ORIGINAL SUBMISSION

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October 31, 2016

Dr. Antonia Mattia
Director, Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Subject: GRAS Notification – DHA Algal Oil

Dear Dr. Mattia:

On behalf of Mara Renewables Corporation, ToxStrategies, Inc. (its agent) is submitting, for FDA review, a copy of the GRAS notification as required. The enclosed document provides notice of a claim that the food ingredient, DHA algal oil, described in the enclosed notification is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to infant formula.

If you have any questions or require additional information, please do not hesitate to contact me at 630-352-0303, or dschmitt@toxstrategies.com.

Sincerely,

(b) (6)

Donald F. Schmitt, M.P.H.
Senior Managing Scientist

GRAS Determination of DHA Algal Oil for Use in Infant Formula

OCTOBER 7, 2016

Tox Strategies

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GRAS Determination of DHA Algal Oil for Use in Infant Formula

SUBMITTED BY:

Mara Renewables Corporation
101 Research Drive
Dartmouth NS B2Y 4T6, Canada

SUBMITTED TO:

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5100 Paint Branch Parkway
College Park MD 20740-3835

CONTACT FOR TECHNICAL OR OTHER INFORMATION

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931 W. 75th St., Suite 137, PMB 263
Naperville, IL 60565

OCTOBER 7, 2016

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List of Acronyms

ARA	arachidonic acid
ARASCO	arachidonic acid single cell oil
ATCC	American Type Culture Collection
BSID	Bayley Scales of Infant and Toddler Development
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CFU	colony-forming unit
cGMP	current Good Manufacturing Practices
COA	Certificate of Analysis
DHA	docosahexaenoic acid
DHASCO	docosahexaenoic acid single cell oil
EC	European Commission
EDI	estimated daily intake
EFSA	European Food Safety Authority
EGFR	epidermal growth factor receptor
EPA	eicosapentaenoic acid
EU	European Union
FAO	Food and Agricultural Organization
FCC	Food Chemicals Codex
FDA	Food and Drug Administration
FR	Federal Register
GMO	genetically modified organism
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
GRN	GRAS Notification
HACCP	hazard analysis and critical control point
IOM	Institute of Medicine
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KOH	potassium hydroxide
LCPUFA	long-chain polyunsaturated fatty acids
LD50	median lethal dose
meq	milliequivalents
mbar	millibar
mM	millimolar
NA	not available
NaCl	sodium chloride
NOAEL	no-observed-adverse-effect level
ONC	Ocean Nutrition Canada Limited
PBS	phosphate-buffered saline
PUFA	polyunsaturated fatty acids
QC	quality control
RBD	refined, bleached, deodorized
SSU-rDNA	small subunit ribosomal DNA
WHO	World Health Organization

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wt%	weight percent

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§ 170.225 Part 1, GRAS Notice: Signed Statements and Certification

(1) GRAS Notice Submission

Mara Renewables Corporation (Mara), through its agent ToxStrategies, Inc., hereby notifies the U.S. Food and Drug Administration (FDA) of the submission of a Generally Recognized as Safe (GRAS) notice for docosahexaenoic acid (DHA) algal oil.

(2) Name and Address

Mara Renewables Corporation
101 Research Drive
Dartmouth NS B2Y 4T6, Canada

(3) Name of Notified Substance

The name of the substance that is the subject of this GRAS determination is DHA algal oil from the wild-type heterotrophic microalgae *Schizochytrium* sp. ONC-T18 (hereinafter referred to as T18).

(4) Intended Use in Food

DHA algal oil is intended for use as a direct ingredient in exempt (pre-term) and non-exempt (term) infant formula (ages from birth to 12 months), in accordance with current good manufacturing practices (cGMP), and in combination with a source of arachidonic acid (ARA). The ratio of DHA to ARA would range from 1:1 to 1:2. The intended use level is similar to all other approved uses for incorporation of DHA in infant formula.

(5) Statutory Basis for GRAS Determination

Mara, through its agent ToxStrategies, Inc., hereby notifies FDA of the submission of a GRAS notice for DHA algal oil, meeting the specifications described herein, has been determined to be GRAS through scientific procedures in accordance with § 170.30(a) and (b).

(6) Premarket Approval Statement

Mara further asserts that the use of DHA algal oil in infant formula, as described below, is exempt from pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act based on a conclusion that the notified substance is GRAS under the conditions of its intended use.

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(7) Availability of Information

The data and information that serve as the basis for this GRAS determination, as well any information that has become available since the GRAS determination, will be sent to the FDA upon request, or are available for the FDA's review and copying during customary business hours from ToxStrategies, Inc., Naperville, IL.

(8) Data and Information Confidentiality Statement

None of the data and information in the GRAS notice is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552, with the exception of non-safety related confidential business-related information found in the certificates of analysis (i.e., the name and address of the original requestor of the analyses) on pages 85, 89, 92, 96, 99, and 103.

(9) GRAS Notice Certification

To the best of our knowledge, the GRAS notice is a complete, representative, and balanced submission. Mara is not aware of any information that would be inconsistent with a finding that the proposed use of DHA-rich algal oil in infant formula (pre-term and term infants) meeting appropriate specifications, and used according to cGMP, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

(10) Name/Position of Notifier

(b) (6)

10/31/16

Donald F. Schmitt, M.P.H.
Senior Managing Scientist
ToxStrategies, Inc.
Agent for Mara

Date

(11) FSIS Statement

Not applicable.

§ 170.230 Part 2, Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

Identity

The DHA product that is the subject of this GRAS determination is a yellow to orange-colored semi-solid to liquid oil that is extracted and refined from the wild-type heterotrophic microalgae *Schizochytrium* sp. ONC-T18 (hereinafter referred to as T18). It is a mixture of triglycerides containing mostly polyunsaturated fatty acids (PUFA) in which the predominant fatty acid (>35%) is DHA.

Empirical Formula and Chemical Structure of DHA

The empirical formula for DHA is $C_{22}H_{32}O_2$. The systematic name is 4,7,10,13,16,19-docosahexaenoic acid, and is often written as 22:6n-3 where the numbers indicate the number of carbon atoms in the molecule (22), the number of double bonds (6), and the number of carbon atoms from the methyl terminus to the first double bond (3). The molecular weight of DHA is 328.488 g/mol. The structural formula for DHA is represented below in Figure 1.

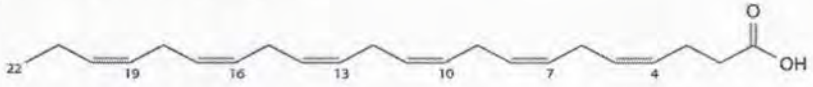

Figure 1. Structural formula of DHA

Common or Chemical Names

The preparation under consideration is referred to as: DHA algal oil, DHA-rich algal oil, *Schizochytrium* sp. oil, omega-3-rich algal oil, omega-3 algal oil, algal oil. CAS No. 68424-59-9; glycerides, C14-C22 and C16-C22 unsaturated.

Characterization of Strain

Schizochytrium sp. are part of the human food chain and they are consumed as a function of eating mussels and clams as well as other marine organisms in general (Hammond et al., 2002). The *Schizochytrium* strain used is naturally occurring and not a product of genetic engineering. The micro-algal family *Thraustochytriaceae* has historically comprised seven genera, *Japanochytrium, Schizochytrium, Ulkenia, Althornia, Diplophrys, Aplanochytrium,* and *Thraustochytrium*, all of which are referred to as thraustochytrids. Under this classification scheme, strain T18 had previously been assigned to the genus *Thraustochytrium* (Burja et al., 2006). The genera *Thraustochytrium, Schizochytrium and Ulkenia* (oils from the latter two are the subject of previous authorizations under EU novel food regulations and are GRAS (FDA, 2010, 2014a)) comprise marine protists commonly found in marine and estuarine environments.

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The taxonomic structure of the family *Thraustochytriaceae* has been the subject of discussion and subsequent redistribution of some of the component organisms into a broader suite of genera, including members of the genus *Schizochytrium* (Yokoyama and Honda, 2007) and the genus *Ulkenia* (Yokoyama et al., 2007). As reported in their 2011 substantial equivalence submission to the UK Food Standards Agency (ONC, 2011), the former Ocean Nutrition Canada Limited (ONC) commissioned an expert review of the relationship between its thraustochytrid strain T18 and *Schizochytrium* sp. ATCC 20888, the parent wild-type strain that was the basis of Commission authorization decision 2003/427/EC. On the basis of morphological characteristics, pigment and fatty acid profiles, and a comparison of small subunit ribosomal DNA (SSU-rDNA) sequences of the two organisms, it was concluded that the two strains were closely related, and the strain T18 was more appropriately considered as falling within the genus *Schizochytrium sensu lato*. In 2012, the UK Food Standards Agency concluded that ONC's algal oil met the criteria for equivalence as defined in Article 3(4) of regulation (EC) 258/97 and that the *Schizochytrium* strain used by ONC was closely related to the organism used in the production of a Martek algal oil (Food Standards Agency, 2012).

The possible presence of microalgae toxins produced by *Schizochytrium* sp. has been previously addressed as part of the substantial equivalence submission referenced above and in GRAS Notification (GRN) No. 553 (FDA, 2014a). Toxin production is unlikely since there are no known reports of toxin production by thraustochytrids, of which *Schizochytrium* is a member (ONC, 2011; Hammond et al., 2002). In addition, T18 oil and algal biomass were screened for the presence of toxins including domoic acid, gymnodimine, desmethyl spirolide C, azaspiracid-1, azaspiracid-2, azaspiracid-3, pectenotoxin-2, okadaic acid, dinophysistoxin-1, dinophysistoxin-2, yessotoxin, prymnesin-1, and prymnesin-2, and none were detected (ONC, 2011). The analytical report for algal toxins can be found in Appendix A.

Manufacturing Process

The following are descriptions of the processes used to manufacture the crude algal oil and then refine the DHA algal oil isolated from the fermentation process (see Figures 2 and 3). The process steps employed to refine the crude algal oil are similar to what is practiced in the refining of vegetable oils.

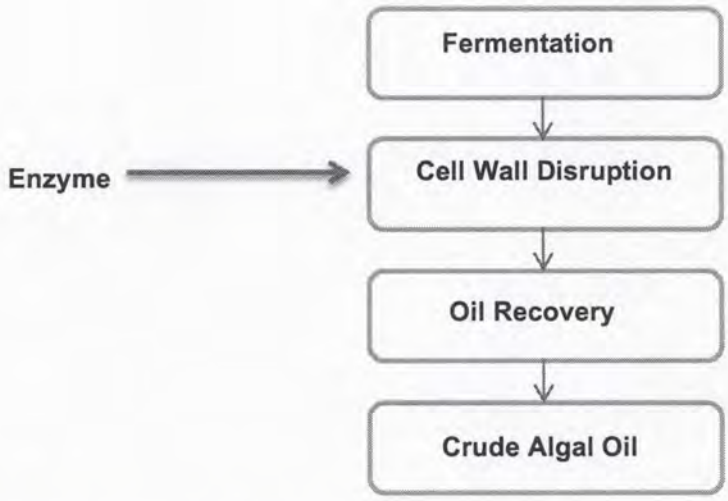

Figure 2. Crude DHA algal oil production

An oil rich in PUFA is produced by a heterotrophic fermentation process with a single cell marine microalgae of the genus *Schizochytrium*, in particular, T18. The fermentation process uses a medium containing carbon and nitrogen sources, bulk and trace mineral nutrients, and vitamins (see Table 1). The microorganism T18 is maintained on nutrient agar plate before production. Following inoculation of the microorganism into a shake flask, the cultivation process is scaled up through multiple stages of transfers, and finally into the production fermentation vessel. All vessels, pipelines, and fermentation media are subjected to a rigorous, timed, and controlled sterilization process prior to the transfer of the microorganism. The fermentation is carried out under axenic conditions (i.e., only one organism present, T18). During the fermentation process, more sterile carbon substrate (i.e., dextrose) is added to the fermentor to allow higher cell growth and more oil synthesis. Operating parameters such as temperature, pH, aeration, and agitation are controlled throughout the process to ensure that results, in terms of cell growth, oil synthesis, and the oil's fatty acid profile, are reproducible. The vessel is operated under positive pressure to prevent any contamination by foreign organisms.

