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Spherix Consulting Group

April 16, 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 GRAS determination titled "Generally Recognized As Safe (GRAS) Notification for Docosahexaenoic Acid-Rich Oil for Use in Non-Exempt Infant Formula and General Foods" constitutes a new notification. The production of Docosahexaenoic Acid-Rich Oil described in this Notice utilizes a new strain of *Schizochytrium* sp.

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

Sincerely,

[redacted]

Claire L. Kruger, PhD, DABT
Managing Partner

Enclosure:

CD containing Form 3667, Cover Letter, GRAS Notification for Docosahexaenoic Acid-Rich Oil For Use in Non-Exempt Infant Formula and General Foods, and all references

Spherix Consulting Group, Inc.
11821 Parklawn Drive, Suite 310, Rockville, MD 20852
301-557-0375; info@spherixgroup.com

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
000934	04/21/2020
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: Tim Zhou		Position or Title: Senior R&D Engineer	
Organization *(if applicable)*: CABIO Biotech (Wuhan) Co., Ltd.			
Mailing Address *(number and street)*: Wuhan Pharmacy Park, Jiangxia Avenue, Jiangxia Economic Development Zone			
City: Wuhan	State or Province: Wuhan	Zip Code/Postal Code:	Country: China
Telephone Number: 027-81309907	Fax Number: 027-67845375	E-Mail Address: tim_zhou@cabio.cn	

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

Name of Contact Person: Claire L. Kruger, PhD, DABT		Position or Title: Managing Partner	
Organization *(if applicable)*: Spherix Consulting Group, Inc.			
Mailing Address *(number and street)*: 11821 Parklawn Drive			
City: Rockville	State or Province: Maryland	Zip Code/Postal Code: 20852	Country: United States of America
Telephone Number: 301-775-9476	Fax Number:	E-Mail Address: ckruger@spherixgroup.com	

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Docosahexaenoic Acid-Rich Oil, or DHA-Rich Oil

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

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

CD containing all files

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 137

☐ 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)* GRN 553; GRN 732

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.

DHA-rich oil is intended for use as an ingredient in non-exempt infant formula that will be consumed by term infants and selected general foods.

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 CABIO Biotech (Wuhan) Co., Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Docosahexaenoic Acid-Rich Oil, or DHA-Rich Oil
(name of notified substance)

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

2. CABIO Biotech (Wuhan) 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)

Wuhan Pharmacy Park, Jiangxia Avenue, Jiangxia Economic Development Zone, Wuhan, 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: 2020.04.10 11:29:38 -04'00'	Claire L. Kruger, PhD, DABT	04/10/2020

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)
	CABIO DHA-rich Oil GRAS to FDA 3-25-2020.pdf	Submission
	Almaas 2016.pdf	Submission
	Almaas Pediatrics 2015.pdf	Submission
	Alshweki Nutrition Journal 2015.pdf	Submission
	Bernhard 2019.pdf	Submission
	Birch 2005.pdf	Submission
	Brenna et al. 2007.pdf	Submission
	Busquets-Cortes 2016.pdf	Submission
	Capo 2014a.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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)
	Capo 2014b.pdf	Submission
	Capo 2015.pdf	Submission
	Capo 2016a.pdf	Submission
	Capo 2016b.pdf	Submission
	Chase Pediatr Diabetes 2015.pdf	Submission
	Clandinin 2005.pdf	Submission
	Colombo Pediatr Res 2011.pdf	Submission
	Columbo 2013.pdf	Submission
	Currie 2015.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Directive 2009-32-EC.pdf	Submission
	Drover 2011.pdf	Submission
	Drover 2012.pdf	Submission
	Duttaroy-2016-European_Journal_of_Lipid_Science_and_Technology.pdf	Submission
	EPA 1990 - Fluoranthene.pdf	Submission
	EPA 1990 - Pyrene.pdf	Submission
	EPA 2009 - Anthracene.pdf	Submission
	EPA 2009 - Phenanthrene.pdf	Submission
	Escamilla-Nunes 2014.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	EC 2003 258-97 DHA rich oil.pdf	Submission
	Falk Food and Chemical Toxicology 2017.pdf	Submission
	Fedorova-Dahms 2011a.pdf	Submission
	Fedorova-Dahms 2011b.pdf	Submission
	Fedorova-Dahms 2014.pdf	Submission
	Florida Dept of Health 2018.pdf	Submission
	FSANZ 2013.pdf	Submission
	Gunaratne 2019.pdf	Submission
	Hammond 2001a.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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	Hammond 2001b.pdf	Submission
	Hammond 2001c.pdf	Submission
	Hammond 2002.pdf	Submission
	Harris 2015.pdf	Submission
	Hoffman 2019.pdf	Submission
	Kamlangdee 2003 J Sci Technol.pdf	Submission
	Kitamura 2016.pdf	Submission
	Koletzko 2014.pdf	Submission
	Koletzko 2008.PDF	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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	Kremmyda 2011.pdf	Submission
	Kroes 2003.pdf	Submission
	Lapillone 2014.pdf	Submission
	Lewis Food and Chemical Toxicology 2016.pdf	Submission
	Leyland Fungal Biology 2017.pdf	Submission
	Maki 2005.pdf	Submission
	Maki 2014.pdf	Submission
	Mallick 2019.pdf	Submission
	Manning 2010 Marine Drugs.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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	Martin 1993.pdf	Submission
	Mulder 2014.PDF	Submission
	Nobili 2013a.pdf	Submission
	Nobili et al 2013b.pdf	Submission
	OKeefe 2019.pdf	Submission
	OECD 408.pdf	Submission
	Pulido 2008 Marine Drugs.pdf	Submission
	Ramakrishnan 2015.pdf	Submission
	Ren 2010.pdf	Submission

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	Ryckebosch 2014 Food Chemistry.pdf	Submission
	Salas Lorenzo Nutrient 2019.pdf	Submission
	Sanders 2006.pdf	Submission
	Schmitt 2012a.pdf	Submission
	Schmitt 2012b.pdf	Submission
	Scholtz 2015.pdf	Submission
	Singhal 2013.pdf	Submission
	Stark 2004.pdf	Submission
	van de Lagemaat 2011.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Voigt 2014.pdf	Submission
	Westerberg 2011.pdf	Submission
	Wu Eurpean Journal of Clinical Nutrition 2006.pdf	Submission
	Yeiser 2016.pdf	Submission
	Yokoyama 2007 - Taxonomic rearrangement of the genus Schizochytrium.pdf	Submission

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION FOR DOCOSAHEXAENOIC ACID-RICH OIL FOR USE IN NON-EXEMPT INFANT FORMULA AND GENERAL FOODS

Prepared for:

CABIO Biotech (Wuhan) Co., Ltd.
Wuhan Pharmacy Park, Jiangxia Avenue
Jiangxia Economic Development Zone
Wuhan, China

Prepared by:

Spherix Consulting Group, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852

March 25, 2020

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LIST OF FIGURES

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

CABIO Biotech (Wuhan) Co., Ltd. is hereby submitting a GRAS notice in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

CABIO Biotech (Wuhan) Co., Ltd.
Wuhan Pharmacy Park, Jiangxia Avenue
Jiangxia Economic Development Zone
Wuhan, China

C. COMMON OR USUAL NAME

Docosahexaenoic Acid-Rich Oil, or DHA-Rich Oil

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

Any trade secret or confidential information will be redacted at the time of notification to the U.S. Food and Drug Administration.

E. INTENDED USE

DHA-rich oil is intended for use as an ingredient in non-exempt infant formula that will be consumed by term infants and selected general foods.

F. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of Docosahexaenoic Acid (DHA)-rich oil as an ingredient in infant formula is based upon scientific procedures as described under 21 CFR §170.30(b). The intake of DHA-rich oil from the intended uses specified above and detailed in the body of the GRAS determination 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.

CABIO Biotech Co., Ltd. is proposing to market DHA-rich oil, produced by CABIO Biotech Co., Ltd. China, as a source of DHA-rich oil used in the manufacture of cow's milk and soy-based infant formula and in general foods. The end-use infant formulas are non-exempt term infant formula and as a source of DHA in select general foods. Consistent with other GRAS sources of DHA-rich oil (GRN 777, 776, 732, 731, 677, 553, and 137), this ingredient is produced by the algae *Schizochytrium* CABIO-A-2 and specifications stipulate a minimum of 35% docosahexaenoic acid in the oil.

The following safety evaluation considers the composition, intake, nutritional, microbiological, and toxicological properties of CABIO DHA-rich oil based on publicly available data from substantially equivalent DHA-rich oils as determined GRAS in GRN 553. Corroborative safety data are described in GRNs 777, 776, 732, 731, 677, and 137, each of which received "no questions" letters from the United States Food and Drug Administration (FDA). The proposed use of CABIO DHA-rich oil as an ingredient in non-exempt term infant formula and general foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based upon the following:

- The DHA-rich oil produced by CABIO is compositionally equivalent to the DHA-rich oil described in GRN 553 in terms of production, product specifications, and strain identity; therefore; information from GRN 553 are relied upon to establish safety of the CABIO DHA rich oil.

- The DHA product that is the subject of this GRAS determination is extracted and refined oil from the microalgae *Schizochytrium* CABIO-A-2. It is a mixture of fatty acids containing mostly polyunsaturated fatty acids in which the predominant fatty acid (>35%) is DHA. The DHA manufacturing process starts with fermentation followed by refining of the crude oil isolated from the fermentation process. The DHA-rich oil product is manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B).

- The proposed uses of the DHA-rich oil from *Schizochytrium* CABIO-A-2 are identical to the uses for other GRAS DHA-rich oils (in combination with ARA) in non-exempt (term) infant formulas (GRN 553) and general foods (GRN 137).

- An estimate of exposure to DHA from its addition to infant formula is based on a target DHA concentration of 0.5% of total fat for term infants. Assuming human infants consume about 100 to 120 kcal/kg body weight/day (term infants) of which fat comprises about 50% of those calories, this corresponds to intakes of DHA of 27 to 33 mg DHA/ kg body weight/day for term infants. This DHA intake estimate is in agreement with current recommendations for DHA consumption by pre-term and

term infants of 18 to 60 mg/kg bw/day (Koletzko et al., 2014; GRN 776) The proposed use levels of the DHA-rich oil in general foods will result in a maximum dietary exposure of less than 1.5 grams of DHA per day.

- DHA-rich oils from numerous sources are considered GRAS for use in food for human consumption and/or infant formula (GRNs 41, 137, 138, 319, 384, 469, 527, 553, 677, 731, 732, 776, 777, and 836). Sources of the DHA-rich algal oils include *Schizochytrium* sp., *Crypthecodinium cohnii, Ulkenia* sp. SAM2179. Other algal oil sources include *Chlorella protothecoides* strain S106, and *Prototheca moriformis* strain S2532. Furthermore, other sources of DHA such as tuna and other fish oil have received "no questions" from the FDA for addition to general food and infant formula.

- Numerous animal safety studies have been conducted over a period of more than a decade on DHA-rich oils derived from *Schizochytrium* sp. The results of unpublished and published subchronic toxicity studies conducted in rats show that administration of algal oil does not result in adverse effects at the highest levels tested (3279 mg/kg bw/day) (GRN 553).

    - Unpublished corroborative toxicity testing has been conducted with the proposed DHA-rich oil product from *Schizochytrium* CABIO-A-2 and includes acute and subchronic toxicity studies. In both studies, no evidence of toxicity was noted at the highest dose levels tested (20 g/kg for the acute toxicity study and 10.2 g/kg/day for the subchronic toxicity study).

Taken together, the available data from studies conducted on DHA-rich oils from *Schizochytrium sp.* establish a strong body of evidence for the safety of DHA-rich oil as a source of DHA for supplementation of non-exempt infant formula and general foods. Therefore, DHA-rich oil is 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.

The GRAS status of DHA-rich oil (compliant with the established food grade specifications), under the intended conditions of use proposed by CABIO 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 DHA-rich oil and the potential human exposure to DHA-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 DHA-rich oil that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when DHA-rich oil is used at levels that might reasonably be expected from the proposed applications. DHA-rich oil is GRAS for use as an ingredient in the manufacture of infant formula.

DHA-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

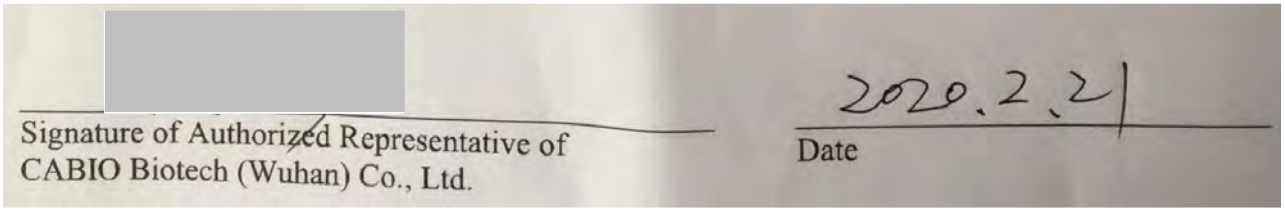
The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, PhD, DABT, Managing Partner, Spherix Consulting Group, Inc., at 11821 Parklawn Drive, Suite 310, Rockville, MD 20852. Telephone: 301-775-9476, 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 CABIO Biotech (Wuhan) Co., Ltd. and pertinent to the evaluation of the safety and GRAS status of the use of this substance.



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

A. COMMON OR USUAL NAME

Docosahexaenoic acid-rich oil, DHA-rich oil

B. TRADE NAME

DHA-rich oil, docosahexaenoic acid oil

C. DESCRIPTION OF DHA-RICH OIL

Docosahexaenoic acid (DHA, 22:6 n-3)-rich oil is a source of DHA in infant formula and general foods, produced by the algae *Schizochytrium* CABIO-A-2 and consists of at least 35% DHA in addition to other long chain saturated and unsaturated fatty acids.

1. Background on Docosahexaenoic Acid

Docosahexaenoic acid (DHA; 22:6 n-3), a polyunsaturated fatty acid (PUFA), is linked to various health benefits in humans, including cognitive and visual development of infants as well as reduced risk of cancer, cardiovascular disease and mental illness in adults (Mallick et al., 2019; O'Keefe et al., 2019).

In 2003, a DHA-rich algal oil produced by the US company Martek Biosciences Corp. obtained marketing authorization as a Novel Food in the EU (EC, 2003). In 2004, the FDA did not object to the Generally Recognized As Safe (GRAS) notification by Martek for its DHA-rich algal oil derived from *Schizochytrium* sp. (GRN 137). Since then, DHA-rich oil has been the subject of multiple GRAS notifications that received "no questions" from the FDA (GRNs 553, 677, 731, 732, 776, 777 and 836). DHA-rich algal oil is now available for use in foods and dietary supplements in both the European Union and the United States.

2. Source

Traditionally, long-chain PUFAs, such as DHA, are obtained from fish such as salmon, mackerel, and tuna. At present, fish oil is the major source of DHA; however, heavy metal pollution and over-fishing jeopardize the sustainability of this source (Ryckebosch et al., 2014). Some marine microalgae such as dinoflagellates and species in the Heterokonta phylum can produce high amounts of DHA, but the majority of those microalgae are photoautotrophic, dependent on light as an energy source and influenced by weather conditions. Heterotrophic

microalgae can derive energy from simple organic substances independent of photosynthesis, making them attractive candidate organisms for generating DHA (Yokoyama and Honda, 2007).

Schizochytrium CABIO-A-2 is a member of the *Schizochytrium* genus, which are heterotrophic microalgae that can be utilized as an alternative to fish oils (Ren et al., 2010). and can produce DHA at up to 49% of its total lipid content.

3. Strain Identity

The genus *Schizochytrium* are spherical, unicellular, heterotrophic microalgae in the family *Thraustochytriacaea*. As described in Yokoyama, et al. 2007, microscopic morphological characteristics of *Schizochytrium* show ectoplasmic nets, formation of zoospores, aplanospores, and amoeboid cells of a size between 10 - 20 μm. The taxonomy details of *Schizochytrium* are described in Table 1.

Table 1. Taxonomy of *Schyzochytrium* CABIO-A-2

Kingdom	*Stramenopila*
Phylum	*Bigyra*
Class	*Labyrinthulomycota*
Order	*Thraustochytrida*
Family	*Thraustochytriaceae*
Genus	*Schizochytrium*
Species	*Schizochytrium* sp.
Strain	CABIO-A-2
Reference: Leyland et al., 2017	

Species within the *Schizochytrium* genus are further classified into the family *Thraustochytriaceae,* characterized by ovoid thalli (undifferentiated vegetative tissue) and an anchoring and feeding network of ectoplasmic threads. This family is a member of the order *Thraustochytrida*, which belongs to the class *Labyrinthulomycota*. The *Labyrinthulomycota* are a class of mostly marine or saprotrophic, fungus-like unicellular organisms. This class is a member of the phylum *Bigyra,* a basal clade of non-plastidial, unicellular organisms within the kingdom *Stramenopila*. The *Stramenopila* are eukaryotic protists characterized by their asymmetrically biflagellated zoospores (Leyland et al., 2017).

Schizochytrium is characterized by biflagellate zoospores and mature cells dividing by repeated binary division to form dyads, tetrads and clusters (Figure 1). Each *Schizochytrium* cell has the potential to develop into a sporangium that produces several zoospores (Kamlangdee and Fan, 2003).

