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

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
Managing Partner
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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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College Park, MD 20740
Phone: 240-402-1327
janet.zhang@fda.hhs.gov

Spherix Consulting Group

October 14, 2020

Jianrong (Janet) Zhang, Ph.D.
FDA/OFVM/CFSAN/OFAS/DST
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
US Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 000934

Dear Dr. Zhang:

In response to your email of September 30, 2020, following are our responses to your request for additional information regarding GRN 000934. Please note that upon further internal review of the GRAS notice, we found a typographical error on page 17, second paragraph, first sentence. The sentence should read:

> "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 0.084 g DHA-rich oil/kg body weight/day in infants."

Also, please correct the GRAS inventory listing for GRN 000934 to read:

> "GRN No. 934
> Algal oil (≥35% docosahexaenoic acid (DHA)) derived from *Schizochytrium sp.* strain CABIO-A-2"

FDA's questions outlined in your letter, dated September 30, 2020, are italicized and our responses are in plain text below.

1. *In Table 6, microbiological specifications and batch analysis data are presented. Due to the intended uses in infant formulas, we recommend you include a specification for Cronobacter sakazakii and provide batch analysis data to support manufacturing can meet the specification.*

   CABIO has updated their product specifications to now include *C. sakazakii* and batch data demonstrating compliance with this specification is provided. The text in Section 1. Product Specifications and Table 4 on Page 13 should now read:

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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. 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
Enterobacter sakazakii (Cronobacter sakazakii)	Absent in 333 g	ISO 22964	-	Absent	Absent	Absent

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. *Please confirm that the manufacturer continuously monitors the fermentation process for contaminants and quality control procedures are taken upon observation of contamination.*

The food ingredient is manufactured in a closed system. CABIO confirms that the fermentation process is monitored for the presence of microbial contaminants every 8 hours by both microscopy and by culturing a sample of the fermentation medium at 37ºC. If a contamination greater than $1x10^8$ CFU/mL is observed, the contaminated fermentation vessel is sterilized and CABIO disposes of the culture. The text on page 9, a. Quality Control of Production, has been updated to accurately represent the quality control procedures that monitor for contamination in the fermentation stage and the cleaning procedures undertaken in the event of a contamination. It shall now read:

"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. The fermentation process is monitored for contamination by sampling every 8 hours. Samples are examined by microscopy and cultured for 24-48 hours at 37°C. Should a contamination greater than $1x10^8$ CFU/mL be observed, the fermentation process is stopped, fermentation vessels are sterilized and the culture is re-started from a glycerol stock. 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."

3. *Please confirm that Schizochytrium sp. CABIO-A-2 is non-pathogenic and non-toxigenic.*

Schizochytrium sp. CABIO-A-2 is confirmed to be non-pathogenic and non-toxigenic. The lack of pathogenicity and toxigenicity for *Schizochytrium sp.* has been documented in GRNs 137, 553, 677, 731, 732, 776, 777, 836, 843, and 844. and there are no published reports of pathogenicity or toxigenicity associated with *Schizochytrium* strains used in the production of DHA algal oils.

4. *You state on Pg. 2 that because DHA-rich oil produced by CABIO is compositionally equivalent to DHA-rich oil described in GRN 553, you are relying upon data and information from GRN 533 for the safety conclusion of your DHA-rich oil. However, we note that there are some quantitative differences in several constituents (e.g., Palmitic acid, Oleic acid, and Eicosapentaenoic) in Table 16 of 30 between your oil and DHA-rich oil described in GRN 533. Please make a brief statement that any observed differences between the two oils are not expected to result in differences in their safety profile*.

The text on page 2 shall now read:

"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. Although there are small differences in the fatty acid profiles of the two oils, these fatty acids are normal components of the diet and the small quantitative differences in constituents such as Palmitic acid, Oleic acid, and Eicosapentaenoic acid will not affect the safety profile of CABIO DHA-rich oil."

5. *You reference the Appendix for the following: sequence alignments to demonstrate species homology between strain Schizochytrium CABIO-A-2 and strain Schizochytrium sp. ATCC 20888, information regarding Food Safety System Certification (FSSC) 22000 compliance and fermentation medium raw materials. However, an Appendix was not submitted with your notice. We request that you provide the referenced Appendix.*

The reference to the appendices was an error. These appendices contain confidential and proprietary manufacturing information and DNA sequences and will not be provided. The following changes remove the reference to the appendices that contain confidential information:

On page 7, last paragraph:

"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. 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."