Table 1. Fermentation Medium Ingredients

Ingredient	CFR Citation
Water	--
Dextrose or Glucose	21 CFR § 184.1857, 184.1865, 184.1866
Soy peptone or yeast extract	21 CFR § 184.1553; 21 CFR § 184.1983
Ammonium sulfate	21 CFR § 184.1143
Ammonium hydroxide	21 CFR § 184.1139
Sodium chloride	21 CFR § 182.1
Magnesium sulfate heptahydrate	21 CFR § 184.1443
Potassium phosphate monobasic	21 CFR § 175.105

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Potassium phosphate dibasic	21 CFR § 182.6285
Ferric chloride	21 CFR § 184.1297
Calcium chloride	21 CFR § 184.1193
Trace element solution	
Copper sulfate	21 CFR § 184.1261
Sodium molybdate	Similar to GRN 384 (FDA no questions letter) (see GRN 553, 2014)
Zinc sulfate	21 CFR § 182.8997
Cobalt (II) chloride	--
Manganese chloride	21 CFR § 184.1446
Nickel sulfate	21 CFR § 184.1537
Vitamins	
Vitamin B12	21 CFR § 184.1945
Biotin	21 CFR § 182.8159
Thiamine hydrochloride	21 CFR § 184.1875
Processing aids	
Sodium hydroxide solution	21 CFR § 184.1763
Silicone- or Vegetable oil-based antifoam	21 CFR § 173.340
Phosphoric acid	21 CFR § 182.1073
Citric acid	21 CFR § 184.1033
Feeding medium	
Dextrose syrup	21 CFR § 184.1866

Once fermentation is complete (i.e., as determined by carbon usage, cell growth, oil synthesis activity, and oil fatty acid profile), the crude oil that accumulates intracellularly is recovered from the fermentation broth via an aqueous extraction process. To release the oil from the cells, the cell wall requires disruption. In the cell wall disruption process, the fermentation broth is pH adjusted with sodium hydroxide and hydrolyzed enzymatically. As a result, no intact algae remain in the oil. The oil is then recovered from the hydrolyzed biomass. In the oil recovery process, the hydrolyzed biomass can be treated and centrifuged to yield the crude algal oil. At each step after cell wall disruption, exposure to air is minimized. Antioxidants (e.g., mixed tocopherols; CAS No. 1406-18-4) can be added. The manufacturing process is represented schematically in Figure 2 and is essentially the same as that described for the production of the currently authorized oil from *Schizochytrium* sp. (DHA-B) (FDA, 2014a). Figure 3 presents the subsequent DHA algal oil refining process.

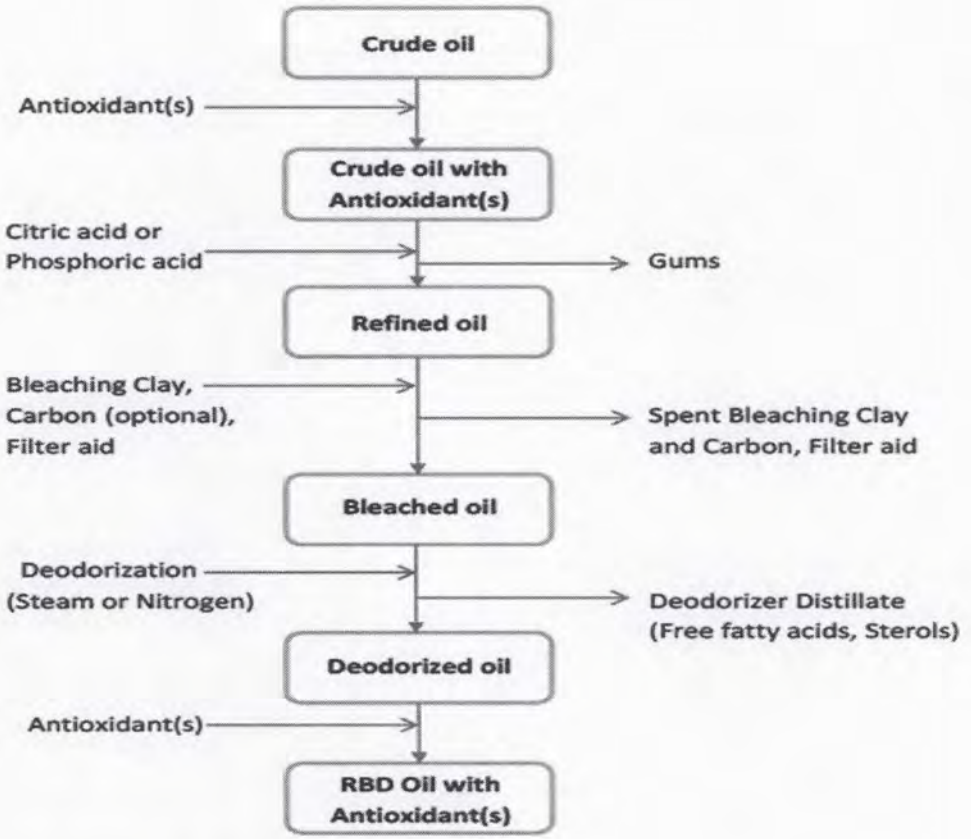

Figure 3. DHA algal oil refining process

An additional optional step that may be employed prior to any of the above steps is fractionation, also known as winterization, in which the semi-solid crude algal oil is cooled and centrifuged or filtered to obtain a crude algal oil that flows easily at room temperature. Winterization can be performed on the crude oil or subsequent to any of the other steps (e.g., after refining, bleaching, deodorization). The resultant fractionated/winterized crude algal oil is a clear liquid at room temperature. Process conditions of the other steps shown in the above flow diagram do not change. Optional steps described below are customer-driven and conducted at a customer's request. The steps in the algal oil refining process are described in more detail in the sections that follow.

Degumming (Optional)

Most crude oils isolated from natural sources contain gums, which after separation from the oil, primarily consist of phospholipids, some entrained oil, traces of soluble sugars, and solid particles. Some of the phospholipids become hydrated and oil-insoluble.

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Hydrating the gums and removing the hydrated gums from the oil prevents the formation of gum deposits in downstream processes. Water degumming is used to remove phosphatides and water-soluble components from the oil. Analysis of the crude algal oil has shown that almost all of the fatty acids are in the form of triglycerides and very little in the form of phospholipids, thus making the degumming step not essential. If the degumming step is skipped, the small amounts of phospholipids present in the crude oil can be removed in the subsequent bleaching step.

If a degumming step is desired, a water degumming process is employed. Crude algal oil is treated with 250 - 2000 ppm of phosphoric acid or citric acid at 60 - 90° C with vigorous stirring, and then gently stirred for a period of 10 - 90 minutes. Water (1 - 5% (w/w)) is added at 60 - 70° C and vigorously stirred. The oil is then gently stirred for 15 - 60 minutes to aid in the hydration of the phospholipids present. An aqueous phase is formed consisting of an emulsion of hydrated phospholipids and entrained oil. The phases are separated from each other by settling and filtration, or by centrifugation, yielding a stream of acid degummed oil and a stream of wet gums. While there is variability among different refining facilities, as noted above, Mara's oils are consistently produced to a set specification as outlined in Table 3.

Bleaching

Bleaching of the algal oil following degumming, is the step of the refining process which removes impurities that adversely affect the appearance, stability, and flavor of the oil. As depicted in Figure 3, the bleaching step is preceded by the degumming and neutralization process, and removes specific impurities that are not effectively removed during degumming. Bleaching effectively removes some of the color, residual soaps and gums, trace metals, and oxidation products. It also has an indirect impact on the color of the deodorized oil. Optionally, carbon may be used along with bleaching clay to improve the quality of the bleached oil, including the color.

The efficiency of bleaching is affected by moisture level, temperature, contact time, vacuum, oil quality, amount and characteristics of the adsorbent, and the type of equipment employed. Bleaching of the degummed algal oil is performed in a batch process. The amount of bleaching clay added depends on the specifications of the bleached oil, such as the residual phosphorus content, fatty acid content, and low soap content. About 0.5 - 5% bleaching clay is used for bleaching the degummed oil. Typically, the oil is vacuum-dried prior to bleaching, and has a moisture content of <0.5%. The operating temperature is from 90 - 125° C, and pressure is between 50 to 125 mm Hg (absolute). The total time the bleaching clay is in contact with the oil typically ranges from 15 minutes to 1 hour. The clay is then separated from the oil by filtration, often with the help of a filter aid, such as diatomaceous earth.

Deodorization

The main purpose of deodorization is to remove oil-derived compounds that cause off-flavors, but the process also removes free fatty acids, tocopherols, squalene, and sterols.

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In addition, other volatile impurities that have undesired off-flavors are removed. The oil also undergoes heat bleaching, where thermal destruction of flavor precursors and certain colored pigments, such as carotenoids, occurs and the oil becomes lighter in color. Deodorization is performed under vacuum to aid in the stripping of specific compounds, and protects the oil from oxidation. Although nitrogen can be used as a stripping agent, superheated steam is frequently used.

The deodorization process is fully defined by temperature, time, pressure, and amount of stripping steam. Deodorization on a commercial scale is a multi-step process comprising de-aeration, multi-stage heating, deodorization-de-acidification, and multi-stage cooling of the oil. The oil after bleaching is de-aerated prior to being heated to deodorizing temperatures in order to avoid oxidation and polymerization. De-aeration can be accomplished in a separate vessel connected to the vacuum system (around 50 mbar), or at even lower pressure in the deodorizer. Sparge steam may be used to improve de-aeration.

Deodorization may be performed either in a batch deodorizer, a semi-continuous system, or a continuous system. Stripping efficiency is superior in the continuous system, which has a column filled with structured packing of a high surface area. Counter-current contact of oil with the stripping steam over the structured packing provides efficient stripping in a short contact time. Various configurations of deodorizers can be used (horizontal or vertical vessels, tray-type, or packed columns). Antioxidants such as mixed tocopherols, ascorbyl palmitate, or other safe and suitable antioxidants are again added, as necessary. In addition, non-genetically modified organism (GMO) sunflower oil can be added as an option in order to standardize the oil for DHA content.

Reagents/processing aids employed in the extraction/refining process are listed in Table 2. The DHA-rich algal oil is manufactured in accordance with hazard analysis critical control point (HACCP) and cGMP, including quality control (QC) checks at every stage of the production process. All steps in the manufacturing process are conducted under conditions that minimize the risk of contamination with foreign materials.