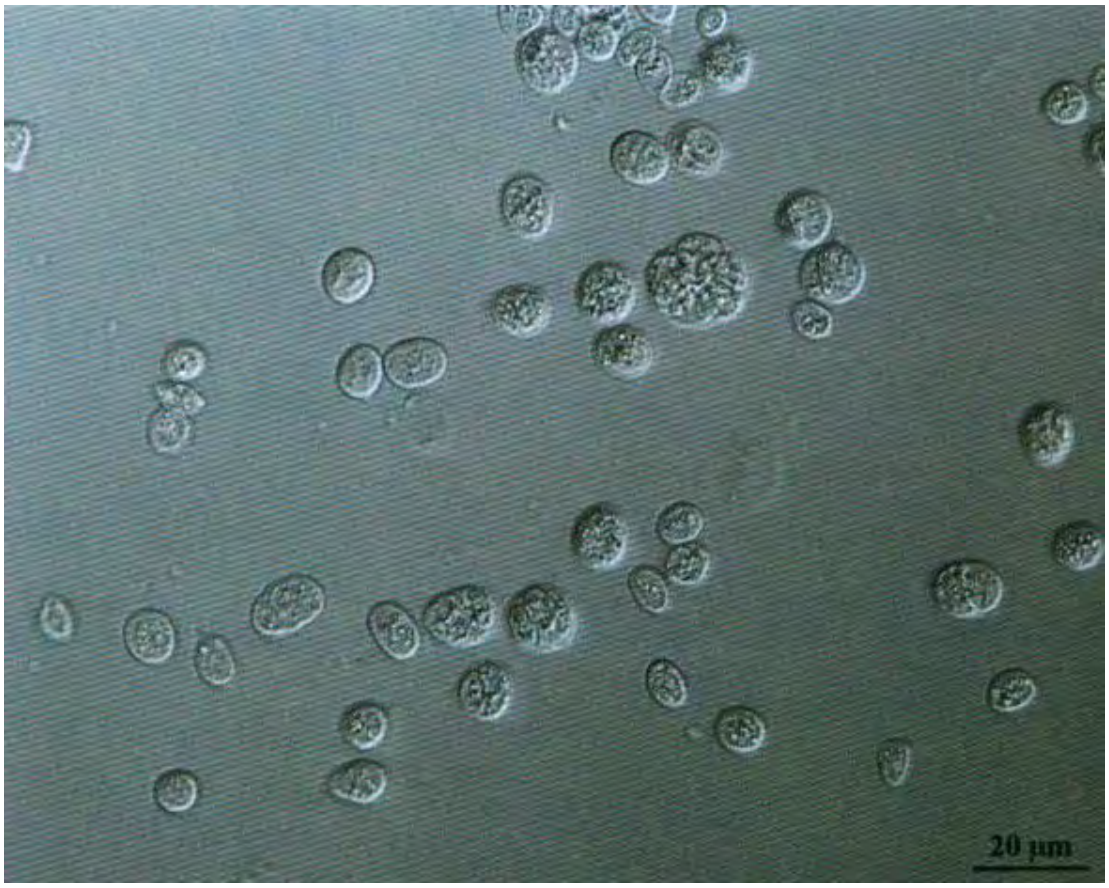


Figure 1. Cell Morphology of *Schizochytrium* CABIO-A-2

The cells are oval or nearly spherical, long axis diameter is 6-20 μm, divided into dyads, triads or clusters. A representative image of lot SS-17-305-1-20170223 is shown.

Schizochytrium CABIO-A-2 is 99.4% homologous to the type strain *Schizochytrium* sp. ATCC 20888, Table 2, as demonstrated by actin gene sequencing. The strain cultivated by CABIO has also been verified to be *Schizochytrium* sp. by *18S* gene sequencing, demonstrating a 99.9% sequence identity with *Schizochytrium* sp. ATCC 20888 (see Appendix for sequence alignments). Figure 2 shows that by actin gene sequencing and phylogenetic taxonomy, *Schizochytrium* CABIO-A-2 is most closely related to *Schizochytrium* sp. ATCC 20888 and not to other species belonging to the family *Thraustochytriaceae*. The actin gene was sequenced as it is the standard according to the China National Accreditation Service for Conformity Assessment. This testing is performed every five years to verify the strain identity.

Table 2. Strain Identity Analysis, % Sequence Alignment for *actin* gene					
Species	***Schizochytrium* CABIO-A-2 Batch Number**				
	SS-17-305-1-20170223	**SS-17-306-2-20170323**	**SS-17-304-2-20170304**	**SS-SF-20170223**	**Average of 4 lots**
Schizochytrium sp. (ATCC 20888)	99.5%	99.3%	99.3%	99.5%	99.4%
Aurantiochytrium mangrovei RCC893	95.6%	95.6%	95.6%	95.6%	95.6%
Schizochytrium aggregatum ATCC28209	94.0%	93.9%	93.9%	94.0%	94.0%
Thraustochytrium aureum	91.0%	91.0%	91.0%	91.0%	91.0%
Thraustochytriidae sp. thel2	90.8%	90.6%	90.6%	90.8%	90.7%
Thraustochytriidae sp. #32	88.9%	88.7%	88.7%	88.9%	88.8%
Thraustochytrium aggregatum KMPBN-BA-110	88.9%	88.7%	88.7%	88.9%	88.8%
Japonochytrium marinum (ATCC 28207)	88.7%	88.6%	88.6%	88.7%	88.7%
Thraustochytrium striatum (ATCC 24473)	87.6%	87.4%	87.4%	87.6%	87.5%

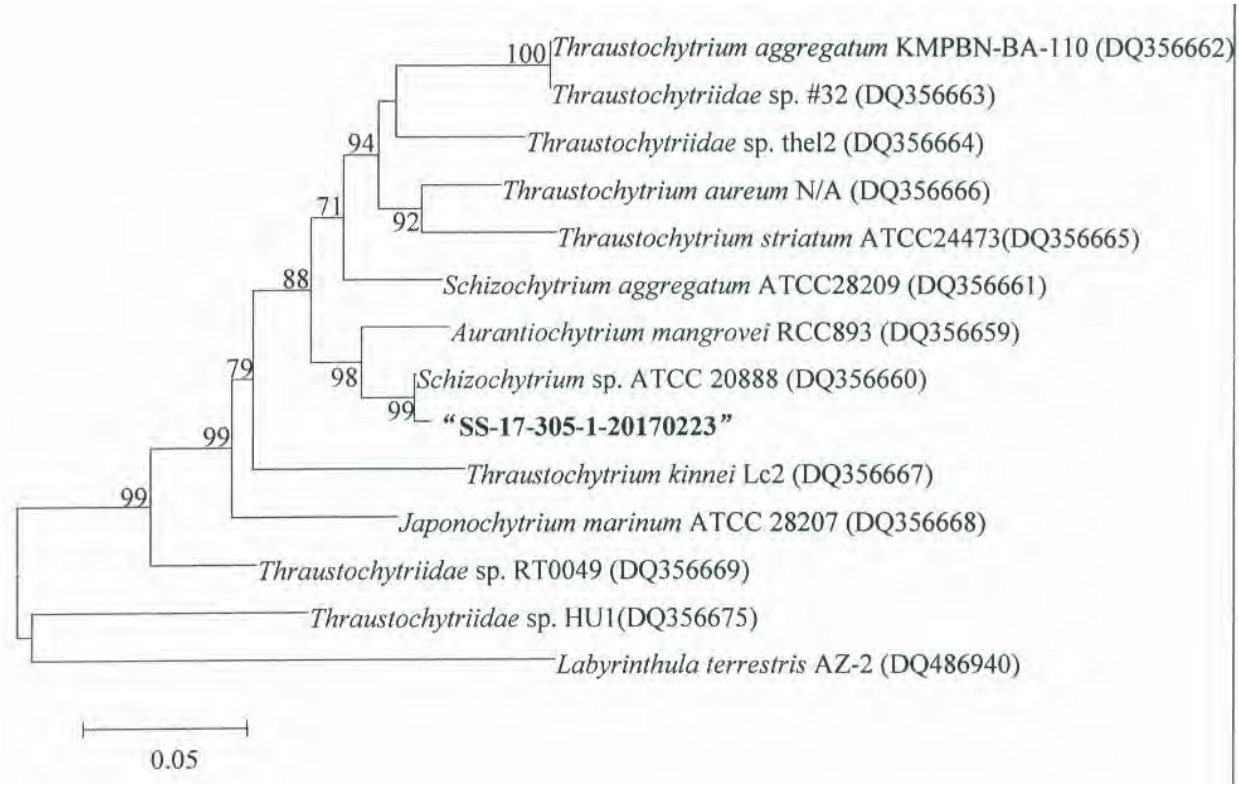


Figure 2. Phylogenetic Tree Mapping of *Schizochytrium* CABIO-A-2

Batch Number SS-17-305-1-20170223 and other reference species, based on *actin* gene sequence by the neighbor-joining in the MEGA program version 5.0. Bootstrap values (>70%) after 1000 replicates are shown at each branch points. Analysis performed by China Center of Industrial Culture Collection, Microbiology Identification Center of CNRIFFI.

D. PRODUCTION PROCESS

1. Production of DHA-rich Oil

CABIO maintains stocks of *Schizochytrium* CABIO-A-2 in glycerol at -80°C at the CABIO Biotech facility. The production process occurs in two main steps: fermentation and oil refining. The fermentation step produces a single batch of crude oil that may then be combined with other batches of crude oil for the oil refining step. All production takes place at the CABIO Biotech facility in Wuhan City, Hubei Province, China. This facility is Food Safety System Certification (FSSC) 22000 compliant (Appendix). As described below, there are several quality assurance (QA) points during the production of DHA-rich oil. If the product fails to meet these QA points, the product is reworked until it meets the quality specification for that step.

Schizochytrium CABIO-A-2 was isolated from seawater in 2005 by Hefei Institute of Physical Science, Chinese Academy of Sciences and was identified by morphology and gene identification as a member of the *Schizochytrium* genus. The original vial of *Schizochytrium* CABIO-A-2 has been preserved at CABIO in an ultra-low temperature refrigerator for over 11 years. CABIO formally marketed its DHA-rich oil in 2012; therefore, the strain used for their DHA-rich oil has been in production for over 7 years.

To generate frozen stocks of Schizochytrium CABIO-A-2, cells are collected by centrifugation from a shake flask cultured at 28℃ for 48 h that is yielding appropriate levels of DHA, according to an internal standard maintained at CABIO. Then glycerol is added to the pelleted cells with a final concentration of 7.5%. The mixture is divided into several sterile tubes and stored at - 80℃. The frozen glycerol stocks are prepared every year.

a. Quality Control of Production

During the production process, operating parameters such as temperature, aeration, agitation and pH are controlled throughout the process to ensure that cell growth and oil production are reproducible. Additional quality parameters are assessed at critical control points throughout the production process and include DHA content, acid value, and peroxide value. All ingredients used in the culture medium are food grade.

b. Fermentation

Fermentation begins with inoculating culture medium a glycerol stock of *Schizochytrium* CABIO-A-2 and culturing until the biomass is ready for crude oil extraction. Protease is added to extract the crude oil from the biomass. After the protease reaction is completed, the protease is heat inactivated and the solids are removed by centrifugation, yielding the DHA crude oil. If the

crude oil does not meet quality control parameters, the batch will be subjected to additional refining steps to ensure the batch complies with quality specifications. The crude oil is then stored in nitrogen flushed HDPE containers at ˉ18 – ˉ13°C for no more than 24 months before proceeding to the refining steps.

c. Oil Refining

Two to four batches of crude oil may be combined for the oil refining step. The crude oil enters the second step of oil refining by mixing with hexane, then acidified and degummed. The oil is then decolorized. The oil is either winterized upon client request or steam deodorized. The oil is finally blended with ascorbyl palmitate, vitamin E, and rosemary extracts as antioxidants, lecithin and sunflower oil. The finished oil is packaged in vacuum, heat sealed food-grade aluminum foil bags or HDPE drums flushed with nitrogen gas to minimize oxidation and stored at -13 – -18°C. Please see Figure 3 for a flow diagram of the production process.

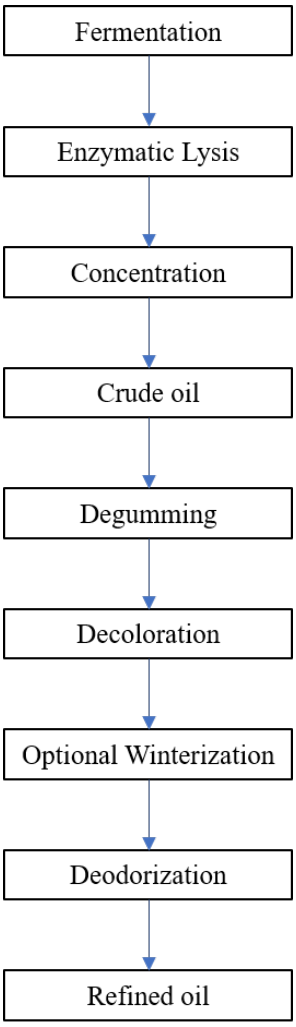


Figure 3: Production Process for CABIO DHA-rich oil

2. Raw Materials, Processing Aids, and Food Contact Substances

Raw materials, processing aids and food contact substances used to manufacture DHA-rich oil are described below in Table 3. Fermentation medium raw materials are listed in the Appendix. All processing aids and food contact materials are either GRAS, United States Pharmacopeia (USP), Food Chemicals Codex grade and/or comply with the US Code of Federal Regulations Title 21 (21 CFR). For hexane, although there are no specific federal regulations stating that it can be used as a processing aid in the extraction of edible oils, GRN 94 and 326 determined it to be safe for use as an extraction solvent for edible oils used in infant formulas, and Directive 2009/32/EC establishes a maximum residue limit for hexanes in the production of fats and oils of 1 mg/kg (1 ppm) fat or oil.

Table 3. Compliance of Processing Aids and Raw Materials with US Laws and Regulations

Role in Production	Processing Aid/Raw Material	Compliance
Refining	Protease (Serine Alkaline)	JECFA, FCC, GRAS, see Appendix
Refining	Silicon Dioxide	FCC 10
Refining	Activated carbon	FCC 11
Refining	Hexane	Acceptable processing aid established in GRN 94 and 326
Refining	Ascorbyl palmitrate	21 CFR §182.3149
Refining	High Oleic Sunflower Oil	USP
Refining	Lecithin	21 CFR §184.1400
Refining	Rosemary extract	FCC
Refining	Citric Acid	21 CFR §184.1033
Refining	Sodium hydroxide	21 CFR §184.1763
Refining	DL-α-Tocopherol	21 CFR §184.1890
Food Contact Material	Polypropylene Filter	21 CFR §177.1520 21 CFR §177.2800 21 CFR §178.3400
Food Contact Material	Aluminum Foil Bags: PET film, PE film	21 CFR §177.1630 21 CFR §177.1500 21 CFR §177.1520 21 CFR §178.2010 21 CFR §175.105 21 CFR §175.300 FCN 424
Food Contact Material	HDPE drum	21 CFR §177.1520 21 CFR §177.2600 21 CFR §178.3297

Abbreviations used: JECFA: Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives, FCC – Food Chemicals Codex; GRAS – generally recognized as safe; CFR: Code of Federal Regulations; USP – United States Pharmacopeia; PET: polyethylene terephthalate; PE: polyethylene; FCN: Food Contact Notification; HDPE – high density polyethylene;.

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Product Specifications

To ensure a consistent food-grade product, CABIO tests each batch of DHA-rich oil for compliance with a defined set of product specifications (Table 4). These parameters are assessed by compendial, validated methods. Microbiological organism specifications are not included, due to the high temperatures used during the production process and low moisture content of the final product (≤ 0.05 %). Also, most recent DHA-rich oil GRNs that received "no questions" from the FDA did not include microbiological specifications as product specifications (see Table 4). Data from three batches of DHA-rich oil demonstrate control of the production process and compliance with the product specifications.