On page 9, first paragraph of Section 1. Production of DHA-rich oil:

"CABIO maintains stocks of *Schizochytrium* CABIO-A-2 in glycerol at -80ºC 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. 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.

On page 12, Section 2. Raw Materials, Processing Aids, and Food Contact Substances, text and Table 3:

"Raw materials, processing aids and food contact substances used to manufacture DHA-rich oil are described below in Table 3. 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
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;.		

6. *In the notice, under the description of DHA-Rich Oil and product specifications (pages 5 and 13, respectively), you list the DHA level in the DHA-Rich Oil of least 35% or ≥35%. However, under the Basis of GRAS Determination (page 2) and Expert Panel Statement (page 71) , you list the level of DHA as >35%. Please provide detailed revisions to describe the specified minimum level of DHA consistently throughout the notice.*

   The text on page 2 shall now read:

   "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 text on page 71 shall now read:

"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)."

7. *On Page 25, Table 14 stated that the category was "As Described in GRN 137, stamped Page 27-28" and the table didn't include meat and poultry products. However, the pages in GRN 137 included both meat and poultry products. Please confirm whether or not the general food category in your notice includes any food products under USDA jurisdiction.*

   CABIO does not intend to add DHA-rich oil to meat products or poultry products. For clarity, the footnote text of Table 14 on page 25 shall now read:

   "As described in GRN 137, stamped pages 27-28, with the exception of meat and poultry products."

8. *On Page 1, Section D. TRADE SECRET OR CONFIDENTIAL INFORMATION, states that "Any trade secret or confidential information will be redacted at the time of notification to the U.S. Food and Drug Administration." Please confirm that the notice does not contain any trade secret or confidential information*.

   The text on page 1 shall now read:

   "This notification does not contain any trade secret or confidential information."

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

Sincerely,

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

References

Hammond BG, Mayhew DA, Kier LD, Mast RW, Sander WJ (2002). Safety assessment of DHA-rich microalgae from *Schizochytrium sp.* IV. Mutagenicity studies. Regul Toxicol Pharmacol 35(2, Part 1):255-265. DOI:10.1006/rtph.2002.1535.

Spherix Consulting Group

November 11, 2020

Jianrong (Janet) Zhang, Ph.D.
Food Technologist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 000934

Dear Dr. Zhang:

In response to your email of November 2, 2020, following is our response to your request for additional information regarding GRN 000934. Your request is in italicized text and our response is below in plain text:

In response to question 1, the product specifications include Cronobacter sakazakii and the citation for the method ISO 22964. However, the sample size you provided for your analyses is 333 g, while the cited method uses 10 g samples. Please address this discrepancy.

The *Cronobacter sakazakii* specification listed in GRN 000934 of absent in 333 g, was determined using an adapted ISO 22964 protocol. Although ISO 22964 specifies the use of 10 g samples, CABIO performed ISO 22964 using 333 g sample in an accordingly adjusted medium volume for analysis (2997 mL of pre-enrichment medium) because the larger sample size is more stringent and therefore more representative of the quality of the batch than 10 g. Use of the larger sample size also meets the standards required by CABIO's international clients.

To demonstrate that the DHA-rich oil produced by CABIO also does not contain *C. sakazakii* following the testing conditions specified by ISO 22964, CABIO repeated the analysis on the three batches of oil using 10 g of sample. *Cronobacter sakazakii* was absent is all three batches. Additionally, CABIO has updated their specifications to reflect the use of a 10 g sample, which is now consistent with ISO 22964. Table 4 on Page 13 shall now read:

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301-557-0375; info@spherixgroup.com
http://spherixgroup.html

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
Enterobacter sakazakii (Cronobacter sakazakii)	Absent in 10 g	ISO 22964	-	Absent	Absent	Absent

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

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

Sincerely,

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

Spherix Consulting Group

March 2, 2021

Jianrong (Janet) Zhang, Ph.D.
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US Food and Drug Administration
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RE: Questions Regarding GRN 000934

Dear Dr. Zhang:

In response to your email of February 19, 2021, following are our responses to your request for additional information regarding GRN 000934. FDA's questions outlined in your email are italicized and our responses are in plain text below.

1. *In the Notice, you describe algal oil (≥35 % docosahexaenoic acid (DHA)) derived from Schizochytrium sp. strain CABIO-A-2 (algal oil (≥35 %DHA)) as the following:*

   "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."