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Table 2. Reagents/processing aids

Reagent/Processing Aid	CAS No.	CFR Citation
Phosphoric acid	7664-38-2	21 CFR § 182.1073
Citric acid	77-92-9	21 CFR § 184.1033
Clay (bleaching)	68515-07-1	21 CFR § 184.1155
Nitrogen	7727-37-9	21 CFR § 184.1540
Alcalase*	9014-01-1	21 CFR § 184.1027
Sodium sulfate	7757-82-6	21 CFR § 186.1797; 21 CFR § 172.615

*Safe and suitable food grade enzyme, that is in compliance with FAO/WHO JECFA and Food Chemicals Codex (FCC) specifications for food grade enzymes and is used for cell wall disruption.

Product Specifications

The specifications for DHA-rich oil from *Schizochytrium* sp. T18 manufactured by the process outlined in Section D are found in Table 3. The specification for unsaponifiables (max. 3.5%) is the same as that of similar DHA algal oils, such as the DHA algal oil notified in GRN 553 (FDA, 2014a) and the EU Novel Food regulation for DHA-B for use in food and infant formula (EU, 2015). Analytical results for six non-consecutive lots of the proposed Mara DHA algal oil compared to another DHA algal oil (the subject of GRN 553) can be found in Table 4 and Appendix B. The proximate analysis of the DHA algal oil is presented in Table 5.

Table 3. Specification for DHA oil from *Schizochytrium sp.* T18

Parameter	Specification
Acid value (KOH/g)	Max 0.5
Peroxide value (meq/kg)	Max 5.0
Moisture (%)	Max 0.05
Unsaponifiables (%)	Max 3.5
Trans-fatty acids (%)	Max 2.0
DHA (% Relative)	Min 35
Arsenic (mg/kg)	<0.1
Copper (mg/kg)	<0.1
Iron (mg/kg)	<0.2
Mercury (mg/kg)	<0.1
Lead (mg/kg)	<0.1

Table 4. Analytical results for six non-consecutive lots of Mara DHA algal oil compared to different algal oil

Parameter	Mara	Mara	Mara	Mara	Mara	Mara	Oil A[1]
Lot Number	**16039**	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**	**08-6530**
Acid value (mg KOH/g)	0.05	0.06	0.05	0.06	0.06	0.06	NA
Peroxide value (meq/kg)	1.0	1.0	1.3	1.06	<0.1	<0.1	0.35
Moisture (%)	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05	<0.1
Unsaponifiables (%)	0.3	0.4	0.3	2.97	2.43	2.50	0.97
Trans-fatty acids (%)	0.20	0.22	0.23	<0.05	<0.05	<0.05	<1.0
DHA (% Relative)	37.10	42.47	41.98	40.54	39.64	39.60	44.35
Arsenic (mg/kg)	<0.1	<0.1	<0.1	<0.01	<0.01	<0.01	<0.1
Copper (mg/kg)	<0.1	<0.1	<0.1	0.08	0.02	0.03	<0.02
Iron (mg/kg)	0.15	<0.1	<0.1	<0.02	<0.02	<0.02	0.04
Mercury (mg/kg)	<0.005	<0.005	<0.005	<0.01	<0.01	<0.01	<0.01
Lead (mg/kg)	<0.05	<0.05	<0.05	<0.01	<0.01	<0.01	<0.1

[1]Analytical data from GRAS Notification No. 553 for DHA-B (10/06/14)

NA — not available

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Table 5. Analysis of six non-consecutive lots of Mara DHA algal oil

Parameter	Mara	Mara	Mara	Mara	Mara	Mara
Lot Number	**16039**	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**
Moisture (%)	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05
Ash (%)	<0.05	<0.05	<0.05	<0.1	<0.1	<0.1
Protein (%)	<0.10	<0.10	<0.10	<0.15	<0.15	<0.15
Fat (%)	100.24	99.17	99.86	101.44	100.31	100.49
Carbohydrate (%)	0	0	0	<0.1	<0.1	<0.1

NA — not available

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As seen in Table 6, all of the fatty acids detected are well-known components of the human diet and are found in both animal and vegetable food sources. The major fatty acids are DHA, myristic acid, palmitic acid, docosapentaenoic, and cis-vaccenic acid. Literature searches did not identify safety/toxicity concerns related to any individual fatty acid or their ratios in the proposed DHA algal oil. The proposed DHA algal oil is similar to other commercially available edible oils. As presented in Table 4, Mara's DHA algal oil is comparable to that of DHA-B that is presented in GRN 553 (FDA, 2014a). When compared to the spectrum of available DHA oils from a variety of sources, including algae and fish that are used in infant formula, the fatty acid profile of the proposed DHA algal oil is comparable to currently marketed DHA oil products, as well as other commercially available oils. The analyzed lots of Mara's DHA algal oil are consistent with those lots submitted previously to the UK Food Standard Agency (ONC, 2011) and found to be substantially equivalent to Martek algal oil (OmegaTech, 2001).

The proposed DHA algal oil can range commercially in form from semi-solid to translucent liquid, and depending on the attributes desired by infant formula manufacturers, the DHA algal oil profile can be managed through the fermentation conditions. Infant formula can be produced by dry blending or wet blending - spray drying. In the dry blending process, the ingredients are received from suppliers in a powdered form and are mixed together to achieve a uniform blend of the macro- and micronutrients. The algal oil must be encapsulated into a powdered form in order to protect the oil from auto-oxidation initiated by oxygen or minerals present during blending or in the finished product. In this application, the semi-solid oil is more advantageous during encapsulation as it is more viscous, which physically aids in the encapsulation process. The viscosity of the oil is directly related to the higher concentration of myristic and palmitic acids in the oil. In the wet blending - spray drying process, ingredients are blended together, homogenized, pasteurized, and spray dried to produce a powdered product. For heat sensitive ingredients such as unsaturated fatty acids (e.g., DHA) or minerals, these ingredients are added after pasteurization. Since the DHA algal oil will not be homogenized, it is required in a liquid form so that it is easily pumped to form a homogenous liquid with the other ingredients, prior to spray drying. Hence, a DHA algal oil with a lower concentration of saturated fatty acids is preferred. Non-consecutive batches of each product form are presented in Tables 4-8 (each with three non-consecutive lots; liquid - 16039, 16040, 16041; semi-solid - N2-006-C, N2-008-C, N2-010-C).

The fatty acid profile presented below for the proposed DHA algal oil has higher myristic, palmitic, docosapentaenoic, and cis-vaccenic acid concentrations, and a lower oleic acid concentration, than algal oil A; however, the fatty acid profile (including myristic and palmitic acids) of the proposed DHA algal oil is similar to that found in other algal oils and fish oils that are currently used in food, including infant formula (FDA, 2000, 2014a). Based on additional analyses conducted by Mara, the increase in the reported combined 18:1 (oleic + cis-vaccenic acid) values in Table 6 are a result of an increase in cis-vaccenic acid (n-7) in batches 16039, 16040, and 16041 as compared to the other three batches of DHA algal oil. Literature searches on the toxicity of cis-vaccenic acid did not reveal any toxicological issues related to the presence and

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consumption of this common monounsaturated fatty acid and isomer of oleic acid. It occurs naturally and can be found in fish oils and krill oil (7%–11%; FDA, 2008a), as well as other fats and oils such as olive oil, sesame oil, and rapeseed oil (FDA, 2008a).

The sterol content of the proposed DHA algal oil was also determined (see Table 7). The detected sterols and stanols are also present in the human diet, from vegetable and animal food sources such as common edible oils. The sterol levels presented in Table 7 for the proposed DHA algal oil are lower in total than the oil used for comparison, and under the intended conditions of use, the total sterol intake from DHA algal oil would be minimal. Additionally, the sterol profile of the proposed DHA algal oil is similar to that found in other algal oils and fish oils that are currently used in food, including infant formula (FDA, 2000, 2014a). The major sterols found in the DHA algal oil are found in human breast milk and commercially available infant formula (Mellies et al., 1976, FDA, 2014a).

It should be noted that numerous other analyses of the proposed DHA algal oil product have been conducted but are not included in the product specifications (e.g., microbiological analyses, chromium, iron, manganese, molybdenum, nickel, phosphorous, silicon, sulfur). Results of these additional analyses are also included in the Certificates of Analysis (COAs) found in Appendix B, and selected results are summarized in Table 8 below. In summary, the analytical results confirm that the proposed DHA algal oil product meets the analytical specifications and confirms that impurities/contaminants are not present at levels of toxicological concern.

Stability Data

Stability testing was conducted on batches of DHA algal oil as presented in Tables 9-10. DHA algal oil is typically shipped and stored in a tightly closed, nitrogen-blanketed, light-resistant container under frozen conditions (–25°C). The results of one study support the stability of the frozen product for a period of 1 year. Proposed labeling will recommend product use (best-before date) within 1 year of the date of manufacture. The batch has also been tested under accelerated stability conditions and found to be stable for a period of 8 weeks (see Table 10). Stability testing will continue for subsequent manufactured lots and will include 24-36 month data points.

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Table 6. Fatty acid profile (area %) of six non-consecutive lots of Mara DHA algal oil compared to different algal oil

Parameter	Mara	Mara	Mara	Mara	Mara	Mara	Oil A[1]
Lot Number	**16039**	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**	**08-6530**
12:0 Lauric	0.92	0.74	0.79	0.97	1.01	1.01	<0.1
14:0 Myristic	12.30	9.0	9.5	13.12	13.63	13.65	1.30
14:1 Myristoleic	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	ND
15:0 Pentadecanoic	0.68	0.45	0.56	0.42	0.51	0.52	0.25
16:0 Palmitic	22.67	21.46	21.76	27.87	29.45	29.39	13.95
16:1 Palmitoleic acid	6.16	3.63	4.21	2.1	2.2	2.23	NA
17:0 Heptadecanoic	0.15	0.12	0.14	<0.10	<0.10	0.10	ND
18:0 Stearic	0.77	0.83	0.78	0.84	0.85	0.85	1.64
18:1 (Oleic + cis-vaccenic acid)	7.49	8.06	7.26	2.17	1.81	1.85	24.52
18:2 Linoleic	0.34	0.78	0.56	<0.10	<0.10	<0.10	2.05
18:3 Gamma-linolenic acid	0.24	0.42	0.33	0.13	0.11	0.12	NA
18:4 Octadecatetraenoic	0.24	0.32	0.30	0.23	0.20	0.21	ND
20:0 Eicosanoic (arachidic)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.32
20:1 Eicosenoic	<0.10	<0.10	<0.10	<0.01	<0.01	<0.01	0.14
20:3 (n-6) Eicosatrienoic	<0.10	<0.10	<0.10	0.15	<0.10	<0.10	ND
20:4 (n-6) Arachidonic	0.65	0.76	0.75	0.74	0.64	0.63	0.67
20:5 (n-3) Eicosapentaenoic	1.08	1.59	1.49	1.12	0.90	0.90	5.90
22:0 Docosanoic	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.32
22:5 (n-6) Docosapentaenoic	7.21	7.65	8.12	8.38	7.73	7.78	2.63
22:6 (n-3) **DHA**	37.10	42.47	41.98	40.54	39.64	39.60	44.35
24:0 Tetracosanoic	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.12

[1]Analytical data from GRAS Notification No. 553 (10/06/14; FDA, 2014a); ND — not detected; NA — not available

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Table 7: Sterol content (% total sterols) of six non-consecutive lots of Mara DHA algal oil compared to different algal oil

