT

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of Hubei Fuxing's ARA-rich oil for the intended use specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b).

Hubei Fuxing Biotechnology Co., Ltd is proposing to market ARA-rich oil, produced by Hubei Fuxing Biotechnology Co., Ltd, China, as a source of ARA-rich oil used in the manufacture of infant formula. The end-use infant formulas are exempt pre-term infant formula and non-exempt term infant formula. Consistent with other GRAS sources of ARA-rich oil (GRN 730; 326), this ingredient is produced by the fungus *Mortierella alpina* and specifications stipulate a minimum of 40% arachidonic acid in the oil.

The following safety evaluation considers the composition, intake, nutritional, microbiological, and toxicological properties of Hubei Fuxing's ARA-rich oil based on publicly available data from essentially equivalent ARA-rich oils as determined GRAS in GRN 326. Corroborative safety data are described in GRNs 730, 41, 80, and 94, each of which received "no questions" letters from the United States Food and Drug Administration (FDA). The proposed use of Hubei Fuxing's ARA-rich oil as an ingredient in non-exempt term infant formula and exempt pre-term infant formula has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based upon the following:

- The compositional data and product specifications are demonstrative of carefully controlled production and purification processes. ARA-rich oil contains no detectable contaminants of concern for human health.

- The morphology, biochemistry, and physiology of *M. alpina* are well-documented and it is not pathogenic or toxigenic. *M. alpina* is not genetically modified.

- The FDA has issued 'no question' letters for five GRAS notices for ARA-rich oils derived from *M. alpina* for infant formula (GRNs 41, 80, 94, 326, and 730). A comparison of the specifications between the ARA-rich oil that is the subject of this notification and those in GRN 326 demonstrates that the product specifications for Hubei Fuxing's ARA-rich oil are comparable to the product specifications for the ARA-rich oil generated from *M. alpina* as described in GRN 326, with some parameters being more stringently controlled, including acid value, anisidine value,

mercury, and moisture. Specifications for ARA rich oils determined GRAS in GRNs 41, 80, 94, and 730 show that they are similar in composition to the ARA rich oil produced by Hubei Fuxing and therefore relevant as corroborative data.

- The intended use for ARA-rich oil is as an ingredient in exempt and non-exempt infant formulas. The functional importance of long-chain polyunsaturated fatty acids (LCPUFA) in pregnancy, lactation, and infancy have been the subject of numerous clinical evaluations, particularly as they relate to the n-6 and n-3 LCPUFA, ARA (C20:4n-6) and docosahexaenoic acid (DHA; C22:6n-3). Studies have suggested 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. Breast milk is the preferred method of providing an exogenous supply of ARA and DHA. However, when for medical or personal reasons, infant formula is chosen as a sole source or supplementary infant food, addition of ARA and DHA to the formula may support the adequate nutritional status of pre-term and term infants. Based on current knowledge regarding the importance of LCPUFA and their presence in human milk, guidelines and recommendations established by the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation collectively recommend that the level of DHA in infant formula be 0.20 to 0.50 weight percent of total fat, with the minimum amount of ARA being equivalent to the DHA content. These recommendations lead to the use of highly refined oils as sources of ARA and DHA for addition to infant formulas.

- 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 proposed use of ARA-rich oil is intended to provide 0.75% and 0.40% ARA by weight of fatty acids in term and pre-term infant formulas, respectively; this is within the range found in human milk. The intended use of ARA-rich oil to deliver this concentration of ARA, corresponds to 1.875% of total fat for non-exempt term infant formula and 1.00% of total fat for exempt pre-term infant formula. This intended use level is consistent with the levels of use cited in GRN 326 (term and pre-term infants) and is also consistent with the same use levels of ARA-rich oils cited in GRNs 80, 94, and 730.

- An estimate of exposure to ARA from its addition to infant formula at target ARA levels of 0.75 g and 0.40 g per l00 g total fat for term and pre-term infant formulas may be calculated as follows: Assuming human infants consume 100 kcal/kg body weight/day (term infants) to 120 kcal/kg body weight/day (pre-term infants), of which

fat comprises about 50% of the energy, an infant will consume 5.6 g (term infants) to 6.7 g (pre-term infants) of fat/kg body weight /day (1 g fat = 9 kcal). These correspond to intakes of ARA of 42 mg and 27 mg ARA/ kg body weight /day (or 104 and 67 mg of ARA-rich oil/kg body weight/day) for term infants and pre-term infants, respectively.

- The source organism, manufacturing process, product specifications, and intended uses of Hubei Fuxing's ARA-rich oil are essentially equivalent to ARA-rich oil cited in GRN 326; therefore, publicly available animal and human safety and tolerance studies of this ARA-rich oil are used as the pivotal data to support the safety of Hubei Fuxing's ARA-rich oil. Data from studies of other ARA-rich oils cited in GRNs 41, 80, 94, and 730 are corroborative of the safety of the Hubei Fuxing's ARA rich oil.
    - The safety of ARA-rich oils as ingredients in infant formula has been reviewed by numerous regulatory bodies worldwide. In these jurisdictions, the conclusions reached were that ARA-rich oils derived from *M. alpina*, meeting appropriate food grade specifications, provide a safe ARA source for supplementation of infant formula. These decisions have led to its availability for this use in at least 50 countries worldwide.
- Numerous animal safety studies have been conducted over a period of more than a decade on ARA-rich oils derived from *M. alpina*. The pivotal study cited in GRN 326 is a subchronic toxicity study with an *in utero* exposure which establishes a no-observed adverse event level (NOAEL) of 5% ARA-rich oil in the diet, equivalent to an average intake of ARA-rich oil of 3170 mg/kg/day (Casterton et al., 2009).
    - The safety is supported by lack of systemic toxicity and reproductive or developmental toxicity reported in corroborative studies described in GRN 730: a 28-day toxicity study, a 90-day subchronic toxicity study with an *in utero* exposure, a 90-day subchronic toxicity study, a reproductive and developmental toxicity study, and a neonatal piglet study (additional corroboration provided from a neonatal piglet study of a blend of ARA- and DHA-rich oils). These studies corroborate the results from the pivotal 90-day toxicology study which established a NOAEL of 3170 mg/kg/day. In addition, studies showed a lack of adverse effects on developmental or reproductive parameters. Tolerance and safety in a neonatal piglet model determined that dietary ARA concentration of up to 96 mg ARA/100 kcal was safe and well tolerated.