Table 4. Product Specifications and Batch Data for DHA-rich Oil Produced by *Schizochytrium* CABIO-A-2

Parameter	Specification	Method	LOQ	Batch No.		
				17120422	18112422	19022322
EPA	≤ 10.0 %	AOCS Ce 1i-07	0.1	0.4	0.20	0.20
Acid Value	≤ 0.5 mg KOH/g	AOCS Cd 3d-63	0.01	0.11	0.12	0.24
Peroxide Value	≤ 5.0 meq/kg	ISO 3960	0.01	0.15	0.34	0.38
Moisture	≤ 0.05 %	ISO 662	0.01	0.0	0.02	0.026
Unsaponifiable Matter	≤ 3.5 %	ISO 3586	0.01	1.2	1.3	1.1
Trans Fatty Acid	≤ 1.0 %	ISO 15304	0.1	0.0745	0.12	0.121
Docosahexaenoic Acid	≥ 35.0%	AOCS Ce 1i-07	0.01	46.9	43.18	43.2
Total Arsenic	≤ 0.1 ppm	AOAC 986-15	0.005	<0.005	<0.005	<0.002
Cadmium	≤ 0.1 mg/kg	AOAC 986-15	0.01	<0.006	<0.01	<0.01
Copper	≤ 0.05 ppm	DIN EN ISO 17294-2-E29	0.05	<0.05	<0.05	<0.05
Iron	≤ 0.2 ppm	DIN EN ISO 17294-2-E29	0.1	0.16	<0.1	<0.1
Mercury	≤ 0.04 ppm	EN 15763	0.003	<0.003	<0.003	<0.003
Lead	≤ 0.1 ppm	AOAC 986-15	0.005	<0.005	<0.005	<0.005

Abbreviations used:
AOCS: American Oil Chemists' Society; ISO: International Organization for Standardization; DIN EN ISO: Deutsches Institut für Normung (German Institute for Standardization) European Standards, International Organization for Standardization; EN: European Standards; AOAC: Association of Official Agricultural Chemists; LOQ: limit of quantitation

2. Other Quality Attributes

To further characterize the quality of DHA-rich acid, CABIO quantified the amounts of fatty acids, microbial organisms and sterols. CABIO also screened for the following contaminants: dioxins and dioxin-like compounds, non-dioxin-like polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pesticides, and domoic acid, and algal toxin.

a. Fatty Acid Analysis

The fatty acid composition of three batches of winterized CABIO DHA-rich oil are shown in Table 5. Table 5 shows that the three CABIO batches have very similar compositions, with only minor differences in levels of individual fatty acids. The fatty acid profile data support the consistency in the fatty acids found within the DHA-rich oil. This analysis is performed annually.

Table 5. Fatty Acid Profiles of CABIO DHA-rich Oil

	Batch No.		
Fatty Acid (%)	**17120422**	**18112422**	**19050112**
C4:0 Butyric acid	ND	ND	0.03
C6:0 Caproic acid	ND	ND	ND
C8:0 Caprylic acid	ND	ND	ND
C10:0 Capric acid	ND	ND	ND
C11:0 Undecanoic acid	ND	ND	ND
C12:0 Lauric acid	0.04	0.07	0.04
C14:0 Myristic acid	0.68	2.56	0.58
C14:1 Myristoleic acid	ND	ND	ND
C15:0 Pentadecanic acid	0.08	0.26	0.10
C15:1 Pentadecenoic acid	ND	ND	ND
C16:0 Palmitic acid	15.97	14.71	19.82
C16:1 Palmitoleic acid	0.10	0.35	0.12
C17:0 Margaric acid	0.06	0.09	0.12
C18:0 Stearic acid	1.23	0.83	1.55
C18:1n9 Oleic acid	9.56	5.52	6.01
C18:2n6 Linoleic acid	0.96	1.35	0.88
C18:3n3 alpha-Linolenic acid	0.18	0.15	0.19
C18:3n6 gamma-Linolenic acid	0.12	0.20	0.12
C18:4 Octadecatetraenoic acid	0.19	0.26	0.18
C20:0 Arachidic acid	0.28	0.17	0.31
C20:3n3 Eicosatrienoic acid	ND	ND	ND
C20:3n6 Eicosatrienoic acid	0.23	0.33	0.23
C20:4n6 Arachidonic acid	0.18	0.44	0.51
C20:5n3 Eicosapentaenoic (EPA)	0.40*	0.63*	0.54
C22:0 Behenic acid	0.26	0.15	0.24
C22:1 Erucic acid	ND	0.33	ND
C22:2n6 Docosadieonic acid	ND	ND	ND
C22:5n3 Docosapentaenoic acid	0.15	0.46	0.16
C22:6n3 Docosahexaenoic acid	43.85*	38.80*	42.71
C24:0 Lignoceric acid	0.17	0.20	0.21
C24:1 Nervonic acid	ND	0.12	ND

Abbreviations used: ND: not detected
Fatty acid profile, EN ISO 15304, ISO 12966-2 and ISO 5508, GC-FID/AOAC 996.06, AOCS Ce 1b-89, Limit of Quantitation (LOQ): 0.02%
*The method used for the fatty acid analysis includes AOCS Ce 1b-89: which determines EPA and DHA in absolute values using a bonded polyglycol liquid phase in a column. This method differs from the method used to measure DHA and EPA in Table 4, AOCS Ce 1i-07, a procedure that separates fatty acid methyl esters (FAME) on a gas chromatography column and is reported as area under the curve. Since these two methods quantify the DHA and EPA content of oils differently, there is discrepancy between the reported values for Table 4 and Table 5.

b. Microbiological Analysis

The refining process edible oils involves incubations of the oil at extreme temperatures (i.e., 200°C), thus greatly reducing the potential for microbial contamination. To confirm there were no microbial contaminants in the finished product, the levels of *Salmonella*, total coliform bacteria, *E. coli*, total aerobic plate count, yeast, molds, and *S. aureus* were quantified in three batches of DHA-rich oil (Table 6). No microbial contaminants were present above the limit of detection. This analysis is performed upon customer request.

Table 6. Microbiological Analysis of CABIO DHA-rich Oil

Organism	Method	Batch No.		
		18112412	19022322	19030512
Salmonella	USP 62	Negative/25 g	Negative/25 g	Negative/25 g
Total Coliform Bacteria	BAM 4	<0.3 MPN/g	<0.3 MPN/g	<0.3 MPN/g
Escherichia coli	USP 62	Negative/1 g	Negative/1 g	Negative/1 g
Total Aerobic Plate Count	USP 61	<10 CFU/g	<10 CFU/g	<10 CFU/g
Yeast	USP 61	<10 CFU/g	<10 CFU/g	<10 CFU/g
Mold	USP 61	<10 CFU/g	<10 CFU/g	<10 CFU/g
Staphylococcus aureus	USP 62	Negative/25 g	Negative/25 g	Negative/25 g

USP: United States Pharmacopeia
BAM: Bacterial Analytical Manual
MPN: most probable number
CFU: colony forming units

c. Sterol Analysis

Sterol content is quantified annually. Sterol content in three batches of CABIO DHA-rich oil is shown in Table 7. Sterols consist of approximately 1% of the total fat in the DHA rich oil, with the most abundant sterols being stigmasterol and cholesterol. This profile is similar to other DHA-rich oils (GRN 776).

Table 7. Sterol content in DHA-rich oil

	Batch No.		
Sterol (mg/100g)	**19022312**	**19033122**	**19050112**
Brassicasterol	80	78	20
Cholesterol	320	403	359
Campesterol	17	24	13
Campestanol	2	3	2
Stigmasterol	149	161	64
Unidentified sterols	340	404	388
Sitosterol	67	73	63
Sitostanol + delta-5-avenasterol	29	42	9
Delta-5,24-stigmastadienol	15	16	12
Delta-7-stigmastenol	43	36	37
Delta-7-avenasterol	10	6	5
Cycloartenol	7	8	4
24-Methylenecycloartanol	1	4	3
Citrostadienol	3	2	3
Total plant sterols + plant stanols in fat	**752**	**843**	**615**

Method: LC-GC-FID
Limit of quantitation: 1 mg/100g

d. Dioxins and Dioxin-Like Compound Screen

Dioxins and dioxin like compounds were measured in three batches of CABIO DHA-rich oil. This analysis is performed annually. Polychlorinated dibenzodioxins (PCDDs) and polychlorinated biphenyls (PCBS) were not detected in any of the batches of DHA-rich oil at the level of detection (Table 8).

Table 8. Dioxins and Dioxin-like Compounds in DHA rich Oil

	LOD	Batch No.		
		17120422	18112422	19050112
Polychlorinated dibenzodioxins (PCDDs)				
1,2,3,4,6,7,8-HeptaCDD	0.265 pg/g	ND	ND	ND
1,2,3,4,6,7,8-HeptaCDF	0.185 pg/g	ND	ND	ND
1,2,3,4,7,8,9-HeptaCDF	0.13 pg/g	ND	ND	ND
1,2,3,4,7,8-HexaCDD	0.126 pg/g	ND	ND	ND
1,2,3,4,7,8-HexaCDF	0.195 pg/g	ND	ND	ND
1,2,3,6,7,8-HexaCDD	0.172 pg/g	ND	ND	ND
1,2,3,6,7,8-HexaCDF	0.179 pg/g	ND	ND	ND
1,2,3,7,8,9-HexaCDD	0.162 pg/g	ND	ND	ND
1,2,3,7,8,9-HexaCDF	0.132 pg/g	ND	ND	ND
1,2,3,7,8-PentaCDD	0.0828 pg/g	ND	ND	ND
1,2,3,7,8-PentaCDF	0.12 pg/g	ND	ND	ND
2,3,4,6,7,8-HexaCDF	0.162 pg/g	ND	ND	ND
2,3,4,7,8-PentaCDF	0.185 pg/g	ND	ND	ND
2,3,7,8-TetraCDD	0.0629 pg/g	ND	ND	ND
2,3,7,8-TetraCDF	0.172 pg/g	ND	ND	ND
OctaCDD	1.92 pg/g	ND	ND	ND
OctaCDF	0.397 pg/g	ND	ND	ND
Polychlorinated biphenyls (PCB) – dioxin-like				
PCB 105	12.9 pg/g	ND	ND	ND
PCB 114	1.75 pg/g	ND	ND	ND
PCB 118	46.4 pg/g	ND	ND	ND
PCB 123	1.32 pg/g	ND	ND	ND
PCB 126	0.828 pg/g	ND	ND	ND
PCB 156	7.28 pg/g	ND	ND	ND
PCB 157	1.36 pg/g	ND	ND	ND
PCB 167	3.64 pg/g	ND	ND	ND
PCB 169	3.97 pg/g	ND	ND	ND
PCB 189	1.32 pg/g	ND	ND	ND
PCB 77	19.3 pg/g	ND	ND	ND
PCB 81	0.894 pg/g	ND	ND	ND
Method: Internal Eurofins (Suzhou) testing method using GC-MS/MS ND: not detected				

e. Non-Dioxin-Like Polychlorinated Biphenyl Screen

The presence of non-dioxin like PCBs was assessed in three batches of DHA-rich oil. This analysis is performed annually. None of the non-dioxin like PCBs were present above the level of detection (Table 9).

Table 9. Non-dioxin Like Polychlorinated Biphenyls (PCB) in DHA-rich Oil				
PCB	**LOD**	**Batch No.**		
		17120422	**18112422**	**19050112**
PCB 101	0.331 ng/g	ND	ND	ND
PCB 138	0.331 ng/g	ND	ND	ND
PCB 153	0.331 ng/g	ND	ND	ND
PCB 180	0.331 ng/g	ND	ND	ND
PCB 28	0.331 ng/g	ND	ND	ND
PCB 52	0.331 ng/g	ND	ND	ND
Method: GC-MS/MS ND: not detected				

f. Polyaromatic Hydrocarbon Screen

The presence of polyaromatic hydrocarbons (PAHs) was assessed in three batches of DHA-rich oil, Table 10. This analysis is performed annually. The following PAHs were detected: anthracene was present in one batch at 0.9 µg/kg, fluoranthene was present in one batch at 0.5 µg/kg and another batch at 0.8 ug/kg, phenanthrene was present in three batches at 3.3, 3.5, and 3.4 µg/kg, and pyrene was detected in one batch at 1.1 µg/kg . When the estimated daily intake is taken into account for the detected PAHs, none of the detected PAHs approach their respective oral reference doses (RfDs); therefore the presence of these PAHs is not at a level that would affect the safety of the DHA-rich oil.

Assuming that DHA-rich oil will be used at 1.25% of the total fat in infant formula (see Chapter III), and that an infant consumes 6.7 g fat/kg body weight/day, the amount of DHA-rich oil that will be consumed will be 0.84 g DHA-rich oil/kg body weight/day in infants. Similarly, the estimated daily intake of DHA in general foods is 1.5 g/person/day. The chronic RfD for anthracene is 0.3 mg/kg/day (US EPA 2009). Anthracene was detected at 0.9 µg/kg DHA-rich oil. Assuming this level of anthracene, then the amount of anthracene consumed in infant formula containing DHA-rich oil would be 7.56×10^{-8} mg/kg body weight/day, seven orders of magnitude less than the RfD. The amount consumed in general foods would be 1.35×10^{-6} mg/kg body weight/day, five orders of magnitude less than the RfD. The RfD for fluoranthrene is listed as 0.04 mg/kg/day in the Integrated Risk Information System (IRIS) by the U.S. E.P.A (US EPA 1990). The highest level of fluoranthene was detected at 0.8 µg/kg DHA-rich oil. Assuming this level of fluoranthrene, then the amount of fluoranthrene consumed in infant formula containing DHA-rich oil would be 6.72×10^{-8} mg /kg body weight/day, six orders of magnitude less than the RfD. The amount of fluoranthrene consumed in general foods would be 1.2×10^{-6} mg/kg body weight/day, four orders of magnitude less than the RfD. The chronic RfD for pyrene is 0.03 mg/kg/day (US EPA 1990). Pyrene was detected at 1.1 µg/kg DHA-rich oil. Assuming this level of pyrene, then the amount of pyrene consumed in infant formula containing DHA-rich oil would be 9.24×10^{-8} mg/kg body weight/day, six orders of magnitude less than the RfD. The amount of

pyrene consumed in general foods would be 1.65 x 10^{-6} mg/kg body weight/day, four orders of magnitude less than the RfD.

The RfD for phenanthrene has not yet been derived by the EPA, but because phenanthrene closely resembles anthracene, the oral RfD for anthracene could be used to represent phenanthrene toxicity. Even though the K_{ow} values are similar between the two compounds, the water solubility is very different. This suggests that toxicological properties also could be different. Therefore, if anthracene values are used to represent phenanthrene, an additional uncertainty factor of at least 10 should be applied in order to conservatively estimate risk (as utilized by the State of Florida Department of Health) (US EPA 2009). Taking this information into account, the RfD for phenanthrene may be estimated as 0.03 mg/kg/day. The highest level of phenanthrene was detected at 3.5 µg/kg DHA-rich oil. Assuming this level of phenanthrene, then the amount of phenanthrene consumed in infant formula containing DHA-rich oil would be 2.94 x 10^{-7} mg/kg body weight/day, five orders of magnitude less than the RfD. The amount of phenanthrene consumed in general foods would be 5.25 x 10^{-6} mg/kg body weight/day, four orders of magnitude less than the RfD.

Table 10: Polyaromatic Hydrocarbons (PAH) in DHA rich oil

	Batch No.			RfD
PAH (µg/kg)	**17120422**	**18112422**	**19050112**	**(mg/kg/day)**
Anthracene	0.9	ND	ND	0.3
Benzo(a)anthracene	ND	ND	ND	-
Benzo(a)pyrene	ND	ND	ND	-
Benzo(b)fluoranthene	ND	ND	ND	-
Benzo(g,h,i)perylene	ND	ND	ND	-
Benzo(k)fluoranthene	ND	ND	ND	-
Chrysene	ND	ND	ND	-
Dibenzo(a,h)anthracene	ND	ND	ND	-
Fluoranthene	ND	0.5	0.8	0.04
Indeno (1,2,3-cd)pyrene	ND	ND	ND	-
Phenanthrene	3.3	3.5	3.4	0.03
Pyrene	ND	<0.5	1.1	0.03
Method: GC-MS LOD: 0.5 µg/kg ND: not detected				

g. Pesticide Screen

An extensive pesticides screen (Table 11) was conducted on two batches of DHA-rich oil. No pesticide was detected above the limit of detection. This screen is performed annually.