Parameter	Mara	Mara	Mara	Mara	Mara	Mara	Oil A[1]
Lot Number	**16039**	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**	**08-6530**
Cholesterol	21.7	14.5	12.6	24.3	32.9	32.2	13.3
Brassicasterol	6.5	4.6	6.3	<0.1	<0.1	<0.1	1.3
24-Methylene cholesterol	2.8	2.3	3.3	3.9	7.1	6.1	1.3
Campesterol	1.5	3.9	3.2	1.2	1.4	2.7	2.0
Campestanol	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Stigmasterol	22.5	23.1	21.7	<0.1	7.2	6.9	64.2
delta-7-campesterol	<0.1	<0.1	<0.1	3.4	7.0	6.7	0.4
delta-5,23-stigmastadienol	3.0	3.8	<0.1	6.9	6.2	7.7	1.0
Clerosterol	14.5	19.3	17.9	8.8	8.2	6.3	1.6
beta-sitosterol	14.8	11.4	13.7	13.4	9.4	11.5	10.2
Sitostanol	<0.1	0.5	<0.1	<0.1	<0.1	<0.1	0.5
delta-5-avenasterol	3.8	4.7	5.7	1.4	1.2	1.3	1.7
delta-5,24-stigmastadienol	4.1	6.8	6.2	7.0	3.9	6.1	0.4
delta-7-stigmastenol	<0.1	<0.1	<0.1	26.1	14.0	11.0	1.7
delta-7-avenasterol	5.0	5.1	9.1	3.6	1.5	1.4	0.3
Total Sterols (mg/kg fat)	**900**	**1070**	**831**	**2310**	**1900**	**1990**	**5600**

[1]Analytical data from GRAS Notification No. 553 (10/06/14; FDA, 2014a)

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Table 8. Selected analytical results for residual contaminants

Elemental Analysis						
Lot Number	**16039**	**16040**	**16041**	**N-2-006-C**	**N-2-008-C**	**N-2-010-C**
Chromium (ppm)	<0.05	<0.05	<0.05	<0.1	<0.1	<0.1
Iron (ppm)	0.15	<0.1	<0.1	<0.02	<0.02	<0.02
Manganese (ppm)	<0.1	<0.1	<0.1	<0.01	<0.01	<0.01
Molybdenum (ppm)	<0.1	<0.1	<0.1	<0.05	<0.05	<0.05
Nickel (ppm)	<0.1	<0.1	<0.1	0.3	0.3	0.3
Phosphorus (ppm)	<3	<3	<3	<2	<2	<2
Silicon (ppm)	51	51	67	79	80	75
Sulfur (ppm)	<2	<2	<2	1.6	<1.0	<1.0
Microbiological Analyses						
Salmonella	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g	Negative/25g
Escherichia coli	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Staphylococci Coagulase+	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Yeast	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Mold	<10 CFU/ml	<10 CFU/ml	<10 CFU/ml	<10 CFU/g	<10 CFU/g	<10 CFU/g
Total Coliforms	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g

CFU — colony-forming unit

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Table 9. Stability study of Mara DHA algal oil

	Specifications	Time (months)		
		0	7	12
Batch No. N2-008C; Frozen				
DHA (%)	Min 35	39.64	39.29	42.62
Peroxide Value (meq/kg)	<5	<1.0	1.3	1.0
Anisidine Value	<15	NA	8.7	9.3

Table 10. Accelerated stability study of Mara DHA algal oil

	Specifications	Time (weeks)		
		0	4	8
Batch No. N-2-008-C; Refrigerated at 5°C				
DHA (%)	Min 35	39.64	38.89	38.41
Peroxide Value (meq/kg)	Max 5.0	<0.1	2.16	1.52
Batch No. N-2-010-C; 25°C at 60% Relative Humidity				
DHA (%)	Min 35	39.6	38.64	38.25
Peroxide Value (meq/kg)	Max 5.0	<0.1	2.84	1.43

§ 170.235 Part 3, Dietary Exposure

DHA algal oil is intended for use as a direct ingredient in exempt (pre-term) and non-exempt (term) infant formula (ages from birth to 12 months), in accordance with current good manufacturing practices (cGMP), and in combination with a source of arachidonic acid (ARA). The ratio of DHA to ARA would range from 1:1 to 1:2. The intended use level is similar to all other approved uses for incorporation of DHA in infant formula.

As presented and discussed in previous GRAS submissions (FDA, 2011a, 2014a), it is assumed that infants consume about 100-120 kcal/kg bw/day, of which fat constitutes approximately 50% of calories, or approximately 5.5–6.7 g fat/kg bw/day (1 g of fat is equivalent to 9 kcal). Assuming incorporation of the proposed DHA ingredient at a maximum use level of 0.5% of fatty acids, the intake of DHA would be 27–33 mg/kg bw/day. This DHA intake estimate is in agreement with current recommendations for DHA consumption by pre-term and term infants of 18–60 mg/kg bw/day (Koletzko et al., 2014).

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§ 170.240 Part 4, Self-Limiting Levels of Use

The use of DHA and DHA algal oil in infant formula is controlled as described in Part 3. As such, there are no self-limiting levels of use.

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§ 170.245 Part 5, Experience Based on Common Use in Food

The statutory basis for our conclusion of GRAS status in the notice is not based on common use in food.

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§ 170.250 Part 6, GRAS Narrative

History of Use/Regulatory Approval of DHA Algal Oil

DHA-rich oils from numerous sources including microalgae are considered GRAS for use in food for human consumption, including infant formula (FDA 2003b; 2011b; 2014a, 2014b). Global infant formula standards in the Food Chemicals Codex, as well as those in the EU, China, and Australia, allow the addition of DHA to infant formula as an optional ingredient (EU Commission, 2006; PRC, 2010; FSANZ, 2014). Sources of the DHA-rich algal oils include *Schizochytrium* sp., *Crypthecodinium cohnii*, *Ulkenia* sp. SAM2179. Other algal oil sources of food ingredients include *Chlorella protothecoides* strain S106, and *Prototheca moriformis* strain S2532. In addition, FDA has approved other sources of DHA for use in human food and/or infant formula, such as menhaden and fish oils. Table 11 provides a list of a number of approvals of DHA from algal sources as well as marine sources for incorporation in pre-term and term infant formula.

Table 11. Regulatory approvals for use of DHA in infant formula

Year Approved	Country	Submission
2001	USA	GRN 41; DHASCO (docosahexaenoic acid-rich single-cell oil) from *Crypthecodinium cohnii* for use in infant formula
2006	USA	GRN 94; Docosahexaenoic acid-rich oil from tuna (DHA-rich tuna oil)
2011	USA	GRN 379; DHA from tuna oil
2015	EU/UK	DHASCO-B (docosahexaenoic acid-rich single-cell oil) from *Schizochytrium* sp. for use in infant formula
2015	USA	GRN 553; Algal oil (40% docosahexaenoic acid) derived from *Schizochytrium* sp.

As summarized above, DHA, produced via fermentation employing various microalgae, has previously been approved and sold for incorporation in infant formula. This includes approval of algal oil from *Schizochytrium* sp. The approvals authorized the addition of DHA at levels up to 0.5% of the total fatty acids in both exempt (pre-term) and non-exempt (term) formulas.

In addition, DHA rich oils from microalgal sources including *Schizochytrium* sp. have been the subject of several authorization decisions and/or notifications under the European Union (EU) Novel Foods and Food Ingredients Regulation 258/97. The first such authorization was Commission Decision 2003/427/EC in June of 2003 which authorized the use of DHA-S oil from the thraustochytrid microalgae *Schizochytrium* sp. in a range of foodstuffs and established a specification for the material. This was followed in December 2003 by a notification under Article 5 of the novel food regulation for placement on the market of a DHA-rich oil derived from a second thraustochytrid

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microalgae *Ulkenia* sp. on the grounds of its substantial equivalence with the oil from *Schizochytrium* sp (Schmitt et al, 2012a). To date, algal oil produced from *Schizochytrium* sp. (DHA-S) has been approved for direct use in foods by the U.S. Food and Drug Administration (FDA), Health Canada, European Union, Food Standards Agency of Australia, China's Ministry of Health, and Brazil's National Health Surveillance Agency (FDA, 2014a). Furthermore, a Novel Food Application was approved for the use of DHA-B in conventional foods, infant formula and follow-up formula, and food supplements (DSM, 2013; EU, 2015). In 2009, Commission Decisions 2009/777/EC and 2009/778/EC authorized extensions to the approved food uses of the oils from *Ulkenia* sp. and *Schizochytrium* sp., respectively. A third DHA-rich oil derived from the microalgae *Crypthecodinium cohnii* was already on the EU market before the Novel Food Regulation came into effect and was therefore legally in use without the need for explicit approval (Schmitt et al., 2012a). It should also be noted that in 2012, the UK Food Standards Agency concluded that T18 algal oil met the criteria for equivalence as defined in Article 3(4) of regulation (EC) 258/97 and that the *Schizochytrium* strain used in the production of T18 oil was substantially equivalent to other *Schizochytrium* sp. DHA-rich algal oils (Food Standards Agency, 2012). In the U.S., the three DHA rich oils described above have also been the subjects of GRAS notifications (GRN Nos. 41, 137, 319) to which the FDA had no objections (FDA, 2000; 2003a; 2010).

Safety

Introduction

DHA is an important component of most cell membranes and tissues. DHA and DHA algal oils are currently marketed for use in food, dietary supplements, and infant formula for human consumption. The oil from *Schizochytrium* sp. T18 has a similar lipid (fatty acid and sterol) profile to that of currently approved/marketed DHA from *Schizochytrium* sp. (see Tables 6 and 7). Regulatory authorities have reviewed the safety of DHA and DHA algal oils and found their use to be safe for use in human food including infant formula. Numerous studies and publications support the safety of DHA and DHA algal oils, including *in vitro* studies, *in vivo* animal studies, and clinical studies in humans. A summary of the most relevant studies on DHA acute and subchronic toxicity, reproductive and developmental toxicity, mutagenicity and genotoxicity, chronic toxicity, carcinogenicity, and irritation/sensitization, along with clinical and epidemiological studies, have been summarized and reviewed (see Tables 11 and 12). Kroes et al. (2003) has reviewed/summarized the well-understood metabolic fate of dietary DHA, which is similar to other dietary fatty acids. The published data, as well as reviews conducted by regulatory authorities, support the conclusion that Mara's DHA-rich algal oil is safe for use in exempt (pre-term) and non-exempt (term) infant formula.

Safety Data

Literature searches were performed to identify available safety data on DHA and DHA algal oil in both adult consumers as well as infants. This included searching sources of information such as publicly available assessments, databases, or reviews from organizations including EFSA, Joint FAO/WHO Expert Committee on Food Additives

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(JECFA), U.S. FDA, and the World Health Organization (WHO), general Internet searching, as well as searching databases such as EMBASE, MEDLINE, TOXLINE, and PubMed.

Human Studies

Numerous algal and marine sources of DHA have been evaluated by the FDA and other global regulatory agencies over the past 15 years for proposed incorporation in food for human consumption, including infant formula. Relevant US GRAS notifications include GRN 41, GRN 94, GRN 379, and GRN 553 (FDA, 2000, 2001, 2011a, 2014a). All of the GRAS notices provided information/clinical study data that supported the safety of the proposed DHA ingredients for use in infant formula. In all of the studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues in infants attributable to DHA-supplemented formulas when compared to control-group infant formulas. The studies reviewed in these notifications supported the safe use of DHA in infant formula up to 1% of total fatty acids.