  - A corroborative, unpublished bacterial reverse mutation assay of Hubei Fuxing's ARA-rich oil was negative.

- There were no test article-related adverse effects reported in clinical studies of infant formula containing ARA-rich oils in pre-term infants when used at levels up to 0.91% total fatty acid content using multiple sources of ARA-rich oil, including *M. alpina*.

- Clinical studies, detailed in GRN 326, using 0.64-0.72% of total fatty acids as ARA also confirmed safety of infant formula containing ARA-rich oil derived from *M. alpina* in term infants.

Taken together, the available data from studies conducted on ARA-rich oils from *M. alpina* establish a strong body of evidence for the safety of ARA-rich oil as a source of ARA for supplementation of infant formula. Therefore, ARA-rich oil is thus safe and GRAS at the proposed levels of ingestion. It is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

Roger Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
School of Pharmacy
University of Southern California

Signature: 

Date: June 15, 2020

A. Wallace Hayes, PhD, DABT, FATS, ERT
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Claire Kruger, PhD, DABT
Scientific Advisor to the Panel
Spherix Consulting Group, Inc.

Signature: 

Date: June 15, 2020

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
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
Rebecca Lee	Export Manager

Organization *(if applicable)*: Hubei Fuxing Biotechnology Co., Ltd.

Mailing Address *(number and street)*: FL. 11, Bldg 23, Baishazhou Avenue

City	State or Province	Zip Code/Postal Code	Country
Baishazhou Enterprise City	Wuhan	43000	China

Telephone Number	Fax Number	E-Mail Address
86-27-83660037		

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

Name of Contact Person	Position or Title
Claire Kruger	Managing Partner

Organization *(if applicable)*: Spherix Consulting Group, Inc.

Mailing Address *(number and street)*: 11821 Parklawn Drive, Suite 310

City	State or Province	Zip Code/Postal Code	Country
Rockville	Maryland	20852	United States of America

Telephone Number	Fax Number	E-Mail Address
301-775-9476		ckruger@spherixgroup.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 326

☐ 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)* GRNs 730, 94, 80. and 41

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 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 among different populations. Therefore, the proposed use of ARA-rich oil is to provide of 0.75% and 0.40% ARA by weight of fatty acids in term and pre-term infant formulas, respectively.

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 Hubei Fuxing Biotechnology Co., Ltd.
(name of notifier)

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

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

2. Hubei Fuxing Biotechnology 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)

FL. 11, Bldg 23, Baishazhou Enterprise City, Baishazhou Avenue, Wuhan District, Wuhan 430000 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)
Claire L. Kruger, PhD Digitally signed by Claire L. Kruger, PhD Date: 2020.06.19 10:48:46 -04'00'	Claire Kruger, Managing Partner	06/15/2020

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)
	H-F ARA-Rich Oil GRAS 6-15-2020.pdf	Submission
	All References	Submission

From:	kbrailer@spherixgroup.com
To:	Morissette, Rachel
Cc:	ckruger@spherixgroup.com; "Jennifer Symonds"; "Dietrich Conze"
Subject:	RE: questions for GRN 000958
Date:	Tuesday, February 2, 2021 3:31:14 PM
Attachments:	image001.png Response to FDA on GRN958 2-2-21.pdf Salas Lorenzo Nutrient 2019.pdf American Academy of Pediatrics 2012.pdf Casterton 2009.pdf Hempenius 2000.pdf Hempenius et al 1997.pdf Hoffman 2019.pdf Koletzko Am J Clin Nutr 2020.pdf Koletzko et al 2014.pdf Lina et al. 2006.pdf Merritt 2003.pdf

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Dear Rachel,

Attached please find our response to your questions on GRN 000958. Also attached are the missing references. Please confirm receipt and let us know if you need anything else.

Best regards,

Kathy Brailer
Director of Administrative Services
Spherix Consulting Group, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852
+1-301-557-0375
kbrailer@spherixgroup.com
www.spherixgroup.com

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, January 7, 2021 11:01 AM
To: kbrailer@spherixgroup.com
Cc: ckruger@spherixgroup.com; 'Jennifer Symonds' <jsymonds@spherixgroup.com>; 'Dietrich Conze' <dconze@spherixgroup.com>
Subject: RE: questions for GRN 000958

Dear Kathy,

Thank you for your email. An extra two weeks will be fine. I'll look for your responses by February 3.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients

Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: kbrailer@spherixgroup.com <kbrailer@spherixgroup.com>
Sent: Thursday, January 7, 2021 10:52 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: ckruger@spherixgroup.com; 'Jennifer Symonds' <jsymonds@spherixgroup.com>; 'Dietrich Conze' <dconze@spherixgroup.com>
Subject: RE: questions for GRN 000958

Dear Dr. Morissette,

We are in receipt of your request for additional information on GRN958. We have corresponded with our client and they will need to generate analytical data to address one of the questions. Would it be possible to extend the deadline for our response by 2 weeks to February 3, 2021?

We appreciate your consideration of this request.

Best regards,

Kathy Brailer
Director of Administrative Services
Spherix Consulting Group, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852
+1-301-557-0375
kbrailer@spherixgroup.com
www.spherixgroup.com

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Wednesday, January 6, 2021 9:07 AM
To: ckruger@spherixgroup.com
Subject: questions for GRN 000958

Dear Dr. Kruger,

Please see attached questions to be addressed for GRN 000958. Let me know if you have any questions.