Table 11. Pesticides Screened on Two Batches of DHA-rich Oil		
Screen performed on batches 18112422 and 19050112 (limit of detection in mg/kg)		
2-Phenylphenol (0.01)	Acetochlor (0.06)	Aclonifen (0.05)
Alachlor (0.01)	Aldrin (0.01)	Ametryne (0.02)
Aminocarb (0.01)	Anthraquinone (0.01)	Aramite (0.06)
Atrazine (0.02)	Azinphos-ethyl (0.05)	Azoxystrobin (0.05)
Benalaxyl (0.05)	Benfluralin (0.01)	Benoxacor (0.01)
Bifenox (0.06)	Bifenthrin (0.01)	Biphenyl (0.01)
Bitertanol (0.05)	Boscalid (0.02)	Bromfenvinfos (0.02)
Bromophos (0.01)	Bromophos-ethyl (0.01)	Bromopropylate (0.01)
Bromuconazole, cis- (0.05)	Bromuconazole, trans- (0.05)	Bupirimate (0.02)
Buprofezin (0.02)	Butachlor (0.06)	Butafenacil (0.06)
Cadusafos (0.02)	Captafol (0.06)	Captan (0.06)
Captan/THPI (Sum calculated as Captan)-	Carbaryl (0.02)	Carbofuran (0.02)
Carbophenothion (0.05)	Carbophenothion-methyl (0.05)	Carbosulfan (0.02)
Carboxin (0.06)	Chlorbenside (0.05)	Chlordane (Sum) (-)
Chlordane, alpha (0.01)	Chlordane, gamma (0.01)	Chlorfenapyr (0.05)
Chlorfenson (0.05)	Chlorfenvinphos (0.01)	Chlormephos (0.05)
Chlorobenzilate (0.06)	Chloroneb (0.06)	Chloropropylate (0.01)
Chlorothalonil (0.01)	Chlorpropham (0.01)	Chlorpyrifos-methyl (0.01)
Chlorthal-dimethyl (0.01)	Chlorthion (0.05)	Chlorthiophos (0.02)
Chlozolinate (0.02)	Clodinafop-propargyl (0.05)	Clomazone (0.02)
Coumaphos (0.02)	Crufomate (0.05)	Cyanazine (0.02)
Cyanofenphos (0.05)	Cyanophos (0.02)	Cycloate (0.05)
Cyfluthrin (0.05)	Cyhalothrin lambda-(incl. Cyhalothrin, gamma-) (0.01)	Cypermethrin (0.05)
Cyphenothrin (0.05)	Cyproconazole (0.02)	Cyprodinil (0.01)
DDD, o,p'- (0.01)	DDD, p,p'-(0.01)	DDE, o,p'- (0.01)
DDE, p,p'- (0.01)	DDT (Sum) (-)	DDT, o,p'- (0.01)
DDT, p,p'- (0.01)	Deltamethrin (0.05)	Demeton-S-methyl-sulfone (0.02)
Diazinon (0.02)	Dichlobenil (0.05)	Dichlofenthion (0.02)
Dichlofluanid (0.02)	Dichlorobenzophenone o,p' (0.02)	Dichlorobenzophenone p,p' (0.02)
Dichlorvos (0.05)	Dicloran (0.05)	Dicofol (Sum) (-)
Dicofol, o,p'- (0.02)	Dicofol, p,p'-(0.02)	Dicrotophos (0.02)
Dieldrin (0.02)	Dieldrin (Sum) (-)	Dienochlor (0.05)
Diethofencarb (0.02)	Difenoconazole (0.05)	Diflufenican (0.02)
Dimethoate (0.05)	Dimethomorph (0.05)	Dimethylvinphos (0.01)
Diniconazole (0.02)	Dinobuton (0.05)	Dioxabenzofos (0.02)
Dioxacarb (0.02)	Dioxathion (0.05)	Diphenylamine (0.01)
Disulfoton (0.05)	Disulfoton sulfoxide (0.05)	Disulfoton-PS-sulfone (0.05)
Ditalimfos (0.02)	Edifenphos (0.02)	Endosulfan (sum) (-)
Endosulfan, alpha- (0.05)	Endosulfan, beta- (0.05)	Endosulfan, sulfat- (0.02)
Endrin (0.02)	EPN (0.05)	Epoxiconazole (0.05)
EPTC (0.05)	Etaconazole (0.05)	Ethion (0.02)
Ethoprophos (0.01)	Etofenprox (0.05)	Etoxazole (0.02)
Etridiazole (0.02)	Etrimfos (0.02)	Fenamiphos (0.05)
Fenarimol (0.05)	Fenazaquin (0.02)	Fenbuconazole (0.05)
Fenchlorphos (0.02)	Fenchlorphos (sum) (-)	Fenchlorphos oxon (0.01)
Fenfluthrin (0.02)	Fenitrothion (0.02)	Fenobucarb (0.02)
Fenoxycarb (0.02)	Fenpropathrin (0.02)	Fenpropimorph (0.05)
Fenson (0.02)	Fensulfothion (0.05)	Fenthion (0.02)

Table 11. Pesticides Screened on Two Batches of DHA-rich Oil		
Fenvalerate & Esfenvalerate (Sum of RR, SS, RS, SR Isomers) (-)	Fenvalerate & Esfenvalerate (Sum of RR&SS Isomers) (0.02)	Fluchloralin (0.05)
Flucythrinate (0.05)	Fludioxonil (0.05)	Flumetralin (0.05)
Fluotrimazole (0.01)	Fluquinconazole (0.02)	Flusilazole (0.02)
Fluvalinate-tau (0.02)	Folpet (0.06)	Folpet/PI (sum calculated as Folpet) (-)
Fonofos (0.02)	Formothion (0.05)	Fosthiazate (0.02)
HCB (0.01)	HCH gamma (Lindan) (0.02)	HCH, alpha-(0.02)
HCH, beta- (0.02)	HCH, delta- (0.02)	HCH, epsilon-(0.02)
Heptachlor (0.01)	Heptachlor (Sum) (-)	Heptachlor epoxide cis (0.01)
Heptachlor epoxide trans (0.02)	Heptenophos (0.02)	Hexaconazole (0.01)
Hexazinone (0.02)	Imazalil (0.05)	Iprobenfos (0.02)
Iprodione (0.02)	Iprovalicarb (0.05)	Isazofos (0.06)
Isocarbophos (0.04)	Isodrin (0.02)	Isoprothiolane (0.02)
Isoxathion (0.05)	Jodfenphos (0.02)	Kresoxim-methyl (0.01)
Landrin (0.02)	Lenacil (0.05)	Malaoxon (0.05)
Malathion (0.02)	Mecarbam (0.04)	Mephosfolan (0.05)
Mepronil (0.06)	Metalaxyl (0.05)	Metconazole (0.05)
Methacrifos (0.02)	Methamidophos (0.02)	Methidathion (0.04)
Methiocarb (0.02)	Methoxychlor (0.02)	Methyl-Pentachlorophenylsulfide (0.06)
Metolachlor (0.02)	Metribuzin (0.04)	Mevinphos (0.02)
Mirex (0.01)	Monocrotophos (0.05)	Myclobutanil (0.02)
Napropamide (0.02)	N-Desethyl-pirimiphos-methyl (0.06)	Nitapyrin (0.06)
Nitrofen (0.02)	Nitrothal-isopropyl (0.06)	Norflurazon (0.05)
Nuarimol (0.02)	Octachlorodipropyl Ether (S-421) (0.05)	Ofurace (0.06)
Omethoate (0.05)	Oxadiazon (0.02)	Oxadixyl (0.05)
Oxychlordane (0.02)	Oxyfluorfen (0.02)	Paclobutrazol (0.04)
Paraoxon (0.05)	Paraoxon-methyl (0.05)	Parathion (0.06)
Parathion-methyl (0.04)	Parathion-methyl (sum)(-)	PCB 101 (0.01)
PCB 118 (0.01)	PCB 138 (0.01)	PCB 153 (0.01)
PCB 180 (0.01)	PCB 28 (0.01)	PCB 52 (0.01)
Penconazole (0.01)	Pendimethalin (0.05)	Pentachloroaniline (0.01)
Pentachloroanisole (0.01)	Permethrin (0.02)	Phenkapton (0.05)
Phenthoate (0.02)	Phorate (0.04)	Phorate (Sum)-
Phorate-sulfone (0.05)	Phorate-sulfoxide (0.05)	Phosalone (0.05)
Phosmet (0.05)	Phosphamidon (0.04)	Phthalimide (PI) (0.06)
Picoxystrobin (0.06)	Piperonyl butoxide (0.05)	Piperophos (0.06)
Pirimicarb (0.02)	Pirimicarb-desmethyl (0.05)	Pirimicarb-Desmethylformamido (0.05)
Pirimiphos-ethyl (0.01)	Pirimiphos-methyl (0.01)	Prochloraz (0.05)
Procymidone (0.01)	Profenofos (0.01)	Profluralin (0.02)
Promecarb (0.02)	Prometryn (0.02)	Propachlor (0.02)
Propanil (0.06)	Propaphos (0.02)	Propargite (0.05)
Propazine (0.01)	Propetamphos (0.02)	Propham (0.05)
Propiconazole (0.05)	Propoxur (0.05)	Propyzamide (0.01)
Prosulfocarb (0.02)	Prothiofos (0.02)	Prothoate (0.05)
Pyraclofos (0.02)	Pyraclostrobin (0.05)	Pyrazophos (0.01)
Pyridaben (0.05)	Pyridalyl (0.06)	Pyridaphenthion (0.02)
Pyrifenox (0.04)	Pyrimethanil (0.01)	Pyriproxyfen (0.02)

Table 11. Pesticides Screened on Two Batches of DHA-rich Oil		
Quinalphos (0.01)	Quinoxyfen (0.02)	Quintozene (0.01)
Quintozene (sum)-	Quizalofop-P-ethyl (0.06)	Sebuthylazine (0.01)
Silafluofen (0.06)	Silthiofam (0.06)	Simazine (0.01)
Sulfotep (0.01)	Sulprofos (0.05)	Tebuconazole (0.02)
Tebufenpyrad (0.06)	Tebutam (0.02)	Tecnazene (0.02)
Tefluthrin (0.02)	TEPP (0.02)	Terbacil (0.05)
Terbufos (0.02)	Terbumeton (0.02)	Terbuthylazine (0.01)
Terbutryn (0.02)	Tetrachlorvinphos (0.02)	Tetraconazole (0.02)
Tetradifon (0.02)	Tetrahydrophthalimide (THPI) (0.06)	Tetramethrin (0.06)
Tetrasul (0.01)	Thiabendazole (0.05)	Thiamethoxam (0.02)
Thionazin (0.02)	Tolclofos-methyl (0.01)	Tolylfluanid (0.02)
Triadimefon (0.02)	Triadimenol (0.05)	Triallate (0.02)
Triazamate (0.06)	Triazophos (0.02)	Trichloronat (0.01)
Trifloxystrobin (0.02)	Triflumizole (0.02)	Trifluralin (0.02)
Triticonazol (0.06)	Uniconazole (0.02)	Vinclozolin (0.02)
Zoxamide (0.01)		

h. Domoic Acid Screen

Domoic acid is a toxin produced by certain alga species, such as the red alga *Chondria armata* and planktonic diatom of the genus *Pseudo-nitzschia* (Pulido 2008, Manning and La Claire 2010). It was not detected in three separate batches of DHA-rich oil (Table 12). This analysis is performed upon customer request.

Table 12. Analysis of Domoic Acid, Algal Toxin, in DHA-rich Oil			
	Batch No.		
	19022312	**19033122**	**19050112**
Domoic acid	N.D.	N.D.	N.D.
N.D.: not detected In house method, LC-GC-FID, limit of detection: 3 µg/g			

F. STABILITY OF DHA-RICH OIL

Three non-consecutive lots of DHA-rich oil were stored at -20^{o}C in vacuum sealed aluminum foil bags with the following parameters measured every 6 months: DHA%, peroxide value, and anisidine value. The stability studies are currently ongoing, with preliminary results supporting the proposed shelf life of 24 months (Table 13). The stability of the DHA-rich oil will be continue to be monitored to support the proposed shelf life. Shelf life will be adjusted to reflect any changes in the stability studies.

Table 13. Stability of DHA-Rich Oil at -20ºC for 24 Months

		Storage Time (Months)				
Lot No	**Specifications**	**0**	**6**	**12**	**18**	**24**
DHA (%)						
17010212	⩾ 35.0 %	42.00	41.76	40.98	42.18	41.41
17051512		39.28	38.59	38.07	39.10	*
18022612		36.20	35.59	36.92	*	*
Peroxide value (meq/kg)						
17010212	⩽ 5.0 meq/kg	0.03	1.83	1.05	1.16	1.18
17051512		0.03	1.14	1.22	2.20	*
18022612		0.03	1.54	1.03	*	*
Anisidine value (AV)						
17010212	⩽ 20 AV	5	5	6	6	6
17051512		4	5	5	5	*
18022612		5	6	6	*	*
*Stability studies currently underway						

III. DIETARY EXPOSURE

The DHA-rich oil produced by CABIO Biotech is isolated from the same species as described in GRN 553. Although the two strains of *Schizochytrium* sp. are slightly different, the DHA-rich oil produced by CABIO Biotech complies with the product specifications described in GRN 553. Therefore, the dietary exposure for this product will be the same as the dietary exposure description from GRN 553, as well as GRNs 677, 731, 732, 776, 777, and 836, more recent DHA-rich oil GRAS notices for DHA-rich oil for infant formula, as well as GRN 137 for use in selected general foods. We incorporate by reference the exposures from these GRAS notices. They are summarized below for convenience.

A. INTENDED EFFECT

DHA-rich oil is intended to be used as a source of docosahexaenoic acid, a fatty acid naturally present in human breast milk and known to play a role in infant development. Human milk provides small quantities of DHA and ARA, usually less than 1% of total fatty acids (Brenna et al., 2007). Briefly, Brenna et al. (2007) conducted a meta-analysis of ARA and DHA concentrations in mature human milk based on published data from 65 studies spanning 1986 to 2006 and involving 2,474 women worldwide. The mean and standard deviation of DHA concentration as a percentage of total fatty acids was 0.32 ± 0.22% (range: 0.06 - 1.4%). The authors noted that the highest concentrations of DHA in human milk were seen in coastal regions and possibly associated with marine-rich diets. This evaluation reveals a broad range of DHA levels in human milk on a worldwide basis and shows the range of possible infant exposure to DHA, which provides a guide for levels of DHA supplementation in infant formulas.

The supplementation of infant formula with DHA at levels consistent with those in human milk is important because the n-3 fatty acids present in human milk have critical roles in membrane structure especially in the brain and retina (Duttaroy et al., 2016).

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 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 to 0.5 weight percent of total fat, with the minimum amount of ARA being equivalent to the DHA content (GRN 667).

B. HISTORY OF USE

The use of long chain DHA-rich polyunsaturated oils derived from the algae *Schizochytrium* sp. for supplementation of infant formula has been assessed by various international bodies. Algal oil produced from *Schizochytrium* sp. has been approved for direct use in foods by Health Canada, the United Kingdom, the European Union, the Food Standards Agency of Australia and New Zealand (FSANZ), China's Ministry of Health, and Brazil's National Health Surveillance Agency (ANVISA).

Algal-derived DHA-rich oils from Schizochytrium sp. (GRNs 137, 553, 677, 731, 732, 776, 777, and 836) are considered GRAS for use in foods and/or infant formula. In addition to algal oils, other sources of DHA such as fish oil (GRN 200, 193, 138, 105) have also received "no questions letters" from the FDA.

C. INTENDED USE

The intended use of DHA-rich oil is to provide a source of DHA in cow's milk and soy-based infant formula at a concentration consistent with that of human milk and as a source of DHA in select general foods. DHA-rich oil is intended to be used as a direct food ingredient to increase dietary intake of the long chain omega-3 fatty acid DHA in food categories and use levels as listed in Table 14. These food ingredient categories are based on the food categories used in GRN 137 (stamped pg 10 – 12, 27 – 28).

The DHA content of human milk varies from 0.06% to 1.4% of total fatty acids among different populations. Therefore, the proposed use of DHA-rich oil is to provide 0.5% DHA by weight of fatty acids in term infants. The intended use of DHA-rich oil is to deliver this concentration of DHA, corresponds to 1.25% total fat for non-exempt infant formula. This intended use level is consistent with levels cited in GRN 553, GRN 677 and GRN 776. The ratio of DHA to ARA would range from 1:1 to 1:2.

D. ESTIMATED DAILY INTAKE

1. Infant Formula

An estimate of exposure to DHA from its addition to infant formula is based on a target DHA concentration of 0.5% of total fat for term infants. Assuming human infants consume about 100 to 120 kcal/kg body weight/day (term infants) of which fat comprises about 50% of those calories, an infant will consume about 5.6 to 6.7 g of fat/kg body weight/day (1 g fat = 9 kcal by convention). These correspond to intakes of DHA of 27 to 33 mg DHA/ kg body weight/day for term infants. This DHA intake estimate is in agreement with current recommendations for DHA

consumption by pre-term and term infants of 18 to 60 mg/kg bw/day (Koletzko et al., 2014); and is cited in GRN 776, pg 16.

2. General Foods

The proposed use levels of the DHA-rich oil are expected to result in a maximum dietary exposure of less than 1.5 grams of DHA per day. The estimated mean intake of DHA from the intended uses at the maximum use levels of DHA-rich oil are listed in Table 14 by U.S. consumers will be approximately 1.5 g/person/day. DHA Algal Oil is intended to be the sole source of DHA in any given food category.

Table 14. Maximum Intended Use Levels of DHA-rich oil from *Schizochytrium* CABIO-A-2

Category of Food	Maximum Intended Use Level (%)
Cookies, crackers	1.45
Breads, rolls	0.29
Fruit pies, custard pies	2.03
Cakes	2.9
Baked goods and baking mixes	1.45
Cereals	1.16
Fats and oils (not including infant formula)	5.8
Yogurt	1.16
Frozen dairy products	1.45
Condiments	1.45
Soup mixes	0.87
Snack foods	1.45
Nut Products	1.45
Gravies and sauces	1.45
Soy protein bars	1.45
Plant protein products	1.45
Processed vegetable drinks	0.29
Hard candy	2.9
Soft candy	1.16
Non-dairy and powdered cream substitutes	1.45
Jams and jellies	2.03
Milk-based meal replacements	0.29
Non-dairy milk, imitation and soy milk	0.3
Dairy product analogs	1.45
Nonalcoholic beverages	0.15
Pastas	0.58
Processed Fruit Juices	0.29
White granulated sugar	1.16
Sugar substitutes	2.9
Chewing gum	0.87
Gelatins and puddings	0.29
Confections and frostings	1.45
Sweet sauces, toppings, and syrups	1.45
As described in GRN 137, stamped pages 27 – 28.	

IV. SELF-LIMITING LEVELS OF USE

This part does not apply.

V. COMMON USE IN FOOD BEFORE 1958

This part does not apply.