   On page 2 of the notice, you also state that algal oil (≥35 % DHA) is compositionally equivalent to algal oil described in GRN 553 in terms of production, product specifications and strain identity. While algal oil (≥35 % DHA) does meet the product specifications of the algal oil in GRN 553, we note differences in the product strain and manufacture of the two ingredients. Due to these differences, we request that you provide information on the identity and composition of algal oil (≥35 % DHA). Specifically, please provide information on the color and tri-, di- and monoglyceride levels. Please base information on the identity of algal oil (≥35 % DHA) solely on your ingredient and independent of the algal oil notified in GRN 553.

   Batch data for color, as well as tri-, di-, and monoacylglycerol content of CABIO DHA-rich oil is presented in Table 1. Color ranges from 2.3 to 7.7 yellow and 0.1 to 0.3 red. Triacylglycerol accounts for approximately 94.98% of the total fat component of CABIO's DHA-rich oil.

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Table 1. Color and Tri-, Di-, and Monoacylglycerol Content of Three Non-consecutive Batches of CABIO DHA-rich Oil					
Parameter	**Method**	**CABIO DHA-rich Oil Batch No.**			
		20052012	**20060212**	**21012912**	**Average ± St. Dev.**
Color (Y/R)	GB 26400	Y=3.2, R=0.2	Y=7.7, R=0.3	Y=2.3, R=0.1	-
Triacylglycerol (%)	AOAC Cd 11c-93	94.58	95.16	95.21	94.98 ± 0.35
Diacylglycerol (%)	AOAC Cd 11c-93	3.21	2.97	3.04	3.07 ± 0.12
Monoacylglycerol (%)	AOAC Cd 11c-93	0.48	0.35	0.29	0.37 ± 0.10
Glycerol (%)	AOAC Cd 11c-93	<1.0	<1.0	<1.0	<1.0

2. *On page 9, under Production Process (Fermentation), you state that fermentation begins with the inoculation of culture medium with a glycerol stock of Schizochytrium CABIO-A-2. In an amendment to the notice, you state that the fermentation process is continuously monitored for the presence of microbial contaminants. Please confirm that algal oil (≥35 % DHA) is produced from a pure culture of Schizochytrium CABIO-A-2.*

   The DHA-rich oil produced by CABIO is produced from a pure culture of *Schizochytrium* CABIO-A-2.

3. *Under the same section noted above, you state that after fermentation is complete a protease is added to extract the crude oil from the biomass. In addition, in Table 3 (Compliance of Processing Aids and Raw Materials with US Laws and Regulations) you describe the protease as a serine alkaline protease. We request that you:*

   a. *Please confirm that the introduced protease is from a non-toxigenic and non-pathogenic source.*

   b. *Please confirm that the protease has the appropriate regulatory status for the specific use.*

   c. *Please indicate if the protease is expected to be present in the final product; if not, provide a narrative on its removal.*

   The introduced protease is an alkaline serine endopeptidase isolated from a strain of *Bacillus longiformis* (IUB# 3.4.21.62), which is non-toxigenic and non-pathogenic. The protease meets Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) specifications for enzyme preparations used in food and is GRAS. The protease is heat inactivated during the production of the crude oil. Due to the harsh conditions of the oil refining process and multiple filtration steps to remove impurities, the protease is not expected to be present in the finished product.

4. *On page 17 of the notice, you provide a specification for EPA as <10% while your batch data is well below that value. Please provide a justification or consider lowering the specification for the level of EPA.*

CABIO will lower the specification for EPA in their oil to 3%.

5. *On page 21 of the notice, you address the stability of algal oil (≥35 % DHA). You provide preliminary stability data for algal oil (≥35 % DHA) stored at -20 °C for 24 months in vacuum sealed aluminum foil bags and noted that stability testing was underway. Please comment on the current status of your stability studies and whether any additional data supports the proposed shelf life of 24 months.*

Since GRN 934 was submitted, CABIO has collected additional timepoints to support the shelf life of DHA-rich oil. Twenty-four-month timepoints have been collected for both Lots 17051512 and 18022612, as well as an 18-month timepoint for Lot 18022612. The new data demonstrates that the DHA content and peroxide and anisidine values still comply with the product specifications, supporting the proposed shelf life of 24 months (Table 2).