Best,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

Spherix Consulting Group

February 2, 2021

Rachel Morissette, Ph.D.
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 000958

Dear Dr. Morissette:

In response to your email of January 6, 2021, following is our response to your request for additional information regarding GRN 000958. Your requests are in italicized text and our responses are below in plain text:

1. *Please provide the following to Part 1 of the notice:*

   a. *A statement that the intended use (Part 1E, p.1) of ARA will include use with a safe and suitable source of DHA at a ratio of ARA:DHA consistent with generally accepted current guidelines for safe infant feeding practices, currently between 2:1 and 1:1 ARA:DHA.*

      The text in Part 1E, p.1 has been updated to accurately represent the intended use of arachidonic acid (ARA). It shall now read:

      "ARA-rich oil is intended for use as an ingredient in exempt infant formula that will be consumed by preterm infants, as well as non-exempt infant formula for term infants. ARA-rich oil will be consumed in combination with a safe and suitable source of DHA, consistent with generally accepted current guidelines for safe infant feeding practices, at a ratio of ARA:DHA between 1:1 and 2:1."

   b. *The reference(s) for the cited (Part 1F, p.3) guidelines and recommendations established by the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation. Although Koletzo et al. (2008) is cited in Part 6 of the notice, there are more recent recommendations available (e.g. Koletzko et al., 2014; Koletzko et al., 2020) that indicate a higher recommended level of DHA than that indicated in the 2008 reference. Please update and/or provide complete citations to support the intended use.*

11821 Parklawn Drive, Suite 310
Rockville, MD 20852
301-557-0375; info@spherixgroup.com
http://spherixgroup.html

The text in Part 1F, p.3 has been updated to accurately represent the latest recommendations for ARA consumption for infants, as described by Koletzko et al. (2014) and Koletzko et al. (2020). The text shall now read:

"The intended use for ARA-rich oil is as an ingredient in exempt and non-exempt infant formulas. The functional importance of long-chain polyunsaturated fatty acids (LCPUFA) in pregnancy, lactation, and infancy have been the subject of numerous clinical evaluations, particularly as they relate to the intake of n-6 and n-3 LCPUFA, ARA (C20:4n-6) and docosahexaenoic acid (DHA; C22:6n-3). 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. Because breastfeeding and human milk are the normative standards for infant feeding and nutrition (American Academy of Pediatrics Policy, 2012), infant formula must be capable of supporting the nutritional needs of pre-term and term infants when infant formula is chosen as a surrogate for human milk. Based on current knowledge regarding the importance of LCPUFA in infant nutrition, their presence in human milk, and the guidelines and recommendations established by the European Academy of Paediatrics, World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation, infant formula should contain 0.3 to 0.5 weight percent of total dietary fat, with the minimum amount of ARA being equivalent to the DHA content."

The text on Part 3A, pg. 26-27 shall now read:

"Based on scientific consensus and current knowledge regarding the importance of long chain PUFAs in the infant diet and their presence in human milk, supplementation of infant formula with ARA together with DHA has been recommended by the European Academy of Paediatrics, World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation (Koletzko et al., 2014; Koletzko et al., 2020). For pre-term infant formula, the recommended intakes of long chain PUFAs are 20-60 mg/kg body weight/day for ARA and 20-40 mg/kg body weight/day for DHA. For term infant formula, the recommended intakes are 20-40 mg/kg body weight/day for ARA and 40 mg/kg body weight/day for DHA."

2. *While the target ARA use levels in GRN 000958 (0.75% and 0.4% ARA in term and pre-term infant formula, respectively) are based on levels cited in previous GRNs (e.g., 000326, 000730), we note that recent GRNs included use of DHA at levels up to 0.5% in pre-term formula. Because the use of 0.4% ARA in an infant formula containing as much as 0.5% DHA would result in a ratio slightly below 1:1, we request that Hubei Fuxing briefly address whether it considered the use of ARA oil in pre-term infant formulas where the ratio of ARA:DHA would be below 1:1. If Hubei Fuxing is considering a lower ratio of ARA: DHA, please discuss and include citations (if applicable) to published, generally accepted data to support this lower ratio.*

Hubei Fuxing is not proposing to lower the ratio of ARA:DHA to what is currently recommended. The text in Part 1.F, 3.C., 3.D., and 7.B. of the Notice have been updated to reflect the current recommended use levels for ARA in pre-term formula.

The text for Part 1.F. Basis for GRAS Determination, second bullet on page 3 shall read:

- "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 proposed use of ARA-rich oil is intended to provide 0.75% and 0.50% ARA by weight of fatty acids in term and pre-term infant formulas, respectively; this is within the range found in human milk. The intended use of ARA-rich oil to deliver this concentration of ARA, corresponds to 1.875% of total fat for non-exempt term infant formula and 1.25% of total fat for exempt pre-term infant formula. This intended use level is consistent with the levels of use recommended by the European Academy of Paediatrics and the Child Health Foundation."

The text for Part 1.F. Basis for GRAS Determination, third bullet on pg. 3 shall now read:

- "An estimate of exposure to ARA from its addition to infant formula at target ARA levels of 0.75 g and 0.50 g per 100 g total fat for term and pre-term infant formulas may be calculated as follows: Assuming human infants consume 100 kcal/kg body weight/day (term infants) to 120 kcal/kg body weight/day (pre-term infants), of which fat comprises about 50% of the energy, an infant will consume 5.6 g (term infants) to 6.7 g (pre-term infants) of fat/kg body weight /day (1 g fat = 9 kcal). These correspond to intakes of ARA of 42 mg and 33.5 mg ARA/kg body weight /day (or 105 and 83.8 mg of ARA-rich oil/kg body weight/day) for term infants and pre-term infants, respectively."