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

The FDA has issued "no question" letters for seven GRAS notices for food uses of DHA-rich oils derived from *Schizochytrium* sp. for infant formula (GRNs 553, 677, 731, 732, 776, 777, and 836) and one for general foods (GRN 137). A comparison of the specifications between the DHA-rich oil that is the subject of this notification and those in the previous GRNs is shown below (Table 15). CABIO's DHA-rich oil and GRN 553 are bolded in the table below to demonstrate that these two product specifications are substantially equivalent, with some parameters in the CABIO DHA-rich oil being more stringently controlled, including acid value, trans fatty acids, and copper. Safety data cited for the DHA-rich oil from GRN 553 may be used as pivotal data to support the GRAS status of CABIO's DHA-rich oil. The product specifications for CABIO's DHA-rich oil are also very similar to GRNs 137, 677, and 776 and data cited for these oils are highly relevant as corroborative data to support the safety of CABIO's DHA-rich oil.

Table 15. Specifications of CABIO DHA-Rich Oil Compared with Previous GRAS Notices for DHA-Rich Oil from *Schizochytrium* sp.

Parameter	**Current Notice**	**GRN 553**	GRN 836	GRN 777	GRN 776	GRN 732	GRN 731	GRN 677	GRN 137
EPA (%)	**≤ 10.0**	**≤ 10.0**	-	-	-	-	-	-	-
Acid Value (mg KOH/g)	**≤ 0.5**	**-**	≤ 1.0	≤ 0.5	≤ 0.5	<0.5	< 0.5	< 0.5	≤ 0.5
Peroxide Value (meq/kg)	**≤ 5.0**	**≤ 5.0**	≤ 5.0	≤ 5.0	≤ 5.0	< 5.0	< 5.0	< 5.0	≤ 5.0
Moisture (%)	**≤ 0.05**	**≤ 0.02**	≤ 0.05	≤ 0.05	≤ 0.05	< 0.1	< 0.1	<0.05	≤ 0.1
Unsaponifiable Matter (%)	**≤ 3.5**	**≤ 3.5**	≤ 4.0	≤ 3.5	≤ 3.5	< 3.0	< 1.0	< 3.5	≤ 4.5
Trans Fatty Acid (%)	**≤ 1.0**	**≤ 3.5**	≤ 1.0	≤ 1.0	≤ 1.0	< 1.0	< 1.0	< 2.0	≤ 2.0
Docosahexaenoic Acid (DHA) (%)	**≥ 35.0**	**≥ 35.0**	50 - 60	≥ 55.0	≥ 35.0	> 45.0	> 45.0	> 35	32 – 45
Total Arsenic (ppm)	**≤ 0.1**	**≤ 0.1**	≤ 0.1	≤ 0.1	≤ 0.1	< 0.2	< 0.1	< 0.1	< 0.5
Cadmium (mg/kg)	**≤ 0.1**	**≤ 0.1**	≤ 0.5	≤ 0.01	≤ 0.01	< 0.1	< 0.1	-	-
Copper (ppm)	**≤ 0.05**	**≤ 0.1**	-	≤ 0.05	≤ 0.05	< 0.1	< 0.5	< 0.1	< 0.1
Iron (ppm)	**≤ 0.2**	**≤ 0.2**	-	≤ 0.2	≤ 0.2	< 0.5	< 0.2	< 0.2	< 0.5
Mercury (ppm)	**≤ 0.04**	**≤ 0.04**	≤ 0.1	≤ 0.04	≤ 0.04	< 0.04	< 0.01	< 0.1	≤ 0.2
Lead (ppm)	**≤ 0.1**	**≤ 0.1**	≤ 0.1	≤ 0.01	≤ 0.01	< 0.2	< 0.1	< 0.1	≤ 0.2
Anisidine Value	-	-	< 10	-	-	-	-	-	-
Total Oxidation Value	-	-	< 20	-	-	-	-	-	-
Free Fatty Acids, as % oleic acid	-	-	-	-	-	< 0.1	< 0.1	-	-
Docosapentaenoic acid (DPA, n-6) (%)	-	-	-	-	-	-	-	-	10 – 20
Residual Hexane (ppm)	-	-	-	-	-	< 5.0	< 5.0	-	< 10
Coliforms (cfu/mL)	-	-	-	-	-	< 1	< 1	-	-
Molds (cfu/mL)	-	-	-	-	-	< 1	< 1	-	-
Yeast (cfu/mL)	-	-	-	-	-	< 1	< 1	-	-
Salmonella (in 25 g)	-	-	-	-	-	N.D.	N.D.	-	-
Ash (%)	-	-	-	-	-	-	< 0.1	-	-

-: parameter not included in listed specifications
N.D.: not detected

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Table 16 is a comparison of the fatty acid profile between CABIO DHA-rich oil and the DHA-rich oil in GRN 553. Some small variations in the composition of the oil are present in palmitic, oleic, and eicosatrienoic acids. These small differences are not expected to pose a risk to the consumer, as these fatty acids are naturally found in the diet. This demonstrates that the fatty acid profile for CABIO-rich oil is similar to the fatty acid profile described in GRN 553, a DHA-rich oil also generated from *Schizochytrium* sp.

Table 16. Comparison of Fatty Acid Analysis between CABIO DHA-Rich Oil and GRN 553 Martek DHA-Rich Oil

Fatty Acid (%)	CABIO DHA-Rich Oil Average ± Standard Deviation (n = 4)	GRN 553 Martek DHA-Rich Oil Average ± Standard Deviation (n = 5)
C4:0 Butyric acid	0.03 ± 0.0	NS
C6:0 Caproic acid	ND	NS
C8:0 Caprylic acid	ND	NS
C10:0 Capric acid	ND	NS
C11:0 Undecanoic acid	ND	NS
C12:0 Lauric acid	0.050 ± 0.014	ND
C14:0 Myristic acid	1.28 ± 0.91	1.18 ± 0.12
C14:1 Myristoleic acid	ND	ND
C15:0 Pentadecanic acid	0.16 ± 0.08	0.240 ± 0.012
C15:1 Pentadecenoic acid	ND	NS
C16:0 Palmitic acid	20.36 ± 8.72	13.78 ± 0.59
C16:1 Palmitoleic acid	0.19 ± 0.11	ND
C17:0 Margaric acid	0.10 ± 0.03	ND
C18:0 Stearic acid	1.30 ± 0.35	1.65 ± 0.080
C18:1n9 Oleic acid	6.58 ± 2.01	25.00 ± 2.43
C18:2n6 Linoleic acid	0.99 ± 0.26	2.01 ± 0.12
C18:3n3 alpha-Linolenic acid	0.18 ± 0.019	0.1 ± 0.0
C18:3n6 gamma-Linolenic acid	0.15 ± 0.039	NS
C18:4 Octadecatetraenoic acid	0.21 ± 0.044	NS
C20:0 Arachidic acid	0.28 ± 0.074	0.32 ± 0.0084
C20:3n3 Eicosatrienoic acid	0.4 ± 0	0.1 ± 0
C20:3n6 Eicosatrienoic acid	0.27 ± 0.048	ND
C20:4n6 Arachidonic acid	0.37 ± 0.14	0.69 ± 0.053
C20:5n3 Eicosapentaenoic (EPA)	0.55 ± 0.11	6.23 ± 0.29
C22:0 Behenic acid	0.22 ± 0.048	0.35 ± 0.033
C22:1 Erucic acid	0.33 ± 0	NS
C22:2n6 Docosadieonic acid	0.68 ± 0	0.53 ± 0.030
C22:5n3 Docosapentaenoic acid	0.26 ± 0.18	0.76 ± 0.20
C22:6n3 Docosahexaenoic acid	42.14 ± 2.28	43.34 ± 1.72
C24:0 Lignoceric acid	0.19 ± 0.021	0.14 ±0.011
C24:1 Nervonic acid	0.17 ± 0.064	ND

NS: Not Specified
ND: Not Detected

Based on a comparison of the manufacturing process and specifications for these products, the DHA-rich oil from CABIO is substantially equivalent to the DHA-rich oil described in GRN 553 and very similar to the DHA-rich oils described in the previously

mentioned GRNs. Therefore, the information and data in GRN 553 are pivotal to the safety determination of CABIO's DHA-rich oil and the data and information from the other cited GRAS notices are corroborative to the safety of the DHA-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 DHA-rich oil from the intended uses and use levels. DHA-rich oil is therefore GRAS as an ingredient in infant formula and general foods at the intended use levels.

This notice incorporates by reference the pivotal and corroborative safety and metabolism studies discussed in previous GRNs (GRN 776, pages 17 – 25; GRN 677, pages 27 – 43; GRN 553, pages 29 – 53; GRN 137, stamped pages 12 – 22) and will not discuss previously reviewed references in detail.

A. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

DHA is mainly found in the form of triglycerides, although they also occur in phospholipids in breast milk, comprising of 0.32% of the total fatty acids (Martin et al., 1993; Brenna et al., 2007). In general, dietary triglycerides undergo enzymatic hydrolysis in the upper intestine to free fatty acids and 2-monoglycerides. These products are then integrated into bile acid micelles for diffusion into the interior of the intestinal epithelial cells for subsequent incorporation into new or reconstituted triglycerides (Kroes et al., 2003). These reconstructed triglycerides enter the lymph in the form of chylomicrons for transport to the blood, which allows distribution and incorporation into plasma lipids, erythrocyte membranes, platelets, and adipose tissue. The chylomicron-contained triglycerides 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. Therefore, long chain fatty acids, such as DHA, may not undergo mitochondrial beta-oxidation to the same extent (Kroes et al., 2003). Instead they are preferentially channeled into the phospholipid pool where they are rapidly incorporated into the cell membranes of the developing brain and retina, among other tissues.

Fatty acids can be desaturated endogenously only up to the Δ9 position due to lack of certain enzymes in humans (Kremmyda et al., 2011). For this reason, linoleic acid (18:2n-6) and linolenic acid (18:3n-3) acids must be obtained from the diet and are termed essential fatty acids. Further elongation and desaturation of these fatty acids to produce long chain polyunsaturated fatty acids is possible, but not very efficient in humans. Examples of polyunsaturated fatty acids include ARA (20:4n-6), eicosapentaenoic (EPA; 20:5n-3), and DHA (22:6n-3). Thus, these fatty acids may be conditionally essential depending on essential fatty acids availability. Genetic variation in human desaturase genes affects fatty acids metabolism, plasma lipid profiles, and risk of disease development.

B. GENOTOXICITY STUDIES

The studies discussed in this section were not performed on the DHA-rich oil manufactured by CABIO. The genotoxicity study described in GRN 553 is on a DHA-rich oil that is similar in production process, source organism, product specifications, and DHA content. The studies described in GRN 553 include published and unpublished studies, including reverse mutation (Ames), in vitro mammalian chromosome aberration, and in vivo mouse micronucleus tests.

1. Genotoxicity of DHA-rich oil from GRN 553

Unpublished studies cited in GRN 553 assessed the genotoxicity of DHA-rich oil (DHASCO®) produced by Martek Biosciences Corporation by performing a reverse mutation (Ames), in vitro mammalian chromosome aberration, and in vivo mouse micronucleus tests. Consistent with the no questions letter for GRN 553, these studies demonstrate a lack of toxicity of DHA-rich oil. These studies were performed in compliance with the respective OECD test guidelines.

The reverse mutation test found no biologically relevant increases in revertant colony numbers in any of the tester strains (*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA) used at any concentration of DHASCO®, regardless of metabolic activation. Positive control agents substantially induced the number of revertant colonies compared to the negative control, confirming the sensitivity of the assay. Based on these findings, the investigators concluded that DHASCO® did not induce gene mutations by base-pair changes or frameshifts in the genomes of the tester strains used and therefore was non-mutagenic.

DHASCO® did not induce chromosomal aberrations in human lymphocytes in both experiments conducted in the absence of metabolic activation. In both the short term (four hour exposure to DHASCO®) and the long term (24 hour exposure to DHASCO®) experiments with metabolic activation, an increase in the frequency of chromosomal aberrations was noted at concentrations of 500 µg/mL and greater; however, no dose-response relationship was observed. Some increases were within the historical control data of the negative controls. In both experiments, positive controls induced distinct and biologically relevant increases in the incidence of cells with structural chromosomal aberrations. No biologically relevant increase in the frequency of polyploidy cells was observed in any experiment. DHASCO® did not induce structural chromosomal aberrations in human lymphocytes in the absence of metabolic activation, but induced an increase in the frequency of chromosomal aberrations in the presence of metabolic activation; however, given that the clastogenic effect was relatively moderate, observed mostly at concentrations beyond the solubility limit and a dose-response relationship was not observed, the study authors concluded that the results of the *in vitro* chromosomal aberration test were equivocal.

A micronucleus test was performed in the immature erythrocytes in the bone marrow of the mouse to investigate the genotoxic potential of DHASCO® *in vivo*. In a preliminary dose-range finding study, Naval Medical Research Institute (NMRI) mice (1/sex) were administered the test article at a single dose of 2000 mg/kg body weight via intraperitoneal (i.p.) injection with no signs of toxicity observed. Therefore, this dose was selected as the maximum feasible dose in the main micronucleus test. In the main micronucleus test, NMRI mice (5/sex) were administered DHASCO® at a single dose of 2000 mg/kg body weight via i.p. injection. The negative and positive control groups were administered cottonseed oil and 40 mg/kg body weight of cyclopiazonic acid (CPA), respectively. No toxicity was observed in animals administered the test article. DHASCO® did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse as no increases in micronuclei was found after the treatment. The incidence of micronuclei in the negative control group was reported to be within the range of historical laboratory control data. A significant increase in micronuclei was observed in the positive control group, thus confirming the validity of the assay. Therefore, DHASCO® was considered to be non-genotoxic as assessed in the *in vivo* mammalian micronucleus test.

2. Genotoxicity of Related *Schizochytrium* sp. Cells and Algal Oil

Whole *Schizochytrium* sp. cells were not mutagenic in a reverse mutation assay, xanthine-guanine phosphoribosyl transferase gene locus assay, in vitro mammalian chromosome aberration assay, and a micronucleus test (Hammond et al., 2002). Algal oil derived from

Schizochytrium sp. was also not mutagenic in Ames, chromosome aberration, and in vivo micronucleus assays in multiple safety assessments (Fedorova-Dahms et al., 2011a; Schmitt et al., 2012a; Lewis et al., 2016).

C. TOXICOLOGY STUDIES

1. Toxicology Studies of DHA-rich Oil from GRN 553

Studies cited in GRN 553 assessed the safety of DHA-rich oil (DHASCO®) produced by Martek Biosciences Corporation by performing a 90-day subchronic toxicity study with an in utero exposure in accordance with the Organization for Economic Cooperation and Development (OECD) Test Guideline No. 408 (OECD, 1998) and U.S. Redbook Guideline IV.C.4.a (U.S. FDA, 2003) (unpublished).

During the in utero phase, DHASCO® was administered at dietary levels of 1.0% (low-dose), 3.0% (mid-dose), or 5.0% (high-dose) % to F_0 rats (13 males and 26 females/group). Two control groups also were included in the study; one that received a standard low fat basal diet and one a basal diet supplemented with 5.0% tuna oil. For the in utero phase, parental males and females received the experimental diet while housed separately for a 28-day pre-mating period, followed by feeding through a 14-day co-habitation period. Upon determination of pregnancy, females were removed to separate cages and continued to be fed through the gestation period of pregnancy and Day 22 of lactation. Males were euthanized following the weaning of their respective litters. In the subsequent 90-day F_1 phase, the test diets were fed to randomly selected offspring from each litter (generally, 1 male and 1 female/litter) according to their respective original in utero treatment. Twenty F_1 animals/sex/group were selected to proceed to the 90-day dietary phase. Parameters evaluated in the F_0 generation included viability, signs of gross toxicity, behavioral changes, body weights, feed consumption, fertility, and selected reproductive and developmental indices. Parameters assessed in the F_1 generation included viability, signs of gross toxicity, behavioral changes, ophthalmology, body weights, feed consumption, functional observation battery, motor activity, hematology, clinical chemistry, urinalysis, organ weights, and gross pathology. Histopathological examination was performed on selected organs and tissues from both control and high-dose groups.

During the pre-mating phase of the study, parental intakes of DHASCO® in the 1.0, 3.0 and 5.0% dietary regimens were equivalent to doses of 757, 2,294, and 3,860 mg/kg body weight/day in males; and 895, 2,613, and 4,320 mg/kg body weight/day in females, respectively. Intakes of the fish oil control (tuna oil) were equivalent to 3,837 and 4,435 mg/kg body weight in males and females, respectively.

No test article-related mortalities were observed during the in utero phase of the study, and no clinical signs of toxicity were observed. No significant differences in body weight, body weight gain, or feed consumption were observed during the pre-mating, mating, or gestation periods compared to basal diet controls.

Fertility and reproductive performance parameters of males and females were comparable between DHASCO® groups and the controls. No significant effects were observed on mean gestation length, gestation index, number of implantation sites, number of corpora lutea, pre-implantation loss, post-implantation loss, stillbirths, live births, or viability indices were observed compared to controls. No significant differences were noted compared to controls in litter loss, litter size, litter or pup weight, sex ratio, time and body weight to attainment of developmental indices and sexual maturity, or pup survival. Taken together, there were no pre-mating, mating, reproductive, or early developmental effects attributed to DHASCO® in the in utero phase of the study, and all indices remained within historical control values for age- and strain-matched rats.