A review of data published since 2010 conducted as part of this GRAS notification supports the summaries provided in previous GRAS notifications. Studies of DHA in infant formulas at concentrations up to 1% did not report serious adverse effects and very often concluded that the addition of DHA to infant formula resulted in benefits to growth and development. While some studies report minor gastrointestinal effects, such as increased gas in infants using DHA-supplemented formula, a review conducted by FDA (2008b) demonstrates that these effects do not warrant concern; therefore, they are not discussed in detail in this notification. In response to a petition, FDA analyzed reported adverse events in the CFSAN Adverse Event Reporting System (CAERS) database for formulas containing DHA oils from 2000 to 2009 (FDA, 2008b). Following their review, FDA concluded that there were no statistically significant increases in the proportion of reported GI adverse events in infants receiving DHA-supplemented formulas over the time during which the market percentage of infant formulas containing algal oils went from 0% to 98%.

GRNs 379 and 553 provided comprehensive summaries of the clinical study literature regarding DHA or long-chain polyunsaturated fatty acids (LCPUFA) relevant to supplementation of infant formula from fish and algal oil sources (FDA, 2011a, 2014a). Therefore, this notification includes only summaries of clinical studies published since the most recent GRN on the supplementation (stand alone or in infant formula) with DHA and/or DHA and ARA for use by exempt (pre-term) and non-exempt (term) infants. A comprehensive literature search for clinical trials evaluating DHA in infant formula (published 2010–present) was performed, and titles and abstracts were reviewed. Only those studies measuring the effects of supplemental DHA on relevant measures of morbidity, growth/development, and metabolism were considered for inclusion. Approximately twenty published clinical trials were identified as meeting these criteria. Given the lack of reported serious adverse events, the clinical studies summarized below were selected to provide a representation of the beneficial effects of DHA supplementation.

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Fetal and Childhood Growth

Two recent clinical studies were identified that investigated the effect of DHA-supplemented formula in infancy on measures of growth. In a randomized, double-blind controlled-trial clinical trial, Currie et al. (2015) fed infants (n=54) formulas containing DHA/ARA in the following ratios: DHA/ARA = 0/0%, 0.32/0.64%, 0.64/0.64%, or 0.96/0.64% of total fatty acids from birth to 12 months. The effects of supplementation on growth parameters up to 6 years of age, compared to a control formula group (n=15), were evaluated based on recall assessments recorded at prescribed intervals over the 6-year study period. In children under 18 months of age, those supplemented with LCPUFAs experienced a higher linear growth curve, but no other differences were noted. However, increased stature- and weight-for-age percentiles, but not body mass index, were observed from two to six years for children who were fed the LCPUFA-supplemented formula when compared to the control formula. The authors note that differences in energy intake did not explain the increased growth parameters. Furthermore, the authors stated that the study results did not suggest any adverse effect on body weight or child growth related to supplementation of formula with LCPUFA, including a predisposition to being overweight.

In a second randomized, double-blind controlled trial, Kitamura et al. (2016) studied the safety and effect of infant formula DHA/ARA ratio on the fatty acid composition of the erythrocyte membrane in low-birth-weight infants (<2,000 g) from a Japanese population (n=35). The content of DHA and ARA in the erythrocyte membrane has been correlated with the fatty acid content in the brain, and therefore, the fatty acid composition of the erythrocyte membrane has been used as an index reflecting the nutritional state of fatty acids in the body. The authors evaluated the safety of an infant formula with a DHA/ARA compounded ratio adjusted to 2:1 to reflect the content of Japanese breast milk (DHA: 9.1 mg/100 mL, ARA: 4.6 mg/100 mL) compared to a control formula (DHA: 9.1 mg/100 mL, ARA: 1.0 mg/100 mL) and administered for 1 month as a supplement to breastfeeding. No significant treatment-related effects were noted in body weight gain, height gain, head circumference gain, or amount of feeding. In addition, no serious adverse events related to consumption of the DHA/ARA formula were noted and included an evaluation of milk allergy, allergy-associated diarrhea, bloody stools, and anaphylaxis. However, an increase in the DHA and ARA contents of the erythrocyte membrane compared to control formula (DHA only) was observed.

Morbidity: Retinopathy of Prematurity

Retinopathy of prematurity (ROP) in extremely preterm infants can cause visual morbidity. Two new studies were identified by Pawlik and colleagues (2011, 2014) examining the effect of intravenous administration of a fish-oil fat emulsion (containing DHA) in premature, very low-birth-weight infants, on retinopathy. While these studies (one retrospective [n= 337] and the other a randomized, controlled trial [n=130]) do not directly investigate infant formula supplementation, the parenteral use of the fish-oil-based emulsions containing DHA was demonstrated to be safe. Supplementation

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significantly reduced the risk of choleostasis and lowered the severity of retinopathy, as well as the need for retinopathy-related laser treatment. The administration of the fish-oil fat emulsion also reduced the incidence of cholestasis and produced a notable increase in plasma and erythrocyte DHA levels at 7 and 14 days of age. There was no difference in the incidence of adverse effects between the fish-oil emulsion and control groups; only elevated triglyceride levels were noted in both groups, and no coagulopathy was reported in any patient.

Neurodevelopment

Several recent clinical trials have demonstrated a benefit to neurodevelopmental parameters with DHA-supplemented infant formula. Supplementation with DHA up to 1% in term infants appeared to improve cognitive function in two studies identified (Drover et al., 2011; Westerberg et al., 2011); one study reported improvements in language and behavior scores (Meldrum et al., 2012).

Drover et al. (2011) conducted a double-blind, randomized, controlled prospective trial in infants (n=181) that were fed DHA-supplemented formulas up to 12 months of age, to assess the effects of supplementation on cognitive outcomes. Infants received formulas containing 0% (no DHA or ARA), 0.32%, 0.64%, or 0.96% DHA, along with 0.64% ARA in the DHA-supplemented formulas. Of the 181 enrolled children, 141 completed the 12-month supplementation period, and of those, 131 were assessed at 18 months of age using the infant development index, the Bayley Scales of Infant Development II. The authors observed enhanced cognitive development as measured by the Mental Development Index, but not in the Psychomotor Development Index or Behavior Rating Scale, at 18 months of age when the DHA-supplemented groups were combined and compared to control-group children. Similarly, combined analysis of the DHA dose groups found that the language facet was increased compared to controls (104.1 vs. 98.4; p=0.02), with no effect observed on cognitive or motor facets. The DHA supplementation was well tolerated, and occurrences of adverse effects (gastrointestinal issues) and serious adverse effects were not significantly different between groups. No adverse events were associated with supplementation in this study, and the authors note that the levels tested were within the range found in breast milk worldwide.

Westerberg et al. (2011) conducted a randomized, double-blind, placebo-controlled trial to evaluate the effects of DHA and arachidonic acid (ARA) supplementation on cognitive function. One-hundred and forty-one very-low-birth-weight infants (<1500 g) received supplementation containing 0.5 mL of oil with 32 mg DHA and 31 mg ARA in human milk from one-week post-birth to an average of 9 weeks. At 20 months, the authors observed positive effects related to attention during free-play sessions in infants receiving supplementation. Cognitive effects were measured using the Bayley Mental Development Index and the Ages and Stages Questionnaire; however, supplementation of human milk with DHA/ARA did not produce a significant change in these other two cognitive function measures. No statistical comparison was provided for a difference between adverse effects related to consumption of DHA/ARA-supplemented human milk; however, the number of infants excluded for this reason was low in both groups

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(supplementation group n = 5/68; control group n = 2/73). The authors note that five and two adverse events occurred in the intervention and control groups, respectively, but no further discussion is provided.

Meldrum et al. (2012) conducted a randomized, double-blind, placebo-controlled study that evaluated the effects of direct supplementation with high-dose fish oil on infant neurodevelopmental outcomes and language. The trial included 420 healthy term infants randomly assigned to receive a DHA-enriched fish-oil supplement (containing at least 250 mg DHA/day and 60 mg eicosapentaenoic acid [EPA]/day) or a placebo (olive oil) from birth to 6 months. Neurodevelopment endpoints were subsequently evaluated at 18 months using the Bayley Scales of Infant and Toddler Development and the Child Behavior Checklist. Language development was assessed using the Macarthur-Bates Communicative Development Inventory and was assessed at 12 and 18 months. After 6 months, supplementation resulted in a significantly higher erythrocyte DHA ($p=0.03$) and plasma phospholipid DHA levels ($p=0.01$) relative to placebo. Children in the supplement group also had "significantly higher percentile ranks in both later developing gestures at 12 and 18 months and total number of gestures"; however, standard or composite scores of the Bayley Scales of Infant and Toddler Development (BSID-III) were not significantly different. Behavior scores from the Categorical Child Behavior Checklist for "Anxious/depressed" illustrated a positive effect, with a significant increase in the supplement group at 18 months, compared to controls. No other significant behavioral effects were observed. Although no statistical comparison was reported between the groups for withdrawal reasons, one subject in the supplement group reported vomiting and diarrhea after taking the capsules, and reflux was reported by a small number of subjects in both groups.

DHA Metabolism/Status

As discussed above, Kitamura et al. (2016) noted an increase in the DHA and ARA contents of the erythrocyte membrane compared to control formula. Collins et al. (2015) conducted a randomized controlled trial to determine the dose of orally administered DHA (via feeding tube) that could be administered to pre-term infants (<30 weeks gestational age) to achieve a DHA status equivalent to that of term infants. Thirty-one pre-term infants were enrolled and randomly assigned to receive an emulsion containing 40, 80, or 120 mg/kg/day of DHA. A non-randomized arm of the study also included two additional groups: infants not receiving supplementary DHA and infants breastfeeding from mothers taking DHA supplements. The 120-mg/kg/day treatment resulted in a significant increase in erythrocyte DHA levels on study day 7 compared to the un-supplemented and maternal-supplemented groups, while the lower supplement groups prevented some of the decline in DHA levels, but the effects were not significant. DHA supplementation did not produce a significant decrease in arachidonic acid erythrocyte phospholipid levels. The emulsion was determined to be well tolerated, and there were no differences between groups with respect to feeding interruptions or time to full enteral feeding, as well as no differences in weight, length, and head circumference at discharge.

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Immune Function

Although not a clinical trial, beneficial effects of DHA supplementation in infant formula on the developing immune system were reported by Lapillonne et al. (2014). In this observational, multi-center, prospective study, infants (n=233) were provided formula containing 17 mg DHA and 34 mg ARA/100 kcal, or a control formula with no supplementation for one year. The DHA/ARA supplemented formula was evaluated for its potential effect on the immune system as measured by the frequency of common illnesses. Reduced incidences of respiratory illnesses (bronchitis/bronchiolitis, nasal congestion, cough, and croup) and diarrhea (requiring medical attention) were reported during the first year of life in healthy infants given formula with added DHA/ARA. No differences were noted in the incidences of eczema or otitis media. Female infants receiving DHA/ARA supplemented formula demonstrated a statistically significant increase in weight gain at 6 and 9 months of age compared to those on control formula; similar results were not observed in male infants at any point during the one-year period. No other differences in growth were noted, as measured by length or head circumference. No increases in frequency of illness or other adverse impacts on the measured illnesses related to consumption of the DHA/ARA formula were observed.

Toxicological Studies

Animal Studies (with Schizochytrium sp. T18-derived algal oil)

Toxicity testing has been conducted with the proposed DHA-rich algal oil product from T18 (Schmitt et al., 2012a,b). Schmitt et al. (2012a) conducted a battery of *in vitro* and *in vivo* genotoxicity tests (microbial reverse mutation assay, *in vivo* rat bone marrow micronucleus assay, and chromosomal aberration assay in cultured human peripheral blood lymphocytes) with DHA-rich algal oil T18. The DHA-rich algal oil was not mutagenic or genotoxic in any of the assays. In addition, the acute oral LD_{50} in rats was estimated to be greater than 5000 mg/kg of body weight.