The text for Part 3.C. Intended Use, pg. 27 shall now read:

"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 among different populations. Therefore, the proposed use of ARA-rich oil is to provide of 0.75% and 0.50% ARA by weight of fatty acids in term and pre-term infant formulas, respectively. The intended use of ARA-rich oil to deliver this concentration of ARA, corresponds to 1.875% of total fat in non-exempt term infant formula and 1.25% of total fat in exempt pre-term infant formula. This intended use level is consistent with the levels of use recommended by the European Academy of Paediatrics and the Child Health Foundation. The ratios of ARA:DHA are expected to be in the range of 2:1-1:1."

The text for Part 3.D. Estimated Daily Intake, pg. 28 shall now read:

"The assumptions upon which this estimation is made are the same as those cited in GRN 326, pg 60 (FDA, 2010), with updated recommendations to provide 0.5% ARA by weight of fatty acids for pre-term infants. An estimate of exposure to ARA from its intended use is

based on target ARA concentrations of 0.75% and 0.50% of total fat in term and pre-term infant formula, respectively. Assuming human infants consume about 100 kcal/kg body weight/day (term infants) to 120 kcal/kg body weight/day (pre-term infants), of which fat comprises about 50% of those calories, an infant will consume about 5.6 g (term infants) to 6.7 g (pre-term infants) of fat/kg body weight/day (1 g fat = 9 kcal). These correspond to intakes of ARA of 42 mg and 33.5 mg ARA/ kg body weight/day (corresponding to 105 and 83.8 mg of ARA-rich oil/kg body weight/day) for term infants and pre-term infants, respectively."

The text for Part 7.B. Expert Panel Statement, second bullet on pg. 61 shall now read:

- "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 proposed use of ARA-rich oil is intended to provide 0.75% and 0.50% ARA by weight of fatty acids in term and pre-term infant formulas, respectively; this is within the range found in human milk. The intended use of ARA-rich oil to deliver this concentration of ARA, corresponds to 1.875% of total fat for non-exempt term infant formula and 1.25% of total fat for exempt pre-term infant formula. This intended use level is consistent with the levels of use recommended by the European Academy of Paediatrics and the Child Health Foundation."

The text for Part 7.B. Expert Panel Statement, third bullet on pg. 61 shall now read:

- "An estimate of exposure to ARA from its addition to infant formula at target ARA levels of 0.75 g and 0.50 g per 100 g total fat for term and pre-term infant formulas may be calculated as follows: Assuming human infants consume 100 kcal/kg body weight/day (term infants) to 120 kcal/kg body weight/day (pre-term infants), of which fat comprises about 50% of the energy, an infant will consume 5.6 g (term infants) to 6.7 g (pre-term infants) of fat/kg body weight /day (1 g fat = 9 kcal). These correspond to intakes of ARA of 42 mg and 33.5 mg ARA/kg body weight /day (or 105 and 83.8 mg of ARA-rich oil/kg body weight/day) for term infants and pre-term infants, respectively."

3. *In the description of the production process (p.11), please provide a statement that the ARA oil is produced in accordance with current good manufacturing practices (cGMPs). We note that although "GMP" is included in the list of abbreviations (p.v), a statement about cGMP is not included in the method of manufacture discussion.*

Hubei Fuxing has been certified as meeting the Food Safety System Certification (FSSC) 22000 standards, which incorporate current Good Manufacturing Practices. The text on page 11 under section 2.D Production Process shall now read:

"ARA-rich oil is produced at Hubei Fuxing Biotechnology Co., Ltd in Wuhan, China. Hubei Fuxing has been certified as meeting the Food Safety System Certification (FSSC) 22000 standards; therefore, Hubei Fuxing manufactures the ARA-rich oil in accordance with current Good Manufacturing Practices (cGMP)."

4. *In the section entitled "ARA-rich oil refining," (p.14) Hubei Fuxing notes that "nitrogen, activated carbon, and activated clay are used to further refine the oil in the decolorization step." Please clarify if Hubei Fuxing is indicating that the refining processes occur under nitrogen. We would not expect nitrogen to play a direct role in the bleaching and deodorization processes other than to prevent oxidation.*

   Hubei Fuxing uses nitrogen during oil refining only to prevent oxidation. The text in Section 2.D.d. ARA-rich oil refining (p.14) in has been clarified to more accurately represent the production process.

   "The oil refining process is shown in Figure 2. To continue with the production process, the phospholipids and free fatty acids in the crude ARA-rich oil are removed with citric acid and EDTA (degumming). The phospholipid content must comply with internal quality specifications by the end of this step and is controlled as a critical control point. All waste materials are discarded via sewage disposal. Next, the oil is deacidified with sodium hydroxide. The acid value must comply with internal quality specifications and is controlled as a critical control point. Next the crude oil is subjected to washing with water to remove saponins and residual soaps. Nitrogen, activated carbon, and activated clay are used to further refine the oil in the decoloration step. Nitrogen is used during the refining process to prevent oxidation of the oil. The oil is deodorized by steam, and a critical control point is in place to control the peroxide value of the oil. Vitamin E and ascorbyl palmitate are used as antioxidants in the finished ARA-rich oil. Following decoloration, the activated carbon and clay are removed by filtration. The refining process has several quality control parameters in place throughout production to ensure a product that complies with product specifications. The finished ARA-rich oil is then subjected to quality control by testing for compliance with the product specifications. The final product is reworked if it fails to meet product specifications for ARA-rich oil. Finished ARA-rich oil is stored in food contact material grade aluminum bottles at -10^{o}C for an average of 3 month until delivered to the customer. The finished ARA-rich oil is stored for no more than 18 months."

5. *Please state if the refining process removes the extraction solvent (butane).*

   The extraction solvent, butane, is removed during the production of the ARA-rich crude oil through repeated solvent removal. To remove the solvent, the biomass + butane mixture is heated to 40-60^{o}C at -0.08 MPa, and the evaporated butane is condensed and collected. Additionally, Hubei Fuxing has a specification set for residual solvents at less than or equal to 1 mg/kg to confirm the butane has been removed from the finished product.