In the 90-day dietary phase of the study, intakes of DHASCO® in the 1.0, 3.0 and 5.0% dietary regimens were equivalent to doses of 645, 1,973, and 3,279 mg/kg body weight/day in mates, and 754, 2,331, and 3,788 mg/kg body weight/day in females, respectively. Intakes of the tuna oil controls were equivalent to 3,237 and 3,761 mg/kg body weight/day in males and females, respectively. No mortalities were observed and no clinical signs of toxicity were noted during the 90-day dietary phase. No test article-related ophthalmoscopic findings or test article-related differences in the functional observational battery or motor activity were observed compared to controls. No test article-related adverse changes in hematology, clinical chemistry, coagulation, or urinalysis parameters were observed, and all differences in these parameters from the basal diet control such as cholesterol concentrations reductions in all dose levels in females and the high dose mates were determined to be within historical control data or without histological correlates and thus were deemed to be incidental.

Moderate granulomatous infiltration of retroperitoneal fat was observed in two high-dose (5% DHASCO®) males. Similar granulomatous infiltration of minimal to slight intensity was noted in the adipose tissue of the mammary gland fat pad in 4 high-dose males and two high-dose females. These were considered to be possibly related to the test article. However, the authors considered these effects to be non-adverse and of little biological relevance.

Slight/moderate cytoplasmic vacuolation of cortical cells in the zona fasciculata of the adrenal glands was noted with increased incidence in the tuna oil control and the high dose DHASCO® males. Vacuolation microscopically was characterized by the presence of large,

single to multiple vacuoles within the cytoplasm of cortical cells consistent with lipid. The increased incidence of this finding in the high dose males was likely due to increased dietary fat content in males fed dietary tuna oil and DHASCO® compared to basal diet control. These histologic changes were not accompanied by any changes in adrenal organ weight or secondary changes in the affected adrenal glands and were therefore, considered non-adverse. The remaining macroscopic and microscopic findings were not considered to be test substance-related and were considered to be incidental.

Compared to the basal diet control, some changes in liver, heart, testes, kidney, and spleen weight were reported; however, these were without histological correlates, without a dose-response relationship, and thus were deemed to be toxicologically insignificant by the authors.

Based on the results of the study, the authors derived a NOAEL of 3,279 and 3,788 mg/kg body weight/day for male and female rats, respectively, the highest doses tested.

2. Toxicology Studies on CABIO DHA-rich Oil

a. Summary

CABIO performed two confirmatory toxicology studies to verify the safety of their DHA-rich oil (unpublished). These acute and subchronic toxicity studies were not OECD compliant but the results serve as corroborative data that further demonstrate the safety of DHA-rich oil. The acute oral toxicity study evaluated one oral dose of 20 g DHA-rich oil/kg body weight in male and female Kunming mice. The mice were then observed for 14 days. No mortality, toxicity, changes in behavior, or weight loss was reported during the observation period. The subchronic toxicity test was performed in male and female Wistar rats administered 0, 2.55, 5.1, and 10.2 g DHA-rich oil/kg body weight by oral gavage every day for 90 days. The rats in the 0 g DHA-rich oil group were fed an equal volume of vegetable oil as a negative control. No mortality, toxicity, changes in behavior, or weight loss was reported during the observation period. There were no statistically significant differences in organ weights, hematology and biochemistry parameters, or histopathology between the DHA-rich oil groups and the negative control. The results of both the acute and subchronic toxicology studies corroborate the NOAEL described in the toxicology studies in GRN 553.

b. Acute Toxicity Study of DHA-rich Oil

i. Materials and Methods

Ten male and ten female Kunming mice were provided by Hubei Laboratory Animal Research Center, (laboratory animal and the forage production license No. SCXK (Hubei) 2008-0005). The animals were kept at 20 to 25℃, with 40 to 70% humidity. The mice were provided water but no feed for 16 hours prior to oral gavage administration of 20 g DHA-rich oil/kg body weight. There were no positive or negative controls for this study. The mice were then observed and weighed once following administration on day 0, then twice daily for 14 days. Any toxicity or mortality was recorded and the mice were weighed on the 14th day of the study.

ii. Results

Weight gain was not affected 14 days after a single oral administration of 20 g DHA-rich oil/kg body weight in either male or female mice (Table 17). No toxicities or mortalities were observed during this period.

Table 17. Body Weight Results from Acute Oral Toxicity Test of DHA-rich oil in Kunming Mice

DHA-rich oil	Sex	Day 0 Weight (g)	Day 14 Weight (g)
20 g/kg body weight	Females (n = 10)	19.5 ± 0.6	30.3 ± 0.9
	Males (n = 10)	19.3 ± 0.6	32.9 ± 1.3

c. Subchronic Toxicity Study of DHA-rich Oil

i. Materials and Methods

Male and female Wistar rats were provided by Hubei Experimental Animal Research Center (production license No. of experimental animals is SCXK (Hubei) 2008-0005). The animals were singly housed at 20 - 25℃, with 40 - 70% humidity. The rats were randomized to one of four groups: a negative control (vegetable oil), 2.55, 5.1 or 10.2 g DHA-rich oil/kg body weight groups (n = 10/group/sex). The rats were administered the indicated amounts of DHA-rich oil by oral gavage for 90 days. Prior to sacrifice, the animals were fasted for 16 hours. The animals were anesthetized with pentobarbital sodium solution and then killed by abdominal aorta puncture.

The following parameters were measured following sacrifice for hematology: hemoglobin, red cell count (RBC), white blood cell count (WBC) including lymphocytes,

monocytes, and granulocytes. The following serum biochemistry parameters were measured following sacrifice: alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), total cholesterol, triglyceride, creatinine, glucose, albumin (Alb), albumin/globulin ratio, and total protein.

Gross pathology was performed after sacrifice, and the following organs were weighed and fixed in 10% formaldehyde: liver, spleen, kidney, and testicles. The following organs in the negative control and 10.2 g/kg/day male and female groups were fixed, dehydrated, embedded in paraffin sectioned and stained with hematoxylin and eosin for histopathology analysis: liver, spleen, kidney, stomach, intestines, ovary and testes.

ii. Results

No abnormal behavior or deaths were recorded during the study. There were no significant differences in rat body weight in either male or female rats fed DHA-rich oil during the study (Figure 5)

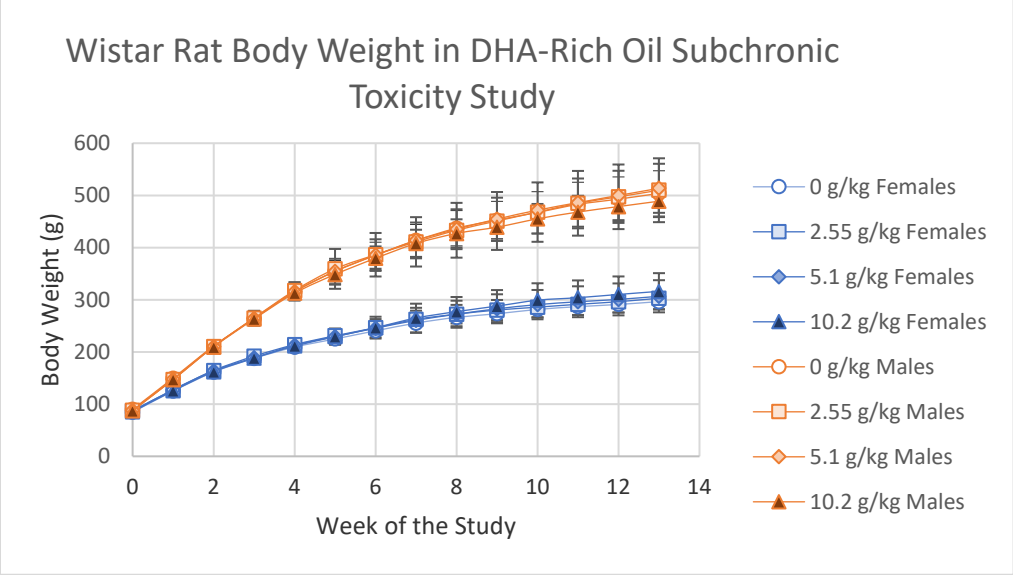

Figure 5. Wistar Rat Body Weight in the CABIO DHA-rich Oil 90 day Subchronic Toxicity Study

N = 10/group, means and standard deviations shown

Similarly, there were no differences in feed consumed during the 90 day subchronic toxicity study in male and female rats fed CABIO DHA-rich oil. (Figure 6)

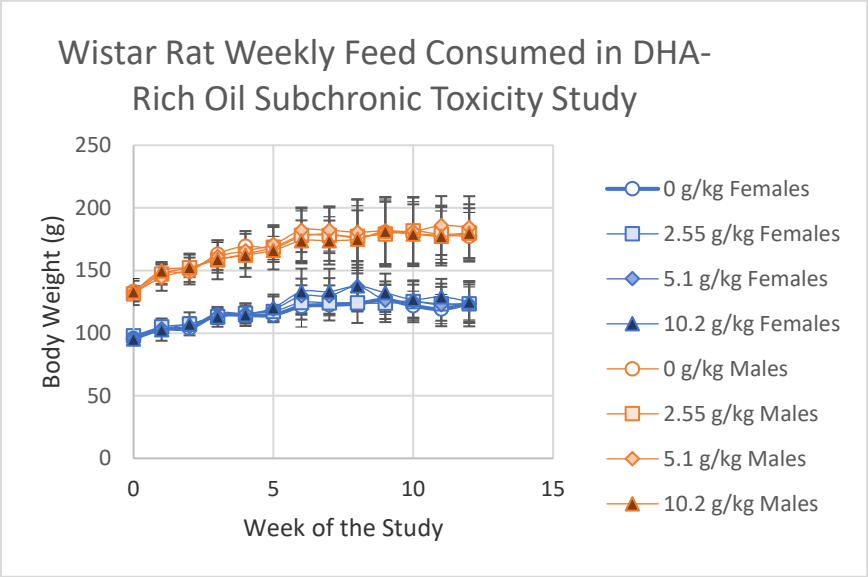

Figure 6. Wistar Rat Feed Consumed in DHA-rich oil Subchronic Toxicity Study

N = 10/group.

No significant differences were observed in the measured hematology and clinical chemistry parameters at the end of the 90 day study in either male or female rats fed 0, 2.55, 5.1 or 10.2 g DHA-rich oil/kg body weight/day (Table 18).

Table 18. Hematology and Clinical Chemistry Results in CABIO DHA-rich Oil Subchronic Toxicity Study

Sex	Males (n=10/group)				Females (n=10/group)			
g DHA-rich oil/ kg/day	**0**	**2.55**	**5.10**	**10.20**	**0**	**2.55**	**5.10**	**10.20**
Hematology Parameters								
WBC (10^9L)	11.3 ± 1.0	11.3 ± 1.1	11.5 ± 1.1	11.3 ± 1.0	11.6 ± 1.6	11.9 ± 1.3	11.9 ± 1.1	11.8 ± 1.1
RBC (10^{12}L)	7.24 ± 0.42	7.19 ± 0.66	7.3 ± 0.8	7.3 ± 0.5	7.0 ± 0.9	6.9 ± 0.5	6.8 ± 0.3	7.0 ± 0.4
Hemoglobin (g/L)	136.1 ± 3.8	133.7 ± 5.4	132.9 ± 7.1	133.0 ± 7.6	133.7 ± 10.1	131.1 ± 6.5	136.7 ± 5.4	129.6 ± 11.1
Lymphocyte (%)	71.6 ± 5.9	71.2 ± 5.9	72.5 ± 6.2	72.4 ± 6.3	70.9 ± 6.5	71.4 ± 8.0	70.0 ± 5.5	71.4 ± 5.6
Monocyte (%)	4.7 ± 0.6	4.6 ± 0.8	4.4 ± 0.6	5.1 ± 0.9	4.6 ± 0.7	4.3 ± 1.4	4.5 ± 1.4	5.2 ± 1.1
Granulocyte (%)	23.6 ± 5.7	24.2 ± 5.8	23.0 ± 6.1	22.5 ± 6.7	24.5 ± 6.1	24.4 ± 8.1	25.5 ± 5.3	23.4 ± 5.9
Clinical Chemistry Parameters								
Glucose (mmol/L)	5.8 ± 0.8	5.4 ± 0.6	5.3 ± 0.9	6.0 ± 0.6	5.7 ± 0.5	5.8 ± 0.7	5.9 ± 0.9	5.6 ± 0.4
BUN (mmol/L)	6.0 ± 0.8	5.9 ± 0.8	6.0 ± 0.8	6.4 ± 0.6	5.9 ± 0.8	6.5 ± 1.6	5.8 ± 0.8	6.2 ± 0.8
Creatine (μmol/L)	50.6 ± 2.8	49.2 ± 2.1	48.8 ± 3.5	49.4 ± 3.4	55.4 ± 3.2	55.1 ± 2.9	54.3 ± 3.6	55.3 ± 3.7
Total Cholesterol (mmol/L)	1.9 ± 0.3	1.8 ± 0.4	1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	1.6 ± 0.4	1.7 ± 0.3	1.9 ± 0.3
Triglycerides (mmol/L)	0.7 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.08	0.6 ± 0.1
ALT(U/L)	42.0 ± 8.3	44.7 ± 7.5	38.7 ± 5.1	41.4 ± 7.1	38.5 ± 5.1	39.2 ± 5.7	40.4 ± 3.7	39.7 ± 4.3
AST (U/L)	107.7 ± 8.7	112.3 ± 7.2	109.2 ± 11.4	112.5 ± 7.0	119.4 ± 13.7	113.6 ± 13.8	112.0 ± 11.4	113.5 ± 14.3
Total Protein (g/L)	61.4 ± 2.2	61.1 ± 2.4	60.1 ± 3.6	62.2 ± 2.4	63.2 ± 3.3	64.3 ± 4.8	63.8 ± 3.4	64.5 ± 2.7
ALB (g/L)	30.7 ± 0.4	30.8 ± 0.7	30.4 ± 0.5	31.0 ± 0.9	32.4 ± 1.0	33.1 ± 1.4	33.2 ± 1.2	33.2 ± 1.0
ALB/GLO	1.0 ± 0.09	1.0 ± 0.07	1.0 ± 0.16	1.0 ± 0.09	1.1 ± 0.06	1.1 ± 0.08	1.1 ± 0.2	1.1 ± 0.09

Abbreviations: WBC: white blood cells, RBC: red blood cells, Glu: glucose, BUN: blood urea nitrogen, TG: triglyceride, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALB: albumin, ALB/GLO: albumin/globulin ratio

No gross abnormalities were observed in the heart, liver, spleen, lung, kidney, stomach, or intestines at sacrifice. No significant differences were observed in the absolute or relative weights of the collected organs at the end of the 90 day study in either male or female rats fed 0, 2.55, 5.1 or 10.2 g DHA-rich oil/kg body weight/day (Table 19).

Table 19. Absolute and Relative Organ Weights in CABIO DHA-Rich Oil Subchronic Toxicity Study

Sex	**Males (n = 10/group)**			
g DHA-rich oil/ kg/day	**0**	**2.55**	**5.10**	**10.20**
Body Weight (g)	489.7 ± 43.6	500.3 ± 63.7	501.7 ± 47.6	476.6 ± 31.9
Liver:				
g	14.4 ± 2.0	15.4 ± 2.6	15.3 ± 2.8	14.7 ± 1.1
% Body Weight	2.9 ± 0.3	3.1 ± 0.3	3.0 ± 0.3	3.1 ± 0.2
Spleen:				
g	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.08	1.0 ± 0.08
% Body Weight (g)	0.2 ± 0.03	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01
Kidney:				
g	3.2 ± 0.3	3.2 ± 0.4	3.1 ± 0.4	3.0 ± 0.3
% Body Weight	0.7 ± 0.07	0.7 ± 0.07	0.6 ± 0.04	0.6 ± 0.05
Testicles:				
g	4.0 ± 0.5	3.9 ± 0.4	4.1 ± 0.4	4.1 ± 0.3
% Body Weight	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.09
Sex	**Females (n = 10/group)**			
g DHA-rich oil/ kg/day	**0**	**2.55**	**5.10**	**10.20**
Body Weight (g)	282.8 ± 11.9	291.8 ± 20.4	295.4 ± 30.3	304.0 ± 36.1
Liver:				
g	9.0 ± 0.8	9.6 ± 1.0	9.0 ± 0.8	9.5 ± 0.8
% Body Weight	3.2 ± 0.3	3.3 ± 0.2	3.1 ± 0.3	3.1 ± 0.2
Spleen:				
g	0.7 ± 0.07	0.8 ± 0.1	0.7 ± 0.07	0.8 ± 0.1
% Body Weight (g)	0.3 ± 0.03	0.3 ± 0.04	0.3 ± 0.03	0.3 ± 0.01
Kidney:				
g	1.7 ± 0.2	1.8 ± 0.1	1.7 ± 0.2	1.9 ± 0.2
% Body Weight	0.6 ± 0.04	0.6 ± 0.05	0.6 ± 0.08	0.6 ± 0.04

Since no abnormalities were observed during the gross pathology, only the negative control and 10.2 g/kg/day groups were analyzed for histopathology for the following organs: liver, spleen, kidney, stomach, intestines, ovary and testes. No pathologies were observed in the spleen, stomach, intestines, ovaries or testes in the 10.2 g/kg/day groups. There were incidences of limited fatty degeneration of the liver in 2/10 male negative control rats and 2/10 female 10.2 g/kg/day rats; fatty degeneration of the liver was not observed in the treated male rats or the untreated female rats. This finding was considered adaptive, due to the large amount of fat being consumed in the test article. Kidney pyelitis was also reported in 1/10 negative control female rats and 2/10 10.2 g/kg/day female rats. This finding was also considered spontaneous and not related to the test article.