In addition, Schmitt et al. (2012a) administered DHA-rich algal oil at concentrations of 0, 10,000, 25,000, or 50,000 ppm in the diet to rats for 13 weeks. The algal oil was well-tolerated and there was an absence of toxicologically significant treatment-related effects on the general condition and appearance of the rats, neurobehavioral endpoints, growth, feed and water intake, ophthalmoscopic examinations, routine hematology and clinical chemistry parameters, urinalysis, and necropsy findings. The no-observed-adverse-effect level (NOAEL) was the highest dietary concentration level of 50,000 ppm, equivalent to 3,305 and 3,679 mg/kg bw/day for male and female rats, respectively. The study results confirmed that the DHA-rich algal oil T18 possessed a toxicity profile similar to other currently marketed algal oils and supported the safety of the proposed DHA-rich algal oil T18 for its proposed use in food.

Schmitt et al. (2012b) conducted both a developmental toxicity study and a 3-month dietary toxicity study with an *in utero* exposure phase of T18 in the rat. Based on the absence of maternal and developmental toxicity at any dose level tested in the

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developmental toxicity study, the high-dose of 2000 mg/kg/day was considered to be the NOAEL for maternal toxicity and embryo/fetal development when DHA-rich algal oil was administered orally by gavage to pregnant Crl:CD(SD) rats during gestation days 6 - 19. In the 3-month dietary toxicity study with an *in utero* phase, the NOAEL for systemic toxicity for F_0 male and female rats and F_1 male rats was considered to be 50,000 ppm (highest concentration administered) and 25,000 ppm for F_1 female rats (based on higher mean body weight, body weight gain, and food consumption).

Mean body weight gain for the 50,000 ppm algal oil group females was similar to the DHA fish oil group during PND 21–35. However, slightly higher mean body weight gain was noted for females in this group beginning on PND 35 and generally continued throughout the remainder of the study; the difference was significant ($p < 0.05$) during PND 77–84 only. As a result, mean body weight gain in the 50,000 ppm algal oil group females was 32 g higher than the DHA fish oil group when the entire generation (PND 21–112) was evaluated and higher mean body weight during PND 70–112 (significant; $p < 0.05$ on PND 84 only). These increases were attributed to algal oil exposure. Mean food consumption in the 50,00 ppm algal oil group females was generally higher than the DHA fish oil throughout the entire generation (PND 21–112); the differences were often significant ($p < 0.05$ or $p < 0.01$). These increases corresponded to the effects on mean body weights observed in this group and therefore, were attributed to test article exposure.

The 50,000 ppm exposure level was equivalent to 3421 and 2339 mg/kg/day for F_0 males during pre-mating 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. Reproductive performance values, estrous cycle length, gestation length, process of parturition, and the numbers of former implantation sites and unaccounted-for sites for the F_0 generation were unaffected by algal oil exposure. F_1 generation postnatal survival and developmental parameters were unaffected by algal oil exposure at all dietary concentrations tested. There were no neurotoxic effects noted at any algal oil exposure level. The authors concluded that the results further supported the safety of DHA-rich algal oil T18 for its proposed use in food. The above studies are summarized in Table 12.

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Table 12. Summary of preclinical toxicological study data on DHA-rich algal oil T18

Findings/Observations	Reference
Acute Toxicity	
Results: DHA-rich algal oil T18; oral LD_{50} in female Sprague-Dawley albino rats only, >5 g/kg.	Schmitt et al., 2012a
Subchronic Toxicity	
Study Design: Male and female Hsd:Sprague-Dawley SD rats were administered 0, 1, 2.5 or 5.0% DHA-rich algal oil T18 in the diet for 13 weeks. **Results:** NOAEL was the highest concentration tested (5% in the diet), equivalent to 3305 and 3679 mg/kg bw/day in male and female rats, respectively.	Schmitt et al., 2012a
Reproductive/Developmental Toxicity	
Study Design: DHA-rich algal oil T18 was tested for reproductive and developmental toxicity in Sprague-Dawley rats following oral gavage administration. **Results (Developmental/Maternal Toxicity):** The DHA algal oil (dosage levels of 400, 1000, and 2000 mg/kg/day) did not produce maternal and developmental toxicity at any dosage level. The high dosage level tested of 2000 mg/kg/day was considered to be the NOAEL for maternal toxicity and embryo/fetal development when DHA-rich algal oil was administered orally by gavage to pregnant Crl:CD(SD) rats during gestation days 6 - 19. **Results (Reproductive Toxicity)**: In a 3-month dietary toxicity study with an *in utero* exposure phase in rats, the NOAEL for F0 male and female and F1 male systemic toxicity was considered to be 50,000 ppm (highest concentration administered) and 25,000 ppm for F1 female systemic toxicity (based on higher mean body weight, body weight gain, and food consumption). Reproductive performance values, estrous cycle length, gestation length, or the process of parturition, and the numbers of former implantation sites and unaccounted-for sites of the F0 generation were unaffected by algal oil exposure. F1 postnatal survival and developmental parameters were unaffected by algal oil exposure at all dietary concentrations. There were no neurotoxic effects noted at any DHA exposure level.	Schmitt et al., 2012b
Genotoxicity/Mutagenicity	
Study Designs: DHA-rich algal oil T18 was tested in a battery of *in vitro* and *in vivo* genotoxicity tests (microbial reverse mutation assays, rat bone marrow micronucleus assay, chromosomal aberration assay in human peripheral blood lymphocytes). **Results:** In all assays, the DHA algal oil did not demonstrate mutagenic or genotoxic potential.	Schmitt et al., 2012a

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Animal Studies (with other DHA algal oil products)

Numerous studies have been conducted with other DHA algal oils and fish oils, including acute toxicity studies (FDA, 2010), subchronic studies (Fedorova-Dahms et al., 2011; Hammond et al., 2001a; Boswell et al., 1996; Wilbert et al., 1997; Arterburn et al. 2000a; Burns et al., 1999; Blum et al., 2007a; Kroes et al., 2003), reproductive and developmental toxicity studies (Hammond et al., 2001b,c; Arterburn et al. 2000b; Kroes et al., 2003; Blum et al., 2007b), genotoxicity and mutagenicity studies (Kroes et al., 2003; Hammond et al., 2002; Arterburn et al., 2000c; Fedorova-Dahms et al., 2011; Blum et al., 2007a), and other safety-related studies (Fedorova-Dahms et al., 2014; Huang et al., 2002; Abril et al., 2003; Turk et al., 2013; Huang et al., 2015; IOM, 2005). No toxicologically significant treatment-related effects were observed in these studies as summarized in Table 13. Only published studies are referenced, although numerous unpublished studies have also been referenced in previous GRAS notifications and support the safety of DHA algal oils. In addition, numerous safety studies of a dried algal biomass were conducted (i.e., *in vitro* and *in vivo* genetic toxicity, subchronic toxicity in rats, reproductive and developmental toxicity in rats and/or rabbits), also without notable toxicity (GRN 553; FDA, 2014a).

The FDA has reviewed the safety information submitted as a part of GRNs for these DHA oil products (e.g., DHASCO-B (FDA, 2000, 2014a); DHA-45 oil (Lonza; FDA, 2010), fish/anchovy oil (FDA, 2003b)). As one example, several published studies were submitted as part of GRN 319 (FDA, 2010) for a DHA algal oil derived from *Ulkenia* sp. SAM2179. Based on the entirety of the regulatory and safety information/data provided, FDA issued a "no questions letter" regarding the proposed use of DHA algal oil (*Ulkenia* sp. SAM 2179) in food. Similar safety studies and resultant FDA "no questions letters" have also been issued for other DHA sources (e.g., fish oils) and GRAS notifications as described in the History of Use section (Section 3.0).

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Table 13. Summary of preclinical toxicological study data on other DHA and DHA algal or fish oil products

Findings/Observations	Reference
Acute Toxicity	
Results: *Ulkenia* DHA oil (45% DHA from *Ulkenia* sp. algae); oral LD_{50} in male ICR mice and male and female Sprague-Dawley (Crj/CD(SD)IGS) rats reported to be >2 g/kg.	FDA, 2010
Subchronic Toxicity	
Study Design: DHA-rich algal oil from *Schizochytrium* sp., containing 40 - 45 wt% DHA and up to 10 wt% EPA, was evaluated in a subchronic (90-day) dietary study in male and female Sprague–Dawley rats with an *in utero* exposure, followed by a 4-week recovery phase. DHA-rich algal oil dietary levels of 0.5, 1.5, or 5 wt% along with two control diets (a standard low-fat basal diet and a basal diet supplemented with 5 wt% of concentrated fish oil) were administered. **Results:** No treatment-related effects were noted in clinical observations, body weight, food consumption, behavior, hematology, clinical chemistry, coagulation, or urinalysis. Increases in absolute and relative weights of the liver, kidney, spleen and adrenals (adrenals and spleen with histological correlates) were observed in both the fish oil- and the high-dose of DHA-rich algal oil-treated females but were not considered to be adverse. The increased slight cytoplasmic vacuolation of adrenal cortical cells of the zona fasciculate and the minimal to slight extramedullary hematopoiesis of the spleen were noted in basal diet, fish oil, and the high-dose DHA-rich algal oil males and females. The intensity was slightly increased in the fish oil group. Additionally, slight enlargement of hepatocytes in the periportal regions in the liver of fish oil control group, but not DHA-rich algal oil group, was observed in both genders. As with organ weight changes, any histological findings in the high dose DHA-rich algal oil treated animals were also noted in fish oil-treated control and were, therefore, not considered to be adverse effects of DHA-rich algal oil but rather physiological adaptations to accommodate the large LC-PUFA load in the diet. The NOAEL for DHA-rich algal oil was the highest dose tested (5% in the diet), equivalent to a DHA algal oil intake of 4122 mg/kg bw/day and 4399 mg/kg bw/day for male and female rats, respectively.	Fedorova-Dahms et al., 2011
Study Design: DHA-rich algal oil from *Schizochytrium* sp. (fermentation biomass) was administered in the diet of male and female Sprague-Dawley Crl:CD(SD)BR rats at doses of 110 to 1090 mg DHA/kg bw/day for 13 weeks. **Results:** No treatment-related adverse effects were noted at any dose.	Hammond et al., 2001a
Study Design: DHA-rich oil from *C.cohnii* was administered to male and female Sprague-Dawley rats both orally and in the diet in two separate 4-week toxicity studies. Doses ranged from 25 to 1250 mg/kg bw/day by gavage and 210 to 1180 mg/kg bw/day in the diet. **Results:** No treatment-related adverse effects were noted. Periportal hepatocellular vacuolation was noted in female rats, but was considered related to the consumption of diets high in fat. The highest doses administered (1250 and 1180 mg/kg/day) were considered the NOAELs.	Boswell et al., 1996; Wilbert et al., 1997