6. *Please clarify the basis for including a mycotoxin analysis (Table 7, p.21) in the notice given that M. alpina is described in the notice as non-toxigenic and food-grade materials are used in the fermentation medium.*

   Mycotoxin analysis is included in the quality attributes of Hubei Fuxing ARA-rich oil due to the precedent established in GRN 730, pgs 8-13. The text for Part 2.E.2.c. Mycotoxins in ARA-rich oil has been updated to reflect that this analysis is performed as a due diligence

exercise and that mycotoxins are not be expected to be present in ARA-rich oil. The text on pg. 21 shall now read:

"Although mycotoxins are not be expected to be present in ARA-rich oil, they are monitored as a part of due diligence consistent with past precedent established in GRN 730. The presence of mycotoxins, including aflatoxins, fumonisins, nivalenol, zearalenone, ochratoxin A, patulin, and sterigmatocystin was analyzed in three batches of ARA-rich oil. These mycotoxins are known to contaminate the food supply and their presence is monitored in ARA-rich oil. None of these toxins were present above the limit of quantitation (Table 7). This analysis is performed every three months."

7. *Please clarify the basis for including polycyclic aromatic hydrocarbons (PAHs) (Table 8, p.22) and polychlorinated biphenyls (Table 10, p.23) in the notice. In the response, please indicate if Hubei Fuxing expects these impurities to be introduced by the fermentation media or controlled method of manufacture of ARA oil.*

Polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) analysis is included in the quality attributes of Hubei Fuxing ARA-rich oil due to the precedent established in GRN 730, pgs 8-13. The text for Part 2.E.2.d. PAH in ARA-rich oil has been updated to reflect that this analysis is performed as a due diligence exercise and that PAHs are not be expected to be present in ARA-rich oil. The text on pg. 23 shall now read:

"Although polyaromatic hydrocarbons (PAHs) are not be expected to be present in ARA-rich oil, they are monitored as part of due diligence consistent with past precedent established in GRN 730. No PAH residues were detected above the limit of quantitation in three batches of ARA-rich oil (Table 8). This analysis is performed every three months."

8. *Hubei Fuxing provides the results of batch analyses for sterols in ARA oil. Please compare this sterol profile to previous GRAS notices for ARA oil cited in this notice and/or to published literature values for the sterol composition of ARA oil.*

It is not possible to compare the sterol profiles of the subjects of GRN 958, GRN41, GRN 80, GRN 94, GRN 326, GRN 730 and the published studies because different sterols were quantitated in the different ARA-rich oils using different methods (Table 1). Additionally, the published studies report sterols extracted from dried mycelia, not sterols extracted from an edible oil rich in ARA (Shimizu et al., 1992; Nes and Nichols, 2006). However, a comparison of total sterol content shows that the total sterol content of the subject of this Notice falls within the range of total sterol content of the ARA-rich oils that are the subjects of GRN 958, GRN41, GRN 80, GRN 94, GRN 326, GRN 730. Furthermore, the unsaponifiable content specification of not more than 3% for the subject of this Notice is consistent with the specifications of other ARA-rich oils that are GRAS for use in preterm and infant formula and, together with appropriate production process controls, ensures that the sterol content of the subject of this Notice is controlled in the finished product (GRNs 41; GRN 80; GRN 94; GRN 326; GRN 730).

Table 1. Comparison of Sterols In the Subject of This Notice with The Subjects Other GRNs, and Published Reports in *M. alpina*							
	Sterols shown as average sterol ± standard deviation (g/100 g oil)					Shimizu et al., 1992[6]	Nes and Nichols, 2006[7]
Sterols	**Current Notice[1]**	**GRN 41 & 80[2]**	**GRN 94[3]**	**GRN 326[4]**	**GRN 730[5]**		
4α-Methyl cholesta-7,24-dienol	-	-	-	-	-	-	0.3%
4α-Methyl zymosterol (4α-Methyl cholesta-8,24-dienol, PubChem ID 22212495)*	-	-	-	0.018 ± 0.005	0.008 ± 0.001	-	1.1%
4,4-Dimethyl-5α-ergosta-8,24(28)-dien-3β-ol (14-Desmethyl lanosterol, PubChem ID 12850767)*	-	-	-	-	-	-	0.1%
7-Dehydrodesmosterol (Cholesta-5,7,24-trienol, PubChem ID: 440558)*	-	-	-	-	-	-	1%
24,25-Methylenecholest-5-en-3β-ol	-	-	-	-	-	1.15 mg/g	-
24,25-Dihydrolanosterol	-	-	-	-	-	-	0.1%
24(28)-Methylene cholesterol	-	-	-	-	-	-	3%
24-Methyl cholesta-5,24(25 or 28)-dien-3β-ol	-	0.206 ± 0.006	-	-	-	-	-
24-methyl cholesta-5,24(25)-dien-3β-ol	-	-	-	0.533 ± 0.130	0.018 ± 0.004	-	-
24-methyl choesta-5,25-dien-3β-ol	-	0.165 ± 0.028	-	-	-	-	-
24-methyl cholesta-5(25)27-dien-3β-ol	-	-	-	0.111 ± 0.050	0.002 ± 0.001	-	-
24-Methyl cholesterol	-	-	-	-	0.016 ± 0.001	-	-
24-Methyl desmosterol	-	-	-	-	-	-	6%
24-Methyl lanosterol	-	-	-	-	-	-	0.1%
24-Methylene cholesterol	-	-	-	0.061 ± 0.022	-	-	-
24,25-methylene cholesta-5-en-3β-ol	-	N.D.	0.025 ± 0.011	-	-	-	-
31-Norlanosterol	-	-	-	0.029 ± 0.003	0.005 ± 0.002	-	-
Brassicasterol	0.157 ± 0.006	-	-	-	0.021 ± 0.009	-	-
Campestanol	N.D.	-	-	-	-	-	-
Campesterol	0.04 ± 0.00	-	-	0.013 ± 0.004	-	-	-
Cholesta-5,25-dien-3β-ol	-	0.023 ± 0.011	-	-	-	-	-
Cholesta-7,24-dienol	-	-	-	-	-	-	0.1%
Cholesterol	N.D.	-	-	-	-	-	0.2%
Codisterol (24β-Methyl-cholesta-5,25(27)-dienol, Ergosta-5,25-dien-3β-ol PubChem ID 13833114)*	-	-	-	-	-	0.56 mg/g	2%
Desmosterol (Cholesta-5,24-dien-3β-ol, PubChem ID: 439577)*	-	1.003 ± 0.010	0.138 ± 0.056	0.083 ± 0.013	0.014 ± 0.002	3.11 mg/g	83%
Delta-5,24-Stigmastadienol	0.01 ± 0.00	-	-	-	-	-	-
Delta-7-Avenasterol	N.D.	-	-	-	-	-	-