This corroborative, non-OECD compliant subchronic toxicity study further supports the safety of DHA-rich oil, as established by the pivotal studies described in GRN 553.

3. Toxicology Studies on Related *Schizochytrium* sp.

Toxicology studies of related *Schizochytrium* sp. are summarized in Table 20. The results of acute, subchronic, and developmental and reproductive toxicology studies of whole cell biomass and DHA-rich oil from *Schizochytrium* sp. are found in Table 20. No systemic toxicity, developmental toxicity or reproductive toxicity was reported in these studies of doses of the biomass up to 22,000 mg/kg/day and at levels in the diet up to 5% or 5,000 ppm of DHA-rich oil, resulting in intakes estimated at 3 - 5 g/kg/day.

Furthermore, the FDA has reviewed numerous GRNs for substantially equivalent or similar products, including three for DHA algal oils from closely related *Schizochytrium* sp. strains and has issued “no questions” letters to these notifications (the following toxicology results are incorporated by reference: GRN 137 stamped pages 15 - 21, GRN 553 stamped pages 35 - 57, and GRN 677 pages 33 - 41).

Table 20. Summary of Corroborative Animal Toxicity Studies Performed using DHA-rich Oil from *Schizochytrium*

Reference	Species	Dose	DHA%	Study Type	NOAEL of DHA-rich oil
Corroborative data from GRN 773, pages 20-24					
Hammond et al., 2001a	Male and female Sprague Dawley rats	0, 400, 1500, 4000 mg/kg/day	Dried *Schizochytrium* sp. whole cell biomass (35% DHA)	90-day subchronic toxicity study	• 4000 mg/kg/day (of Dried *Schizochytrium* sp. whole cell biomass)
Hammond et al. 2001b	Male and female Sprague Dawley rats	0.6, 6, 30%	Dried *Schizochytrium* sp. whole cell biomass (35% DHA)	Developmental toxicity	• 30% (equivalent to 22000 mg/kg Dried *Schizochytrium* sp. whole cell biomass for maternal and developmental toxicity)
	Male and female New Zealand White rabbits	180, 600, 1800		Developmental toxicity	• 600 mg/kg/day for maternal toxicity (reductions seen in food consumption and body weight) • 1800 mg/kg/day for developmental toxicity of Dried *Schizochytrium* sp. whole cell biomass
Hammond et al. 2001c	Male and female Sprague Dawley rats	0, 0.6, 6, 30%	Dried *Schizochytrium* sp. whole cell biomass (35% DHA)	One-generation reproductive toxicity	• 30% (equivalent to 17,800 and 20,700 mg/kg/day for F_0 males and females, respectively) of Dried *Schizochytrium* sp. whole cell biomass
Fedorova-Dahms et al. 2011a	Male and female Sprague-Dawley rats	0.5% (312 mg/kg/day), 1.5% (965 mg/kg/day), 5% (3246 mg/kg/day)	37% DHA	90-day subchronic toxicity Study	• 5% of the diet (equivalent to 3149 mg/kg/day for males and 3343 mg/kg/day for females)
Fedorova-Dahms et al. 2011b	Male and female Sprague-Dawley rats	0.5% (5000 ppm), 1.5% (15000 ppm), 5% (50000 ppm)	42.6% DHA	90-day subchronic toxicity study with 28-day in utero exposure, and a 30-day recovery	• 5% of the diet (equivalent to 4122 mg/kg/day for males and 4399 mg/kg/day for females)
Schmitt et al., 2012a	Male and female Sprague-Dawley rats	5000 mg/kg	39-42% DHA	Acute toxicity	• Not applicable, LD_{50} was greater than 5000 mg/kg.
		Control: tuna oil (50000 ppm), 10000 ppm, 25000 ppm, 50000 ppm		Subchronic toxicity study with 28 day recovery period	• 50000 ppm (equivalent to 3305 mg/kg/day for males and 3679 mg/kg/day for females)

Table 20. Summary of Corroborative Animal Toxicity Studies Performed using DHA-rich Oil from *Schizochytrium*

Reference	Species	Dose		DHA%	Study Type	NOAEL of DHA-rich oil
Schmitt et al., 2012b	Male and female rats (Sprague-Dawley)	DHA fish oil	0 ppm, 50000 ppm	26 – 27% DHA	90-day subchronic toxicity study with 28 day in utero exposure	• F_0 male and females: 50000 ppm • F_1 males: 50000 ppm (equivalent to 3421and 2339 mg/kg/day for F_0 males, premating and after mating, respectively; 3558, 3117 and 7464 mg/kg/day for F_0 females during pre-mating, gestation and lactation, respectively and 3526 and 4138 mg/kg/day for F_1 males and females, respectively) • F_1 females: 25000 ppm (higher body weight and food consumption, intake on mg/kg basis not reported)
		Algal oil	10000 ppm, 25000 ppm, 50000 ppm	42% DHA		
		Algal oil	400, 1000, 2000 mg/kg/day	42% DHA	Developmental toxicity study days 6 - 19 of gestation	• NOAEL for maternal and embryofetal toxicology of 2,000 mg/kg/day
Fedorova-Dahms et al. 2014	Domestic Yorkshire Crossbred Piglets	0.32 % and 0.96% DHA as % of total fatty acids (dose volume of formula 500 ml/kg/day)		41.5% DHA (in combination with ARA oil)	21-day repeat dose toxicity, oral (diet)	• Well-tolerated at up to 0.96% DHA (equivalent to 700 mg DHA/L).
Lewis et al., 2016	Female Wistar rats	5000 mg/kg/day		41.37% DHA (in combination with ARA)	Acute toxicity	• Not applicable, LD_{50} was greater than 5000 mg/kg.
	Male and female Wistar rats	0 mg/kg/day, 1000 mg/kg/day, 2500 mg/kg/day, 5000 mg/kg/day			28-day repeat dose toxicity	• Not applicable, no treatment related adverse effects at any dose.
					90-day subchronic toxicity study with 28 day recovery period	• 5000 mg/kg/day
Falk et al., 2017	Male and female Wistar rats	Vehicle control (corn oil), 1000 mg/kg/day, 2500 mg/kg/day, 5000 mg/kg/day		41.37% DHA (in combination with ARA)	Developmental toxicity study days 6 - 20 of gestation	• 5000 mg/kg/day
					Reproductive toxicology study; administration through mating, pregnancy, nursing and lactation	• 5000 mg/kg/day

Abbreviations: DHA: docosahexaenoic acid; GRN: GRAS Notice; NOAEL: no observed adverse event level; ppm: parts per million; LD_{50}: 50% of the lethal dose; ARA: arachidonic acid.

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D. CLINICAL STUDIES

1. Clinical Studies Reviewed in Published GRAS Notices

Numerous clinical trials have been summarized in GRNs 41, 94, 379, and 553 on DHA-containing fish and marine-based oils as well as from the algal *C. cohnii;* the data indicate that the source of DHA does not impact the safety of DHA or DHA-oils. In this GRAS dossier, only studies performed on *Schizochytrium sp.* derived DHA-rich oil in term infants and in general foods are reviewed.

Studies in term infants of DHA-rich oil derived from *Schizochytrium sp.* that have been summarized in previous GRAS Notices are provided in Table 21. These studies evaluate safety of infant formulas delivering: 0.11 - 1.0% of total fatty acids as DHA (0.36% - Birch et al., 2005, 0.36 – 0.96% - Colombo et al., 2011; 0.36 – 0.96% Drover et al., 2011; 0.36% - Westerberg et al, 2011; van de Lagemaat et al., 2011; 0.36 – 0.96% - Drover et al., 2012; 0.36 – 0.96% Columbo et al., 2013; 0.36 – 0.96% - Currie et al., 2015; 0.86% - Almaas et al., 2015; 0.3 – 0.37% - Alshweki et al., 2015; 0.86% - Almaas et al., 2016; 0.11% - Salas Lorenzo et al., 2019) or 17 mg/100 kcal DHA (Clandinin et al., 2005; Lapillone et al., 2014, Yeiser et al., 2016; Hoffman et al., 2019). The studies demonstrated the safety and tolerance of these levels of intake in term infants. None of these studies reported test article related adverse effects of DHA or DHA-rich oil. We incorporate by reference the clinical studies described in GRN 553 stamped pg 55 – 57, GRN 677 pg 28 – 33, GRN 731 pg 35 – 40, and GRN 776 pg 24 – 25 (Table 21).

2. Clinical Studies of Algal-derived DHA-rich Oil in Infant Populations not Summarized in GRAS Notices

The latest *Schizochytrium* sp. DHA-rich oil GRAS notice that received no questions from the FDA was GRN 776 in 2019. Since GRN 776, three clinical studies have been published on the oral administration of *Schizochytrium* sp. derived DHA in term (Hoffman et al., 2019; Salas Lorenzo et al., 2019) and preterm infants (Bernhard et al., 2019). These studies are summarized below and in Table 21.

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 a prebiotic (n = 20). The results of the study describe 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 (Salas Lorenzo et al., 2019). This cohort was a 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 experimental formula, with 15.8 mg/100 mL fungal oil 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 safety parameters were reported in this study.

One study in preterm infants has been published since GRN 776. No safety parameters were reported. Bernhard et al. (2019) reported a randomized, singly blinded single center trial in infants < 32 weeks post menstrual age to determine if combined choline and DHA supplementation would increase the levels of plasma choline and DHA-phosphatidylcholine to that observed in term infants. Infants were given either standard nutrition (control), standard nutrition with 30 mg/kg/day choline, standard nutrition with 60 mg/kg/day DHA or standard nutrition with both 30 mg/kg/day choline and 60 mg/kg/day DHA. Infants in the test groups including DHA had increased DHA-phosphatidylcholine measured in the serum. This study in pre-term infants is summarized in Table 21.

Table 21. Corroborative Term and Preterm Infant Clinical Studies in sp. Derived DHA-Rich Oil			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Studies discussed in GRNs 776, 731, 677, and 553			
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)	• 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. • Evaluation of sweep visual evoked potential (VEP) acuity in the long chain poly unsaturated fatty acid (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).
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 21. Corroborative Term and Preterm Infant Clinical Studies in sp. Derived DHA-Rich Oil			
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 in 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.
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.

Table 21. Corroborative Term and Preterm Infant Clinical Studies in sp. Derived DHA-Rich Oil

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters
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.
Drover et al., 2012	Double-masked, randomized, controlled, prospective trial in 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.
Colombo et al., 2013	Randomized, double-blind, controlled trial in term infants from birth to 12 months (54 infants). 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)	• DHA/ARA supplementation did not influence performance on standardized tests of language and performance at 18 months. • Significant positive effects observed at 3 – 5 years old on rule-learning and inhibition tasks, the Peabody Picture Vocabulary test and the Weschler Primary Preschool Scales of Intelligence. • No safety parameters were reported.

Table 21. Corroborative Term and Preterm Infant Clinical Studies in sp. Derived DHA-Rich Oil			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Lapillone et al., 2014	Multicenter, prospective, observational, open-label study in healthy term infants.	• Control: infant and follow-on formula with no added DHA/ARA. • Test group: infant and follow on formula with 17 mg/100 kcal DHA and 34 mg/100 kcal ARA.	• DHA/ARA consumption was associated with lower incidence of respiratory illnesses, lower incidence of diarrhea requiring medical attention, no difference in the incidence of eczema. • 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.
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.
Chase et al., 2015	Multicenter, two-arm, randomized, double-blind pilot trial in the first 5 months after birth (57 infants)	• Control group: 3.4 mg DHA/ounce of infant formula • Test group: 10.2 mg DHA/ounce of infant formula	• Infants that receive DHA supplementation had a 20% increase in DHA levels in red blood cells.
Currie et al., 2015	Randomized, double-blind, controlled trial in term infants from birth to 12 months (54 infants). 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 adverse effect on body weight or child growth in children 6 years of age. • Increased stature and weight for age percentiles, but not body mass index, were observed in the test groups compared to the control. • No safety parameters were reported.

Table 21. Corroborative Term and Preterm Infant Clinical Studies in sp. Derived DHA-Rich Oil			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
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. • No adverse events occurred. • 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
Yeiser et al., 2016	Multicenter, double blind, randomized controlled parallel trial for 106 days in healthy term infants	• Control group: cow milk-based formula with 17 mg/100kcal DHA rich oil from *Crypthecodinium cohnii* • Test group: cow milk-based formula with 17 mg DHA-rich oil from *Schizochytrium* sp. • Both control and test formula included ARA, galactooligosaccharides, and a prebiotic blend of polydextrose	• No study related adverse events reported. • No significant differences in adverse events reported. • No significant difference in subjects who discontinued the study due to formula intolerance.

Table 21. Corroborative Term and Preterm Infant Clinical Studies in sp. Derived DHA-Rich Oil			
Reference	**Study Design and Population**	**Treatment Groups**	**Outcomes and Safety Parameters**
Studies not included in GRN 776: term infants			
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 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.
Salas 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

Table 21. Corroborative Term and Preterm Infant Clinical Studies in sp. Derived DHA-Rich Oil

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters
Studies not included in GRN 776: pre-term infants			
Bernhard et al., 2019	Randomized, singly blinded singe center trial in 24 infants < 32 weeks post menstrual age	• Control: standard nutrition • Test group 1: standard nutrition + 30 mg/kg/day choline • Test group 2: standard nutrition + 60 mg/kg/day DHA • Test group 3: standard nutrition with both choline and DHA	• No safety parameters reported • Infants in test groups with DHA had increased plasma DHA-phosphatidylcholine.

Abbreviations: GRN: GRAS Notice Number; ARA: arachidonic acid; DHA: docosahexaenoic acid, VEP: visual evoked potential; LCPUFA: long chain poly unsaturated fatty acids, PMA: post-menstrual age; MDI: Bayley mental development index; PDI: psychomotor development index; DIAMOND: DHA Intake And Measurement of Neural Development; EPA: eicosapentaenoic acid

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3. Algal-derived DHA-rich Oil in Adult Populations

Clinical studies in adults have reported safe use of algal-derived DHA-rich oil at a range of 1.52 g to 6 g of DHA-rich oil/day (Table 22), supporting the safety of the intended use of not more than 1.5 g DHA-rich oil/day. Many of these studies investigated the role of DHA-rich oil on cardiovascular health endpoints, especially cholesterol and triglycerides in healthy subjects (Maki et al., 2004; Stark and Holub, 2004; Sanders et al., 2006; Wu et al., 2006; Singhal et al., 2013; Maki et al., 2014). A series of studies were performed in professional Spanish athletes to determine if DHA supplementation would have beneficial effects following acute exercise performance (Capo et al., 2014a,b, 2015, 2016a, 2016b; Busquets-Cortes et al., 2016). Algal-derived DHA-rich oils also provide a vegetarian source of polyunsaturated fatty acids, as demonstrated by both Wu et al., (2006) and Maki et al., (2014). Sanders et al. (2006) and Singhal et al. (2013) noted that the DHA supplementation was well tolerated, and Maki et al. (2014) found no changes in hematology and liver function; otherwise no safety parameters were reported for the studies described above.

Some of the studies summarized in Table 22 were performed in specific cohorts of subjects. Many studies have investigated the potential benefit of DHA supplementation in pregnant women on gestation and birth outcomes (Escamilla-Nunez et al., 2014; Mulder et al., 2014; Harris et al., 2015; Ramakrishnan et al., 2015; Scholtz et al., 2015). A few clinical studies have been published investigating the role of algal-derived DHA on non-alcoholic fatty liver disease (250 and 500 mg DHA/day, Nobili et al., 2013a,b) and autism (200 mg/day DHA, Voigt et al., 2014) in children. No safety parameters were reported for these studies.

None of the studies below reported any adverse events that were related to the test articles. For the purposes of this GRAS notice, only clinical trials performed with *Schizochytrium* sp – derived DHA-rich oil (and algal-derived DHA-rich oil, if the species of algae is not specified) are included in Table 22. A further discussion of these studies is incorporated by reference from GRN 732, pages 38 – 45.