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Findings/Observations	Reference
Study Design: DHA-rich oil from *C.cohnii* was administered by oral gavage to male and female Sprague-Dawley rats for 13 weeks. Doses ranged from 500 to 1250 mg/kg bw/day. **Results:** No treatment-related adverse effects were observed.	Arterburn et al. 2000a
Study Design: DHA-rich oil (DHA-Arachidonic Acid (ARA) blend) from *C.cohnii* was administered in the diet to male and female Sprague-Dawley rats for 13 weeks (including an in utero phase). Doses ranged from 410 to 3290 mg/kg bw/day. **Results:** The DHA oil did not produce treatment-related adverse effects in rats when administered via the diet *in utero* and for a subsequent 90 days.	Burns et al., 1999
Study Design: DHA-rich oil (from *Ulkenia* sp. algae) was administered to male and female Sprague-Dawley Crj:CD(SD)IGS rats by gavage for 13 weeks. Doses ranged from 540 to 900 mg DHA/kg bw/day. **Results:** No treatment-related adverse effects were noted in clinical observations, food and water consumption, mortality, gross pathology, and histopathology. Increased body weights and liver weights in DHA oil-treated groups were observed. The changes were considered to be related to the large lipid load administered, and thus not regarded as toxicologically significant.	Blum et al., 2007a; Kroes et al., 2003
Reproductive/Developmental Toxicity	
Study Design: In a single-generation reproduction toxicity study, DHA-rich algal oil from *Schizochytrium* sp. (fermentation biomass) was administered in the diet of male and female Sprague-Dawley rats at doses ranging from 130 to 5625 mg DHA/kg bw/day for 13 weeks. **Results:** No treatment-related adverse effects were noted (e.g., in estrus cycle duration, fertility, gestation length, pups per litter).	Hammond et al., 2001b
Study Designs: In two developmental toxicity studies in female Sprague-Dawley rats and New Zealand White rabbits, DHA-rich algal oil from *Schizochytrium* sp. (fermentation biomass) was administered during gestation to rats via the diet and by oral gavage to rabbits. Doses ranged from 130 to 5900 mg DHA/kg bw/day (rats) or 49 to 490 mg DHA/kg bw/day (rabbits). **Results:** No maternal or developmental toxicity was observed (e.g., no adverse effects on reproductive performance, postnatal survival) in rats. In rabbits, the high-dose DHA oil and fish oil treatment groups demonstrated reduced food consumption and body weight gain, and a slight increase in abortions when compared to the control group. However, the authors considered the effects to be related to the consumption of high-fat diets. No effects were noted in offspring in either study.	Hammond et al., 2001c
Study Design: In a developmental toxicity study, DHA-rich oil from *C.cohnii* was administered by oral gavage to pregnant Sprague-Dawley rats during gestation, at doses ranging from 260 to 645 mg/kg bw/day. **Results:** No maternal/developmental toxicity was noted.	Arterburn et al. 2000b

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Findings/Observations	Reference
Study Design: In a single-generation reproduction toxicity study, DHA-rich algal oil from *Ulkenia* sp. was administered by oral gavage to male and female Sprague-Dawley rats at doses ranging from 360 to 5040 mg DHA/kg bw/day. **Results:** No treatment-related adverse effects were noted on parameters of reproduction (e.g., estrus cycle duration, fertility, gestation length, pups per litter).	Kroes et al., 2003; Blum et al., 2007b
Genotoxicity/Mutagenicity	
Study Designs: DHA45-oil was evaluated in several *in vitro* genetic toxicity assays. Fujii and Suwa (1998a (unpublished), as cited in Kroes et al., 2003) investigated the potential mutagenicity of DHA45-oil in the Ames assay using *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 at concentrations of 0.5 - 5 mg DHA45-oil/plate, in the presence and absence of S9 fraction from the livers of Aroclor-induced rats. Bruijntjes-Rozier and van Ommen (2001 (unpublished), as cited in Kroes et al., 2003) evaluated the potential mutagenicity of DHA45-oil in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and in *Escherichia coli* WP2 uvrA at concentrations of 0.06 - 5 mg DHA45-oil/plate, with and without metabolic activation. The ability of DHA45-oil to induce chromosomal aberrations was evaluated using Chinese hamster fibroblast cells, with and without metabolic activation (Kashima and Sarwar, 2000, (unpublished), as cited in Kroes et al., 2003). **Results:** No evidence of mutagenicity was detected in any of the *in vitro* studies. DHA45-oil also did not induce chromosome aberrations under the conditions of the study.	Kroes et al., 2003
Study Designs: Numerous *in vitro* assays were conducted with and without mammalian metabolic activation. **Results:** DHA-rich microalgae were not mutagenic in the Ames reverse mutation assay employing five different *Salmonella* strains. Similarly, DHA-rich microalgae was tested and found not to be mutagenic in the CHO AS52/XPRT gene mutation assay. It was not clastogenic to human peripheral blood lymphocytes in culture and did not induce micronucleus formation in mouse bone marrow in *vivo*.	Hammond et al., 2002
Study Designs: Docosahexaenoic acid single cell oil (DHASCO), a microbially-derived triglyceride rich in docosahexaenoic acid was tested for mutagenic activity in three different *in vitro* mutagenesis assays. All assays were conducted with and without metabolic activation. **Results:** DHASCO oil was not mutagenic in the Ames reverse mutation assay using five different *Salmonella* tester strains, nor was DHASCO mutagenic in the mouse lymphoma TK(+/-) forward mutation assay. The oil also was not clastogenic in a chromosomal aberration assay performed with Chinese hamster ovary cells.	Arterburn et al., 2000c
Study Designs: DHASCO-B oil was tested in the Ames reverse mutation assay, in an *in vitro* mammalian chromosome aberration test in human lymphocytes, and in an *in vivo* mouse micronucleus study in immature erythrocytes of the bone marrow. **Results:** DHASCO-B oil was found to be non-mutagenic/non-genotoxic.	Fedorova-Dahms et al., 2011

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Findings/Observations	Reference
Design/Results: DHA-algal oil (*Ulkenia* sp.) was non-mutagenic in various bacterial strains (Ames assay), and did not induce chromosomal aberrations in Chinese hamster fibroblast cells.	Blum et al., 2007a
Additional Safety-Related Studies	
Study Design: Bioequivalence study in domestic Yorkshire Crossbred neonatal pigs. Diets containing DHASCO-B or DHASCO blended with ARASCO (ARA single cell oil) were administered from day 2 to 22 after birth. **Results:** Both diets were well-tolerated and diets were found to be bioequivalent.	Fedorova-Dahms et al., 2014
Study Design: The effect of administration of DHA to female pigs/piglets was measured by changes in clinical chemistry and organ weights. **Results:** No treatment-related differences between groups of piglets receiving DHA and control diets were noted.	Huang et al., 2002
Study Design: Male early-weaned pigs were fed the fermentation biomass of the DHA-producing organism *Schizochytrium* sp. **Results:** No effects were noted in hematology parameters, organ weights, or histopathology (liver, heart, and spleen) compared to animals receiving control diets. No attempt was made to balance fat between the control and treatment group diets in the study.	Abril et al., 2003
Study Design: A mouse immortalized colonocyte model study was conducted. Mice were fed either a corn oil-, DHA-, or EPA-enriched diet prior to intestinal wounding (2.5% dextran sodium sulfate for 5 days followed by termination after 0, 3, or 6 days of recovery). **Results:** DHA uniquely reduced epidermal growth factor receptor (EGFR) ligand-induced receptor activation (wound healing events), whereas DHA and its metabolic precursor EPA reduced wound-induced EGFR transactivation compared with the control group (no fatty acid or linoleic acid). The results indicate that, during the early response to intestinal wounding in this mouse colonocyte model, DHA and EPA delay the activation of key wound-healing processes in the colon.	Turk et al., 2013
Study Design: A proteomics study was conducted in an effort to provide insights into PUFA-regulated hepatic protein expression in apoE-knockout mice. The control group was given normal laboratory mouse diet *ad libitum* and 1.1% ethanol in phosphate-buffered saline (PBS) (150 mM NaCl, 20 mM sodium phosphate, pH 7.4) by gavage every day for 10 weeks. Similarly, the four test groups were fed the same normal diet *ad libitum* plus 200 mg/kg of DHA, EPA, ARA, or linoleic acid in 1.1% ethanol/PBS every day by gavage for 10 weeks. The mice were then euthanized and blood and liver samples were collected. **Results:** The results provided evidence that PUFAs may act as either pro-inflammatory or anti-inflammatory agents.	Huang et al., 2015

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Findings/Observations	Reference
Review	
IOM reviewed studies of DHA and noted that DHA administration to animals via the diet has produced an increase in lipid peroxidation and oxidative damage in erythrocytes, liver, and kidney membranes, and bone marrow DNA. However, IOM noted that the effects were reduced or mitigated with co-administration of vitamin E (Ando et al., 1998; Song and Miyazawa, 2001; Umegaki et al., 2001; Yasuda et al., 1999; Leibovitz et al., 1990 as cited by IOM, 2005).	IOM, 2005

Safety Data Summary

DHA and DHA algal oils are currently marketed for use in infant formula, food, and dietary supplements for human consumption. The oil from *Schizochytrium* sp. T18 has a similar proximate composition and lipid (fatty acid and sterol) profile to that of currently approved/marketed DHA oils from *Schizochytrium* sp. and other algal and marine sources. Regulatory authorities have reviewed the extensive safety study database of DHA and DHA algal oils and found their use to be safe for use in human food and infant formula. Numerous studies have been conducted and published in support of the evaluation of the safety of DHA and DHA algal oils, including *in vitro* studies, *in vivo* animal studies, and clinical studies in humans including infants. The most relevant studies on DHA acute and subchronic toxicity, reproductive and developmental toxicity, and mutagenicity and genotoxicity, along with clinical and epidemiological studies have been reviewed/summarized above.

In summary, the available published scientific data on the safety of DHA from algae and other sources (e.g., fish oil) including Mara's proposed algal source are extensive. The compositional profile of the DHA-rich algal oil ingredient presents no obvious safety concerns. The totality of published study data, as presented in previous GRNs, reviewed by FDA (2008b), and summarized here, support the safe use of Mara's DHA algal from *Schizochytrium* sp. in infant formulas up to 1% of total fatty acids. Additionally, FDA has already reviewed numerous GRAS notifications for similar products and their use in infant formulas and issued "no questions" letters in those previous cases. Lastly, DHA products have been reviewed and approved around the world for addition to food, including infant formula, and for use as a dietary supplement.

Basis for the GRAS Determination

Introduction

The regulatory framework for determining whether a substance can be considered generally recognized as safe (GRAS) in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic (FD&C) Act (21 U.S.C. § 301 et. Seq.) ("the Act"), is set forth at 21 CFR 170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information.

These criteria are applied in the analysis below to determine whether the use of DHA-rich algal oil for use in infant formula (pre-term and term infants) is GRAS based upon scientific procedures. All data used in this GRAS determination are publicly available and generally known, and therefore meet the "general recognition" standard under the FD&C Act.

Safety Determination

DHA and DHA algal oils are currently marketed for use in food for human consumption, including infant formula, as well as dietary supplements. The proposed DHA algal oil from *Schizochytrium* sp. T18 has a similar composition and lipid (fatty acid and sterol) profile to that of currently approved/marketed DHA oils from *Schizochytrium* sp. and other algal and marine sources. Regulatory authorities have reviewed the extensive safety study database of DHA and DHA algal and fish oils and found no issues of concern with respect to their use in human food including infant formula. Numerous studies have been conducted and published in support of the evaluation of the safety of DHA and DHA algal and fish oils, including *in vitro* studies and *in vivo* animal studies (i.e., acute and subchronic toxicity, reproductive and developmental toxicity, mutagenicity and genotoxicity, chronic toxicity, carcinogenicity, irritation/sensitization), as well as clinical studies in infants and adults.

DHA-rich oils from numerous sources including microalgae are considered GRAS for use in food for human consumption, including infant formula (FDA 2003b; 2011b; 2014a, 2014b). Sources of the DHA-rich algal oils include *Schizochytrium* sp., *Crypthecodinium cohnii*, *Ulkenia* sp. SAM2179. Other algal oil sources of food ingredients include *Chlorella protothecoides* strain S106, and *Prototheca moriformis* strain S2532. In addition, FDA has approved other sources of DHA for use in human food and/or infant formula, such as menhaden and fish oils.