Table 1. Comparison of Sterols In the Subject of This Notice with The Subjects Other GRNs, and Published Reports in *M. alpina*

Sterols	Sterols shown as average sterol ± standard deviation (g/100 g oil)					Shimizu et al., 1992[6]	Nes and Nichols, 2006[7]
	Current Notice[1]	GRN 41 & 80[2]	GRN 94[3]	GRN 326[4]	GRN 730[5]		
Delta-7-Stigmastenol	N.D.	-	-	-	-	-	-
Ergosta-5,24(25)-dien-3β-ol	-	-	-	-	-	0.5 mg/g	-
Lanosterol (4α,4β,14-trimethyl-8,24-dien-3β-ol, PubChem 246983)*	-	0.029 ± 0.017	-	0.038 ± 0.008	0.005 ± 0.001	-	3%
Sitostanol+Delta-5-Avenasterol	0.02 ± 0	-	-	-	-	-	-
Sitosterol (β-sitosterol, PubChem ID 222284)*	0.077 ± 0.012	-	-	0.034 ± 0.005	0.013 ± 0.006	-	-
Stigmasterol	0.01± 0	-	-	-	-	-	-
Zymosterol	-	-	-	0.012 ± 0.005	0.005 ± 0.001	-	-
Unidentified Sterols	0.217 ± 0.078	-	-	0.045 ± 0.022	0.060 ± 0.003	-	-
Total Sterols (g/100 g oil) (average ± standard deviation, number of batches indicated)	0.49 ± 0.06 (n=3)	1.42 ± 0.04 (n=2)	0.16 ± 0.06 (n=3)	0.98 ± 0.08 (n=5)	0.17 ± 0.01 (n=3)	-	-

*Synonyms identified by PubChem

-: not described

N.D.: not detected

[1] Current Notice: sterols quantified by gas chromatography methods (NMKL 198:2014-2025), data reported as g sterol/100 g oil. Limit of quantitation is 0.01 g/100 g oil. Strain used: *M. alpina* AF

[2] GRN 41 & 80 (see stamped page 31, GRN 41): sterols quantified by two independent laboratories: Weete, Auburn University, Alabama, United States and Volkman, CSIRO Division of Oceanography, Australia. Limit of quantitation was not described. Data presented in GRN 80 was percent of total sterols. The non-saponifiable fraction of ARASCO, which is comprised primarily of sterols, is 1.5% by weight. To compare among other GRAS notices, the reported percent of total sterols was multiplied by 0.015 to determine g sterols/100 g oil. Strain used: *M. alpina* ATCC 32222.

[3]GRN 94 (see stamped page 92): method for sterol quantification not described, data presented as g/100 g oil. Strain used: *M. alpina* 1S-4.

[4]GRN 326 (see stamped page 29): size exclusion chromatography used to quantify sterols, data presented as weight/weight%. Strain used: *M. alpina* I_{49}-N_{48}.

[5]GRN730 (see pg 59, 66, 73): internal Eurofins liquid chromatography-mass spectrometry method used to quantify sterols, data presented as mg/100 g. To compare among other GRAS notices, the reported sterols as mg/100 g were converted to g/100 g. Strain used: *M. alpina* I_{49}-N_{48}

[6]Shimizu S, Kawashima H, Wada M, Yamada H. (1992). Occurrence of a novel sterol, 24, 25-methylenecholest-5-en-3β-ol, in *Mortierella alpina* 1S-4. *Lipids*, *27*(6): 481-483. Methods used to identify and quantify sterols in the dried mycelia: gas-liquid chromatography or high performance liquid chromatography.

[7]Nes W and Nichols S. (2006). Phytosterol biosynthesis pathway in *Mortierella alpina*, Phytochemistry, 67(16):1716-1721. Data reported as percent of total sterols with sterols accounting for approximately 0.07% of the mycelial dry weight. Gas chromatograph-mass spectrometry methods were used to identify and quantify sterols. Strain used: *M. alpina* CBS 210.32

9. Please provide specifications for Cronobacter sakazakii and three non-consecutive batch analyses, as well as the method employed for the analyses.

Hubei Fuxing has set a product specification for *C. sakazakii* of "not detected in 100 mL". This specification is assessed by the mandatory Chinese national standard issued by the Standardization Administration of China compendial method GB 4789.40-2016. *C. sakazakii* was not detected in three non-consecutive batches of ARA-rich oil, see Table 2 below.

Table 2. *Cronobacter sakazakii* Specifications and Batch Data for Three Batches of Hubei Fuxing's ARA-Rich Oil

Parameter	Specification	Method	LOQ	Batch Number		
				A1905 0301J	A1905 0501J	A1905 0701J
Cronobacter sakazakii	N.D. in 100 mL	GB 4789.40-2016	-	N.D.	N.D.	N.D.