Table2. Stability of DHA-Rich Oil at -20°C for 24 Months

Lot Number	Specifications	Time (months)				
		0	6	12	18	24
DHA (%FAME)						
17010212	Min. 32.0	42.00	41.76	40.98	42.18	41.41
17051512		39.28	38.59	38.07	39.10	39.56*
18022612		36.20	35.59	36.92	36.21*	35.79*
Peroxide Value (meq/kg)						
17010212	Max. 5	0.03	1.83	1.05	1.16	1.18
17051512		0.03	1.14	1.22	2.20	3.85*
18022612		0.03	1.54	1.03	3.09*	2.94*
Anisidine value (AV)						
17010212	Max. 20	5	5	6	6	6
17051512		4	5	5	5	6*
18022612		5	6	6	6*	6*
*Stability data points completed since the submission of GRN 934.						

6. *On page 23 of the notice, you state that the dietary exposure to algal oil (≥35 % DHA) will be the same as that described in GRN 553 and other GRNs, for use in infant formula, and in GRN 137 for use in selected general foods. Please indicate if the intended uses of algal oil (≥35 % DHA) are substitutional for other DHA-containing oils on the market.*

The intended uses of CABIO's DHA-rich are substitutional for other DHA-containing oils on the market.

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

Sincerely,

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

From: ckruger@spherixgroup.com
To: Zhang, Janet
Cc: "Dietrich Conze"; "Jennifer Symonds"; "Kathy Brailer"
Subject: [EXTERNAL] RE: GRN 000934
Date: Wednesday, March 10, 2021 1:51:12 PM
Attachments: image001.png

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Dear Janet:
With respect to your question on the identity (≥35 % docosahexaenoic acid (DHA)) of algal oil in GRN 000934, color is identified by a visual check using the Chinese compendial method GB 26400 which specifies it is light yellow to orange yellow; this product is not intended for use as a color additive.

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: Wednesday, March 10, 2021 9:38 AM
To: ckruger@spherixgroup.com
Subject: GRN 000934

Good morning Dr Kruger,

In response to our request on the identity (≥35 % docosahexaenoic acid (DHA)) algal oil in GRN 000934, you submitted batch data for color. Please provide a statement on the color of the ingredient. In addition, please indicate whether the method used to identify is validated.

Best regards,
Janet

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

janet.zhang@fda.hhs.gov

Spherix Consulting Group

June 4, 2021

Jianrong (Janet) Zhang, Ph.D.
FDA/OFVM/CFSAN/OFAS/DST
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
US Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 000934

Dear Dr. Zhang:

In response to your email of May 13, 2021, following are our responses to your request for additional information regarding GRN 000934. FDA's questions outlined in your email are *italicized* and our responses are in plain text below.

a. *In your notice on page 10, you state that rosemary extract is used as an antioxidant during the refining process for algal oil (≧35% DHA). In Table 3 of the notice, you list rosemary extract as a processing aid/raw material and note that it is FCC compliant. Please provide information that substantiates that rosemary extract is authorized for use as an antioxidant in infant formula.*

CABIO has clarified that rosemary extract is not used in the production of DHA-rich oil for infant formula. The text in Section c. Oil Refining (page 10) and Table 3 on Page 12 should now read:

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 as an antioxidant, 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.

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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
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	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.		

b. *In Table 4 of the notice, you list the product specifications and batch data for algal oil (≧35% DHA) and list the specification for trans fatty acid as ≤ 1.0%. Please clarify if the percent fatty acids are based on weight percent of ingredient or based on the percent of total fatty acids.*

The trans fatty acid specification is set at ≤1.0% of the weight percentage of the ingredient, not the percent of total fatty acids.

c. On page 15 of the notice, you state that the sterol profile of algal oil (≧35% DHA) is similar to other DHA-rich oils. However, in Table 7 we note the high levels of unidentified sterols. Please provide a narrative to explain the components of these unidentifiable sterols are and discuss their levels in algal oil (≧35% DHA) relative to other DHA-rich oils.

Upon further internal review of the GRAS notice, we found an error in the footnote for Table 7, page 15. The correct method for this analysis is NMKL 198:2014. The table shall now read:

Table 7. Sterol content in DHA-rich oil

	Batch No.		
Sterol (mg/100 g)	**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: NMKL 198:2014 Limit of quantitation: 1 mg/100 g			