Table 22. Corroborative non-infant Clinical Studies with Algal-Derived DHA-Rich Oil

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters
Maki et al., 2004	Randomized, double-blind, controlled clinical trial of 57 healthy men and women with below-average levels of HDL cholesterol	• Control: olive oil • Test group: 1.52 g/day DHA from DHA-rich algal triglycerides	• No safety parameters reported • Supplementation with DHA raised the LDL cholesterol level, but had favorable effects on triglycerides, the triglyceride/HDL cholesterol ratio and the fraction of LDL cholesterol carried by small, dense particles.
Stark and Holub 2004	Randomized, double-blind, placebo-controlled cross-over trial with a 6 week washout period between cross-over in women both receiving and not receiving hormone replacement therapy	• Control: Corn and soil oil • Test group: 2.8 g DHA/day from algal derived DHA-rich oil	• No safety parameters reported. • DHA supplementation was associated with the following significant changes: lower serum triacylglycerol, HDL-cholesterol, lower overall ratio of serum triacylglycerol: HDL-cholesterol, decreased resting heart rate.
Sanders et al., 2006	Double-blind randomized placebo-controlled parallel-design trial in healthy men and women.	• Control: 4 g/day refined olive oil • Test group: 4g refined DHA-rich triacylglycerol derived from *Schizochytrium* sp.	• Treatment was well tolerated and did not adversely affect cardiovascular risk • No significant differences in hematology, liver function tests, or self-reported adverse events.
Wu et al., 2006	Single-blind, randomized, placebo-controlled trial in 27 post-menopausal vegetarian women	All subjects received 2 weeks of 6 g corn oil/day, prior to randomization • Control: 6 g corn oil/day • Test group: 6 g algal DHA-rich oil/day (2.14 g DHA/day)	• Plasma LDL-DHA and EPA levels significantly increased in test group. • DHA supplementation significantly decreased plasma cholesterol. • No safety parameters reported
Nobili et al., 2013a	Randomized placebo-controlled trial in 60 children with non-alcoholic fatty liver disease	• Control: 290 mg linoleic acid • Test group 1: 250 mg DHA/day • Test group 2: 500 mg DHA/day	• No safety parameters reported • DHA supplementation in subjects with the I148M variant of Patatin-like phospholipase domain-containing protein-3 had decreased probability of severe steatosis of the liver

Table 22. Corroborative non-infant Clinical Studies with Algal-Derived DHA-Rich Oil

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters
Nobili et al., 2013b	Randomized placebo-controlled trial in 60 children with non-alcoholic fatty liver disease	• Control: 290 mg linoleic acid • Test group 1: 250 mg DHA/day • Test group 2: 500 mg DHA/day	• No safety parameters reported • Both levels of DHA supplementation improves liver steatosis in children with non-alcoholic fatty liver disease as assessed by ultrasound
Singhal et al., 2013	Double-blind, parallel group, placebo controlled randomized trial in healthy adults aged 18 – 37 years.	• Control: 4 g/day olive oil • Test group: 1.6 g DHA/day with 2.4 g/d carrier oil	• DHA supplementation did not improve endothelial function in healthy young adults. • There were no serious adverse events in either group, both diets were well tolerated. No participant dropped out of the study due to adverse effects of the study.
Capo et al., 2014a	Randomized, placebo-controlled trial in 15 Spanish male football players	• Control: Almond-based beverage with 0.8% olive oil • Test group: almond-based beverage with 0.2% DHA-rich oil and 0.6% olive oil (1.16 g DHA/day)	• No safety parameters reported • Subjects consuming the DHA supplemented beverage had increased DHA content in erythrocytes
Escamilla-Nunez et al., 2014	Double-blind randomized placebo-controlled trial in 1094 pregnant women from 18 – 22 weeks of gestation to delivery	• Control: placebo • Test group: 400 mg/day of algal DHA	• No safety parameters reported • DHA supplementation during pregnancy may decrease the incidence of respiratory symptoms in children with a history of maternal atopy
Maki et al., 2014	Double-blind, parallel trial in 93 healthy adults with hypertriglyceridemia.	• Control: 4 1g Corn oil/soy oil softgel capsules/day with meals for 14 weeks • Test group 1: 2.5 g/day 2.7:1 ratio of marine algal derived DHA and EPA • Test group 2: 2 g/day 0.7:1 fish oil derived DHA and EPA	• No significant differences in systolic and diastolic blood pressures, heart rate, or body weight changes. • No safety issues arose from routine screening of serum chemistry and hematology • The frequencies of any treatment-emergent adverse events were not significantly different among treatment groups. • Ingestion of algal-derived DHA and EPA lowered triacylglycerol levels to a similar degree as the fish oil derived product.

Table 22. Corroborative non-infant Clinical Studies with Algal-Derived DHA-Rich Oil

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters
Mulder et al., 2014	Randomized double-blind, single center prospective study of term gestation single birth healthy infants born to women given either placebo or DHA	• Control: corn and soybean oil blended to reflect the dietary 18:2n-6 and 18:3n-3 ratio • Test group: 400 mg algal DHA	• No safety parameters reported • Infants born to mothers supplemented with DHA had decreased risk of lower language development assessed as words understood and produced at 14 month and word understood and sentences produced at 18 months.
Voigt et al., 2014	Randomized, double-blind placebo-controlled trial in children aged 3 – 10 with autism	• Control: 250 mg corn oil and 250 mg soybean oil • Test group: 200 mg DHA and 300 mg high oleic sunflower oil	• No significant differences in adverse effects were reported between control and test group • Dietary DHA supplementation did not improve autism symptoms
Capo et al., 2014b	Randomized, placebo-controlled trial in 15 Spanish male football players	• Control: Almond-based beverage with 0.8% olive oil • Test group: almond-based beverage with 0.2% DHA-rich oil and 0.6% olive oil (1.16 g DHA/day)	• No safety parameters reported
Capo et al., 2015	Randomized, placebo-controlled trial in 15 Spanish male football players	• Control: Almond-based beverage with 0.8% olive oil • Test group: almond-based beverage with 0.2% DHA-rich oil and 0.6% olive oil (1.16 g DHA/day)	• No safety parameters reported • Diet supplementation with DHA significantly increased the antioxidant protein expression after and acute exercise, and reduced the production of reactive oxygen species in peripheral blood mononuclear cells after acute exercise
Harris et al., 2015	Randomized double-blind placebo-controlled trial in 564 pregnant women, aged 18 – 40 years, and 505 woman and infant pairs	• Control: olive oil placebo • Test group 1: 300 mg DHA from *Schizochytrium* sp • Test group 2: 600 mg DHA from *Schizochytrium* sp.	• No safety parameters reported • Gestational length was significantly increased by 4 – 4.5 days in women supplemented with 600 mg DHA. • The rate of early preterm birth was lower in women who received DHA.
Ramakrishnan et al., 2015	Randomized, double-blind, placebo-controlled trial in offspring of women given DHA during the latter half of pregnancy.	• Control: mix of corn and soy oil • Test group: 400 mg/day algal DHA	• No safety parameters reported • Prenatal DHA supplementation in a population with low intakes of DHA had no effects on offspring development at 18 months of age

Table 22. Corroborative non-infant Clinical Studies with Algal-Derived DHA-Rich Oil

Reference	Study Design and Population	Treatment Groups	Outcomes and Safety Parameters
Scholtz et al., 2015	Randomized placebo-controlled trial in pregnant women at a mean 14.5 weeks of gestion	• Control: mix of corn and soybean oil • Test group: 600 mg algal DHA	• No safety parameters reported • Increasing DHA intake increased DHA as measured in red blood cells
Busquets-Cortes et al., 2016	Randomized, double blind trial in 23 male professional and federated Spanish football players	All subjects received a beverage containing almond, sucrose, water, lemon, cinnamon and vitamin E • Control: the beverage supplemented with 0.8% refined olive oil • Test group: the beverage supplemented with 0.2% DHA-rich oil and 0.6% olive oil	• No safety parameters reported • Subjects consuming the DHA supplemented beverage had increased DHA content in erythrocyte membranes,
Capo et al., 2016a	Randomized, placebo-controlled trial in 15 Spanish male football players	• Control: Almond-based beverage with 0.8% olive oil • Test group: almond-based beverage with 0.2% DHA-rich oil and 0.6% olive oil (1.16 g DHA/day)	• No safety parameters reported • DHA supplementation and exercise acted synergistically to increase plasma prostaglandin E2.
Capo et al., 2016b	Randomized, placebo-controlled trial in 15 Spanish male football players	• Control: Almond-based beverage with 0.8% olive oil • Test group: almond-based beverage with 0.2% DHA-rich oil and 0.6% olive oil (1.16 g DHA/day)	• No safety parameters reported • DHA supplementation attenuated cytokine production in an invitro assay of subject peripheral mononuclear cells.

Abbreviations: HDL: high density lipoprotein; LDL: low density lipoprotein; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid

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E. ALLERGENICITY

A search performed on January 16th, 2020, on PubMed using the term “Schizochytrium” and “allergy” yielded no results. No reports were found in the literature of allergic responses to any members of the family Chromista, including the thraustochytrids. Searching for “docosahexaenoic acid” and “allergy” found a single study on the possible effect of DHA supplementation during infancy for pre-term infants had on the incidence of allergy seven years later (Gunaratne et al., 2019). DHA supplementation in pre-term infants did not affect the allergy incidence later in life. No other reports describing an allergic reaction to DHA were found.

F. REGULATORY APPROVALS ACROSS THE WORLD

DHA-rich oil produced from *Schizochytrium* sp. has been listed as a novel food by Health Canada, the European Union, approved for use in infant formula by the Food Standards Agency of Australia and New Zealand (FSANZ, 2013), China's Ministry of Health, and Brazil's National Health Surveillance Agency (ANVISA), as described in GRN 553 stamped pg 33.

VII. SUPPORTING DATA AND INFORMATION

A. REFERENCES

All information included in the following list of references is generally available.

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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 CABIO Docosahexaenoic Acid (DHA)-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).

CABIO Biotech Co., Ltd. is proposing to market DHA-rich oil, produced by CABIO Biotech Co., Ltd. China, as a source of DHA-rich oil used in cow's milk and soy-based non-exempt infant formula and general foods. Consistent with other GRAS sources of DHA-rich oil (GRN 777, 776, 732, 731, 677, 553, 137), this ingredient, produced by the algae *Schizochytrium* CABIO-A-2, contains specifications that stipulate a minimum of 35% docosahexaenoic acid in the oil.

The safety evaluation considers the composition, intake, nutritional, microbiological, and toxicological properties of CABIO DHA-rich oil based on publicly available data from an equivalent DHA-rich oil (GRN 553). Corroborative safety data are described in GRNs 777, 776, 732, 731, 677, and 137, each of which received "no questions" letters from the United States Food and Drug Administration (FDA). The proposed use of CABIO DHA-rich oil as an ingredient in non-exempt term infant formula and general foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based upon the following:

- The DHA-rich oil produced by CABIO is compositionally equivalent to the DHA-rich oil described GRN 553 in terms of production, product specifications, and strain identity; therefore; information from GRN 553 are relied upon to establish safety of the CABIO DHA rich oil.

- The DHA product that is the subject of this GRAS determination is extracted and refined oil from the microalgae *Schizochytrium* CABIO-A-2. It is a mixture of fatty acids containing mostly polyunsaturated fatty acids in which the predominant fatty acid (>35%) is DHA. The DHA manufacturing process starts with fermentation followed by refining of the crude oil isolated from the fermentation process. The DHA-rich oil product is manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B).

- The proposed uses of the DHA-rich oil from *Schizochytrium* CABIO-A-2 are identical to the uses for other GRAS DHA-rich oils (in combination with ARA) in non-exempt (term) infant formulas (GRN 553) and general foods (GRN 137).

- An estimate of exposure to DHA from its addition to infant formula is based on a target DHA concentration of 0.5% of total fat for term infants. Assuming human infants consume about 100 to 120 kcal/kg body weight/day (term infants) of which fat comprises about 50% of those calories, this corresponds to intakes of DHA of 27 to 33 mg DHA/ kg body weight/day for term infants. This DHA intake estimate is in agreement with current recommendations for DHA consumption by pre-term and term infants of 18 to 60 mg/kg bw/day (Koletzko et al., 2014; GRN 776) The proposed use levels of the DHA-rich oil in general foods are expected to result in a maximum dietary exposure of less than 1.5 grams of DHA per day.

- DHA-rich oils from numerous sources are considered GRAS for use in food for human consumption and/or infant formula (GRNs 41, 137, 138, 319, 384, 469, 527, 553, 677, 731, 732, 776, 777 and 836). Sources of the DHA-rich algal oils include Schizochytrium sp., Crypthecodinium cohnii, Ulkenia sp. SAM2179. Other algal oil sources include Chlorella protothecoides strain S106, and Prototheca moriformis strain S2532. Furthermore, other sources of DHA such as tuna and other fish oil have received “no questions” from the FDA for addition to general food and infant formula.

- Numerous animal safety studies have been conducted over a period of more than a decade on DHA-rich oils derived from *Schizochytrium* sp. The results of unpublished and published subchronic toxicity studies conducted in rats show that administration of algal oil does not result in adverse effects at the highest levels tested (3279 mg/kg bw/day) (GRN 553).

    - Unpublished corroborative toxicity testing has been conducted with the proposed DHA-rich oil product from *Schizochytrium* CABIO-A-2 and includes acute and subchronic toxicity studies. In both studies, no evidence of toxicity was noted at the highest dose levels tested (10.2 g/kg/day).

Taken together, the available data from studies conducted on DHA-rich oils from *Schizochytrium sp.* establish a strong body of evidence for the safety of DHA-rich oil as a source of DHA for supplementation of non-exempt infant formula and general foods. Therefore, DHA-rich oil is 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: [redacted]
Date: March 25, 2020

A. Wallace Hayes, PhD, DABT, FATS, ERT
GRAS Expert Panel Member
Harvard School of Public Health

Signature: [redacted]
Date: March 25, 2020

Thomas E. Sox, PhD, JD
GRAS Expert Panel Member
Principal, Pondview Consulting LLC

Signature: [redacted]
Date: March 25, 2020

Claire Kruger, PhD, DABT
Scientific Advisor to the Panel
Spherix Consulting Group, Inc.

Signature: [redacted]
Date: March 25, 2020

From:	ckruger@spherixgroup.com
To:	Zhang, Janet
Cc:	"Kathy Brailer"; "Dietrich Conze"; "Jennifer Symonds"; "Fred Lozy"
Subject:	RE: GRAS Notice No. GRN 000934
Date:	Friday, August 7, 2020 12:42:18 PM
Attachments:	image001.png image002.jpg image003.jpg image004.jpg image005.jpg image006.jpg

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Dear Janet,
Yes, as discussed, we agree with the edits as stated below in your e-mail.

Best regards,
Claire

Claire Kruger, Ph.D., DABT, CFS
Managing Partner
Spherix Consulting Group
11821 Parklawn Drive
Suite 310
Rockville MD 20852
+1-301-775-9476

From: Zhang, Janet <Janet.Zhang@fda.hhs.gov>
Sent: Friday, August 7, 2020 10:23 AM
To: ckruger@spherixgroup.com
Cc: 'Kathy Brailer' <kbrailer@spherixgroup.com>; 'Dietrich Conze' <dconze@spherixgroup.com>; 'Jennifer Symonds' <jsymonds@spherixgroup.com>; 'Fred Lozy' <flozy@spherixgroup.com>
Subject: RE: GRAS Notice No. GRN 000934

Dear Claire,

Thank you and your team for joining the conference call yesterday.

As we discussed yesterday, below is the updated version regarding the intended uses in the 2nd paragraph of the filing letter of GRN 000934:

The subject of the notice is algal oil (>35% docosahexanoic acid (DHA)) derived from *Schizochytrium* sp. strain CABIO-A-2, for use as an ingredient, at up to 5.8 % (w/w) in food categories as listed in 21 CFR 184.1472(a)(3), and at up to 0.5% (w/w) of fatty acids as DHA in non-exempt infant formula for term infants.

Please send me your concurrence response. The email will be kept as an amendment for this notice.

Thanks,
Janet

Jianrong (Janet) Zhang, Ph.D.
FDA/OFVM/CFSAN/OFAS/DST
College Park, MD 20740
Phone: 240-402-1327
janet.zhang@fda.hhs.gov

From: ckruger@spherixgroup.com <ckruger@spherixgroup.com>
Sent: Friday, July 31, 2020 11:46 AM
To: Zhang, Janet <Janet.Zhang@fda.hhs.gov>
Cc: 'Kathy Brailer' <kbrailer@spherixgroup.com>; 'Dietrich Conze' <dconze@spherixgroup.com>; 'Jennifer Symonds' <jsymonds@spherixgroup.com>; 'Fred Lozy' <flozy@spherixgroup.com>
Subject: RE: GRAS Notice No. GRN 000934

Dear Janet:
We are available on Thursday August 6 at 1 – 2 pm for a call with your chemistry reviewers. Should we send a meeting invitation or will you send one to us?

Best regards,
Claire

Claire Kruger, Ph.D., DABT, CFS
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Rockville MD 20852
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From: Zhang, Janet <Janet.Zhang@fda.hhs.gov>
Sent: Friday, July 31, 2020 11:05 AM
To: ckruger@spherixgroup.com
Subject: RE: GRAS Notice No. GRN 000934

Good morning, Dr. Kruger. I'd like to schedule a conference call with you and our chemist reviewers to get clarification of the intended uses of GRN 000934. Will 11am to 12pm next Wed or 1 to 2pm

Thursday work for you? Please let me know your preference.

Best regards,
Janet

From: Zhang, Janet
Sent: Monday, July 20, 2020 3:28 PM
To: ckruger@spherixgroup.com
Subject: GRAS Notice No. GRN 000934

Dear Dr. Kruger, attached is the acknowledgement letter for GRAS Notice No. GRN 000934. Please let me know if you have any questions or concerns.

Thanks,
Janet

Jianrong (Janet) Zhang, Ph.D.
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