GB: mandatory Chinese national standard issued by the Standardization Administration of China
N.D.: not detected

10. On pg.36 of the notice, Hubei Fuxing states, "The safety of the ARA-rich oil manufactured by Hubei Fuxing is supported by the following studies in rats: two 28-day repeat dose toxicity studies, a 90-day subchronic toxicity study, three 90-day subchronic toxicity studies with an in utero exposure, a productive and developmental toxicity study and a neonatal piglet study (additional corroboration provided from a neonatal piglet study of a blend of ARA- and DHA-rich oils)." Please provide appropriate citations for each of the studies mentioned in this statement and, if necessary, update Table 15 on p.37 of the notice.

The text on pg. 36 has been updated to include the appropriate citations for each study. The text shall now read:

"Each ARA-rich oil used in the corroborative studies described below is compositionally similar to Hubei Fuxing's ARA-rich oil and also has at least 40% ARA. These studies were discussed in GRN 730, pages 31–35. The safety of the ARA-rich oil manufactured by Hubei Fuxing is supported by the following studies in rats: two 28-day repeat dose toxicity studies (Hempenius et al., 1997; Lewis et al., 2016), a 90-day subchronic toxicity study (Lewis et al., 2016), a 90-day subchronic toxicity study performed on the biomass of *M. alpina* (Nisha et al., 2009), three 90-day subchronic toxicity studies with an *in utero* exposure (Hempenius et al., 2000; Lina et al., 2006; Gao et al., 2014) , a reproductive and developmental toxicity study (Falk et al., 2017), and a neonatal piglet study (Merritt et al., 2003) (additional corroboration provided from a neonatal piglet study of a blend of ARA- and DHA-rich oils, Tyburczy et al., 2012). All corroborative data supporting the safety of Hubei Fuxing's ARA-rich oil are summarized in Table 15. Table 15 also summarizes corroborative data discussed in GRN 326, pages 149-153."

11. On p.43 of the notice, Hubei Fuxing describes two clinical studies that were published since FDA's most recent review of an ARA-rich oil (i.e., 2017). However, full details of the literature search were not provided. Please provide the results of an updated literature search, through at least June 2020, for studies relevant to the safety of ARA-rich oil. As part of this discussion, please include search terms, time frames, and databases utilized for the search.

The updated literature search was performed using PubMed on January 18, 2021 to identify publications relevant to the safety of ARA-rich oil. The search terms were "arachidonic acid" AND "infant," with the results constrained to clinical trials published between 2017 and the present. Publications that are not relevant to assessing the safety of ARA in infant formula (i.e. those that studied non-infant ARA supplementation or studied ARA as a supplement and not as an ingredient in an infant formula), or did not use *M. alpina*-derived ARA were not reviewed. Publications that did not report safety parameters were not reviewed. The remaining publications, Hoffman et al. (2019) and Salas Lorenzo et al. (2019) were reviewed in the GRAS notice on pg. 43 and Table 17. No new publications with safety endpoints were found with this updated search.

12. Please review all in text citations for accuracy and completeness and ensure that each reference has a full citation in the "References" section at the end of the notice.

We have reviewed the in text citations and found two typographical errors in the citations for Carnielli et al., 2007 and Merritt et al., 2003 in Section VI., and several references missing from the references list. The typographical errors in text citations for Carnielli et al., 2007 and Merritt et al., 2013 have been corrected (see below) and the citations for the missing references are provided below.

On pg. 36: the reference for Merrit et al., 2013 is a typographical error and should read Merritt et al., 2003.

On pg. 42: the reference for Merritt et al., 2013 is a typographical error and should read Merritt et al., 2003.

On pg. 42: the reference for Carnelli et al., 2007 contains a typographical error, it should read Carnielli et al., 2007.

On pg 46: the reference for Carnelli et al., 2007 in Table 17 is a typographical error and should read Carnielli et al., 2007.

The citations for the missing references follow and electronic copies are provided:

American Academy of Pediatrics. Policy Statement: Breastfeeding and the Use of Human Milk. Pediatrics 2012;129:e827–e841.

Casterton PL, Curry LL, Lina BA, Wolterbeek AP, Kruger CL. 2009.90-Day feeding and genotoxicity studies on a refined arachidonic acid-rich oil. Food Chem Toxicol. 2009 Oct;47(10):2407-18

Hempenius R, JMH van Delft, M Prinsen and BAR Lina. 1997. Preliminary safety assessment of an arachidonic acid-enriched oil derived from *Mortierella alpina:* Summary of toxicological data. Food and Chemical Toxicology 35: 573-581.

Hempenius RA, BAR Lina and RC Haggit. 2000. Evaluation of a sub-chronic (1 3-week) oral toxicity study, preceded by an in utero exposure phase, with arachidonic acid oil derived from *Mortierella alpina* in rats. Food and Chemical Toxicology 38: 127-139.

Hoffman DR, Harris CL, Wampler JL, Patterson AC, Berseth CL. 2019. Growth, tolerance, and DHA and ARA status of healthy term infants receiving formula with two different ARA concentrations: Double-blind, randomized, controlled trial. Prostaglandins, Leukotrienes and Essential Fatty Acids 146:19-27, https://doi.org/10.1016/j.plefa.2019.04.007.

Koletzko B, Boey C, C, M, Campoy C, Carlson S, E, Chang N, Guillermo-Tuazon M, A, Joshi S, Prell C, Quak S, H, Sjarif D, R, Su Y, Supapannachart S, Yamashiro Y, Osendarp S, J, M: Current Information and Asian Perspectives on Long-Chain Polyunsaturated Fatty Acids in Pregnancy, Lactation, and Infancy: Systematic Review and Practice Recommendations from an Early Nutrition Academy Workshop. Ann Nutr Metab 2014;65:49-80. doi: 10.1159/000365767

Koletzko B, Bergmann K, Brenna JT, Calder PC, Campoy C, Clandinin MT, Colombo J, Daly M, Decsi T, Demmelmair H, Domellöf M, FidlerMis N, Gonzalez-Casanova I, van Goudoever JB, Hadjipanayis A, Hernell O, Lapillonne A, Mader S, Martin CR, Matthäus V, Ramakrishan U, Smuts CM, Strain SJJ, Tanjung C, Tounian P, Carlson SE. Should formula for infants provide arachidonic acid along with DHA? A position paper of the European Academy of Paediatrics and the Child Health Foundation. Am J Clin Nutr. 2020 Jan 1;111(1):10-16. doi: 10.1093/ajcn/nqz252. PMID: 31665201.