As stated in the corrected Table 7, the method used to quantify the sterols in the subject of GRN 934 is NMKL 198:2014, which defines "unidentified sterols" as those that elute from the gas chromatography column between stigmasterol and sitosterol. The method also states that the components eluting between stigmasterol and sitosterol are most likely delta 7-campesterol, delta 5,23-stigmastadienol, and clerosterol, which have all been detected in other DHA-rich oils that have received "no questions" from the FDA regarding their GRAS status (GRN 913, GRN 862, GRN 677, GRN 553). Delta-4 sitosterol also elutes between stigmasterol and sitosterol (NMKL 198:2014); however, it does not occur naturally and has not been reported in any of the DHA-rich oils that are the subjects of GRAS Notices. Importantly, NMKL 198:2014 is the same method used to quantify the sterols in the DHA-rich oil that is the subject of GRN 933, which received a "no questions" letter from FDA. A comparison of the sterols in the subject of this GRAS Notice and the subject of GRN 933 shows that the sterol profiles are similar, with the most abundant sterols being cholesterol, unidentified sterols, stigmasterol, and sitosterol (Table 1). Additionally, as stated in Chapter II, Section C.3, the *actin* and *18S* gene sequences of the strain used to manufacture the subject of this GRAS Notice are >99%

identical to the *actin* and *18S* gene sequences of the type strain *Schizochytrium* sp. ATCC 20888, which is used to manufacture the DHA-rich oil that is the subject of GRN 137. The strain of *Schizochytrium* sp. used produce the oil that is the subject of GRN 933 was also confirmed to be *Schizochytrium* sp. by morphology and *18S* gene sequencing. It is therefore likely that the unidentified sterols in the DHA-rich oil that is the subject of GRN 934 are equivalent to those detected in GRN 933 because both production organisms used are strains of *Schizochytrium* sp. and the oils are manufactured using similar processes. The sterols present in the subject of this GRAS notice, as well as those of the DHA-rich oils that are the subjects of GRNs that received "no questions" letters from the FDA, are ubiquitous in food, commonly present in sources of fatty acids in infant formula, including corn, palm, safflower, soybean, and sunflower oil, and do not pose a safety concern. Therefore, CABIO concludes that the unidentified sterols in the DHA-rich oil described in GRN 934 do not pose a safety concern.

Table 1. Percent Sterols in GRN 933 and GRN 934

Sterols*	GRN 934 (current notice, n=3 batches)	GRN 933 (n=5 batches)
24-Methylenecycloartanol	0.24 ± 0.13%	0.64 ± 0.31%
Brassicasterol	5.20 ± 2.81%	2.67 ± 0.45%
Campestanol	0.21 ± 0.03%	0.39 ± 0.33%
Campesterol	1.60 ± 0.29%	1.89 ± 0.63%
Cholesterol	32.70 ± 3.56%	36.73 ± 1.29%
Citrostadienol	0.25 ± 0.08%	0.33 ± 0.01%
Cycloartenol	0.56 ± 0.13%	0.54 ± 0.15%
Delta-5,24-stigmastadienol	1.29 ± 0.08%	1.44 ± 0.30%
Delta-7-avenasterol	0.64 ± 0.25%	0.87 ± 0.56%
Delta-7-stigmastenol	3.53 ± 0.59%	4.25 ± 0.58%
Sitostanol + delta-5-avenasterol	2.31 ± 1.25%	1.50 ± 0.30%
Sitosterol	6.13 ± 0.31%	9.75 ± 2.06%
Stigmasterol	11.02 ± 3.93%	4.22 ± 0.91%
Unidentified Sterols	34.32 ± 4.51%	34.95 ± 2.51%
Calculated total sterols in %DHA-rich oil**	1.11 ± 0.14	0.50 ± 0.19

*Sterols quantified by Eurofins using the NMKL 198:2014 method for both GRNs 933 and 934.
**To assess the similarity in unidentified sterol content of GRN 933 and GRN 934, a percent sterol calculation was performed based on the total of quantified sterol residues, including unidentified sterols. It is notable that the certificates of analysis issued by Eurofins did not include unidentified sterols in the reported total sterol content in the analysis performed on both GRN 934 and GRN 933's DHA-rich oils. The total amount of sterols in GRN 933 and GRN 934 were calculated by adding each sterol quantity, including unidentified sterols, based on the information provided in the certificates of analysis. The sterol quantities were originally provided in mg sterol residue/100 g DHA-rich oil. These values were converted to percent total sterols by dividing the reported sterol quantity by the total calculated sterols and multiplying by 100%. The means and standard deviations were calculated from the converted percent sterol values, and the number of batches included in the analysis are indicated in the header of the columns.

d. For clarification in the method of manufacture description, is the hexane used in extraction or degumming of ARA oil?

To clarify your question, the subject of this notice is a DHA-rich oil.

Hexane is added during extraction of the DHA-rich oil.

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

Sincerely,

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