Lina BAR, Wolterbeek APM, Suwa Y, Fujikawa S, Ishikura Y, Tsuda S, Dohnalek M. 2006. Subchronic (13-week) oral toxicity study, preceded by an in *utero* exposure phase, with arachidonate-enriched triglyceride oil (SUNTGA40S) in rats. Food and Chemical Tox. 44: 326- 335.

Merritt RJ, Auestad N, Kruger C, Buchanan S. 2003. Safety evaluation of sources of docosahexaenoic acid and arachidonic acid for use in infant formulas in newborn piglets. Food and Chemical Tox. 41: 897-904.

Salas Lorenzo I, Chisaguano Tonato AM, de la Garza Puentes A, Nieto A, Herrmann F, Dieguez E, Castellote AI, López-Sabater MC, Rodríguez-Palmero M, Campoy C. The Effect of an Infant Formula Supplemented with AA and DHA on Fatty Acid Levels of Infants with Different FADS Genotypes: The COGNIS Study. *Nutrients*. 2019; 11(3):602. https://doi.org/10.3390/nu11030602.

Should you need any additional information, please feel free to contact me at 301-775-9476 or ckruger@spherixgroup.com.

Sincerely,

Claire L. Kruger, Ph.D. D.A.B.T.
Managing Partner

133 pages have been removed in accordance with copyright laws. The removed reference citations are:

- Lorenzo, "The Effect of an Infant Formula Supplemented with AA and DHA on Fatty Acid Levels of Infants with Different FADS Genotypes: The COGNIS Study", Nutrients (2019)
- Eidelman, "Breastfeeding and the Use of Human Milk", American Academy of Pediatrics (2012)
- Casterton, "90-Day feeding and genotoxicity studies on a refined arachidonic acid-rich oil", Food and Chemical Toxicology (2009)
- Hempenius, "Evaluation of a Subchronic (13-Week) Oral Toxicity Study, Preceded by an In Utero Exposure Phase, with Arachidonic Acid Oil Derived from Mortierella alpina in Rats", Food and Chemical Toxicology (2000)
- Hempenius, "Preliminary Safety Assessment of an Arachidonic Acid-enriched Oil derived from Mortierella alpina: Summary of Toxicological Data", Food and Chemical Toxicology (1997)
- Hoffman, "Growth, tolerance, and DHA and ARA status of healthy term infants receiving formula with two different ARA concentrations: Double-blind, randomized, controlled trial", Prostaglandins, Leukotrienes and Essential Fatty Acids (2019)
- Koletzko, " Should formula for infants provide arachidonic acid along with DHA? A position paper of the European Academy of Paediatrics and the Child Health Foundation", Am J Clin Nutr (2020)
- Koletzko, "Current Information and Asian Perspectives on Long-Chain Polyunsaturated Fatty Acids in Pregnancy, Lactation, and Infancy: Systematic Review and Practice Recommendations from an Early Nutrition Academy Workshop", Annals of Nutrition & Metabolism (2014)
- Lina," Subchronic (13-week) oral toxicity study, preceded by an in utero exposure phase, with arachidonate-enriched triglyceride oil (SUNTGA40S) in rats", Food and Chemical Toxicology (2006)
- Merritt, "Safety evaluation of sources of docosahexaenoic acid and arachidonic acid for use in infant formulas in newborn piglets", Food and Chemical Toxicology (2003)

From:	kbrailer@spherixgroup.com
To:	Morissette, Rachel
Cc:	ckruger@spherixgroup.com; "Dietrich Conze"; "Jennifer Symonds"; "Fred Lozy"
Subject:	[EXTERNAL] FW: follow-up from today re: GRN 958
Date:	Monday, March 22, 2021 2:19:06 PM
Attachments:	image001.png GRN958 Withdrawal Letter 3-22-21.pdf

Dear Rachel,

Thank you for today's call and your follow-up email below. Attached please find our cease-to-evaluate request. Please confirm receipt.

Best regards,

Kathy Brailer
Director of Administrative Services
Spherix Consulting Group, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852
+1-301-557-0375
kbrailer@spherixgroup.com
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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Monday, March 22, 2021 10:39 AM
To: ckruger@spherixgroup.com; Dietrich Conze <DietrichC@chromadex.com>
Subject: follow-up from today re: GRN 958

Dear Claire and Dietz,

Thanks again for the phone call this morning. As we discussed, as soon as we receive your cease-to-evaluate request for GRN 000958, we'll send along the remaining questions and appropriate references to help with a revised submission. Please let me know if you have any other questions at this time.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients

Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

March 22, 2021

Rachel Morissette, Ph.D.
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Withdrawal of GRN 000958

Dear Dr. Morissette:

In response to your email of March 15, 2021, and subsequent teleconference on March 22, 2021, our client, Hubei Fuxing Biotechnology Co., Ltd., requests that the Agency cease the evaluation of GRN 000958, "Generally Recognized As Safe (GRAS) Notification for the Use of ARA-Rich Oil as an Ingredient in Exempt and Non-Exempt Infant Formula". Please confirm receipt of this request.

Should you need additional information, please feel free to contact me at 301-775-9476 or ckruger@spherixgroup.com.

Sincerely,

Claire L. Kruger, PhD, DABT
Managing Partner

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