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DHA, produced via fermentation employing various microalgae, has been approved and marketed commercially for incorporation into infant formula. This includes approval of algal oil from *Schizochytrium* sp. The approvals authorized the addition of DHA at levels up to 0.5% of total fatty acids in both pre-term and term formulas.

In Europe, DHA rich oils from micro-algal sources have been the subject of several authorization decisions and/or notifications under the EU Novel Food Regulation 258/97. Most recently, a Novel Food Application was approved for the use of DSM's DHASCO-B from *Schizochytrium* sp. in conventional foods, infant formula and follow-up formula, and food supplements (DSM, 2013; EU, 2015). The first authorized the use of DHA-rich oil from the thraustochytrid microalgae *Schizochytrium* sp. in a range of foodstuffs and established a specification for the material. The second was for a DHA-rich oil derived from a second thraustochytrid microalgae *Ulkenia* sp. on the grounds of its substantial equivalence with the oil from *Schizochytrium* sp. The other decisions authorized extensions to the approved food uses of the oils from *Ulkenia* sp. and *Schizochytrium* sp., respectively. An additional DHA-rich oil derived from the microalgae *Crypthecodinium cohnii* was already on the EU market before the Novel Food Regulation came into effect and was therefore legally and safely in use without the need for explicit approval. It should also be noted that in 2012, the UK Food Standards Agency concluded that T18 algal oil met the criteria for equivalence the currently marketed DHA algal oils as defined in Article 3(4) of regulation (EC) 258/97 and that the *Schizochytrium* strain used in the production of T18 oil was closely related to the organism used in the production of other *Schizochytrium* sp. DHA-rich algal oils (Food Standards Agency, 2012). To date, algal oil produced from *Schizochytrium* sp. has been approved for direct use in foods by the U.S. FDA, Health Canada, European Union, Food Standards Agency of Australia, China's Ministry of Health, and Brazil's National Health Surveillance Agency (FDA, 2014a).

The safety of orally administered DHA from many different sources (e.g., fish oil) including Mara's proposed algal source (*Schizochytrium sp.* T18) have been extensively characterized in the publicly available preclinical and clinical study literature. The compositional profile of the proposed DHA-rich algal oil from T18 presents no obvious safety concerns. Finally, similar DHA products have been reviewed and approved around the world for addition to infant formula food as well as food.

General Recognition of the Safety of DHA Algal Oil

The intended use of DHA-rich algal oil has been determined to be safe through scientific procedures as set forth in 21 CFR § 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination and is based on the following:

- The DHA product that is the subject of this GRAS determination is extracted and refined oil from the wild-type heterotrophic microalgae *Schizochytrium* sp. T18. It is a mixture of triglycerides containing mostly PUFA in which the predominant fatty acid (>35%) is DHA. The DHA manufacturing process starts with fermentation followed by refining of the crude DHA algal oil isolated from the

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fermentation process. The DHA algal oil product is manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes.

- The possible presence of microalgae toxins from *Schizochytrium* sp. has been previously addressed as part of a substantial equivalence submission (ONC, 2011) and in GRAS Notification (GRN) No. 553 (FDA, 2014a). Toxin production is unlikely since there are no known reports of toxin production by thraustochytrids, of which *Schizochytrium* is a member (ONC, 2011; Hammond et al., 2002). In addition, T18 oil and algal biomass were screened for the presence of toxins including domoic acid, gymnodimine, desmethyl spirolide C, azaspiracid-1, azaspiracid-2, azaspiracid-3, pectenotoxin-2, okadaic acid, dinophysistoxin-1, dinophysistoxin-2, yessotoxin, prymnesin-1, and prymnesin-2, and none were detected (ONC, 2011).

- There is common knowledge of a long history of human consumption of DHA from food and foods containing added DHA such as infant formula, and other products such as dietary supplements. It will be added to infant formula for pre-term and term infants in order to supplement the dietary intake of the omega-3 fatty acid DHA.

- Numerous algal and marine sources of DHA have been evaluated by the FDA and other global regulatory agencies over the past 15 years for proposed incorporation in food for human consumption including infant formula. Relevant US GRAS notifications include GRN 41, GRN 94, GRN 379, and GRN 553 (FDA, 2000; 2001; 2011; 2014). All of the GRAS notices provided information/clinical study data that supported the safety of the proposed DHA ingredients for use in infant formula. In all of the studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues in infants attributable to DHA supplemented formulas when compared to control group infant formulas. The studies supported the safe use of DHA in infant formula up to 1% of total fatty acids.

- Literature searches did not identify safety/toxicity concerns related to any individual fatty acid or their ratios in the proposed DHA algal oil. The proposed DHA oil is similar to other commercially available edible oils incorporated in infant formulas. While the fatty acid profile for the proposed DHA algal oil has higher myristic, palmitic, docosapentaenoic, and cis-vaccenic acid and lower oleic acid concentrations when compared to other algal oil products, it is similar to that found in other algal oils and fish oils (e.g., krill oil) that are currently used in food and/or infant formula.

- The proposed uses of the DHA algal oil from *Schizochytrium* sp. T18 are identical to the approved uses for other GRAS DHA (and/or in combination with ARA)

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products incorporated in exempt (pre-term) and non-exempt (term) infant formulas.

- 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). 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/fish oil is approved by the FDA for addition to human food and infant formula.

- Toxicity testing has been conducted with the proposed DHA-rich algal oil product from *Schizochytrium* sp. T18 and includes acute and subchronic toxicity studies, a battery of genotoxicity studies, and developmental and reproductive toxicity studies. In all of the studies, no evidence of toxicity was noted at the highest dose levels tested, doses approximately 100x or more higher than those proposed for infant formula (i.e., 27-33 mg.kg/day).

- The publicly available scientific literature on the consumption and safety of DHA and DHA algal oil ingredients, in clinical studies in infants and adult humans as well as animals, is extensive and sufficient to support the safety and GRAS status of the proposed DHA algal oil product.

Since this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Determination of the safety and GRAS status of the DHA-rich algal oil that is the subject of this self-determination has been made through the deliberations of an Expert Panel convened by Mara and comprised of Michael Carakostas, DVM, Ph.D., Lewis P. Rubin, MD, and I. Glenn Sipes, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that the proposed DHA-rich algal oil, produced consistent with cGMP and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concluded that these uses of the DHA algal oil product are GRAS based on scientific procedures, and that other experts qualified to assess the safety of foods and food additives would concur with these conclusions. The Panel's GRAS opinion is included as Exhibit 1 to this document.

It is also Mara's opinion that other qualified scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Mara has concluded that DHA-rich algal oil is GRAS under the intended conditions of use on the basis of scientific procedures; and therefore, it is excluded from the definition of a

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food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

Mara is not aware of any information that would be inconsistent with a finding that the proposed use of DHA-rich algal oil in infant formula (pre-term and term infants) meeting appropriate specifications, and used according to cGMP, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

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§ 170.250 Part 7, Supporting Data and Information

The following references are all generally available, unless otherwise noted. Appendices A and B, and Exhibit 1 (Algal toxin analytical report, analytical COAs for DHA algal oil, signed Expert Panel report) are not generally available but are attached for reference.

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APPENDIX A

Algal Toxin Analytical Report

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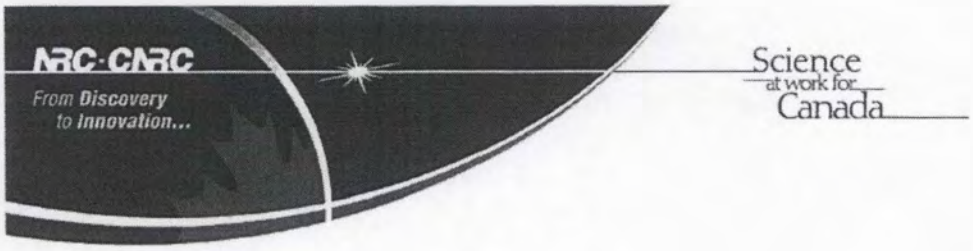

National Research Council Canada

LC-MS screening of algal toxins

October 13, 2010

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Summary

Liquid chromatography - mass spectrometry (LC-MS) was employed to screen for multiple classes of marine toxins in both dried algal biomass and processed oil. An established extraction procedure was employed for algal biomass and an extraction protocol for oil samples was developed and tested. Recovery of the liquid/liquid extraction procedure was measured to be 80% by spiking known amounts of a typical lipophilic toxin into an oil sample void of any toxins and measuring levels by mass spectrometry. Using an in-house high-resolution LC-MS method, no toxins were detected in either the algal biomass nor the oil sample. Therefore, toxin levels were reported as less than the instrumental limits of detection (LOD) for each toxin, corrected for the recovery and the dilution factor of the extraction procedure.

A. SAMPLE INFORMATION

One algal oil sample and one dried biomass sample were received in 15 mL sample vials.

Date Samples received:	August 20, 2010
Client Sample Codes:	ONC-T18 Oil 3T4505 Lot# 22740 (10 grams)
	ONC-T18 Freeze-dried Biomass Lot # 22740 (5 g)
Date Analysis Completed:	August 26, 2010

B. EXPERIMENTAL PROCEDURES

1. Extraction Method

Two sub-samples of algal oil (~ 4 g) and one sub-sample of dried biomass (~4 g) were extracted for marine toxins. To each sample were added three aliquots of 6 mL of methanol/water (1:1, v/v). Samples were placed in the vortex for 10 minutes and centrifuged for 15 minutes @ 3000 ppm. Supernatants were decanted and combined into 25 ml volumetric flasks with 1:1 methanol:water. An aliquot (0.5 mL) was removed from each extract solution and filtered through a centrifugal "spin-filter" (0.45 µm) prior to mass spectrometric analysis.

2. Liquid Chromatographic (LC) Conditions

HPLC column: Waters Acquity HSS T3 1.8 µm 2.1×100 mm
Mobile phase A: Water 0.1 % formic acid
Mobile phase B: Acetonitrile 0.1 % formic acid
Flow rate: 0.4 mL/min, Temperature: 40°C, Injection volume: 3 µL
Gradient elution: 0-30% B in 6 min followed by 30-100% in 4 min.

3. Mass Spectrometric (MS) Conditions

LC-MS instrumentation consisted of a Thermo Accela quaternary pump coupled to a Thermo Exactive mass spectrometer equipped with a HESI-II probe for electrospray ionization. Alternating positive and negative polarity scans were acquired and data was collected at a resolution setting of 50,000 at 2 Hz over a mass range of 100 – 2500 *m/z*.

C. METHOD DEVELOPMENT AND QUALITY CONTROL

Precision and recovery test of extraction protocol

The recovery of the liquid/liquid extraction procedure was determined by spiking known amounts of okadaic acid into sunflower oil, which is presumably void of any marine toxins. Okadaic acid was chosen as a model lipophilic toxin Portions of ~ 4 grams of sunflower oil were spiked at three different levels and extracted with 1:1 methanol:water. Precision and recovery test results are shown in **Table 1**. Recovery was greater than 80% in all cases with precision of roughly 10%. It should be noted, that previous work in our laboratory using an identical liquid/liquid extraction procedure for the highly polar domoic acid also yielded recovery levels greater than 80%. Therefore, this extraction procedure is suitable for a wide polarity range and was chosen to extraction the range of toxins investigated in this study.

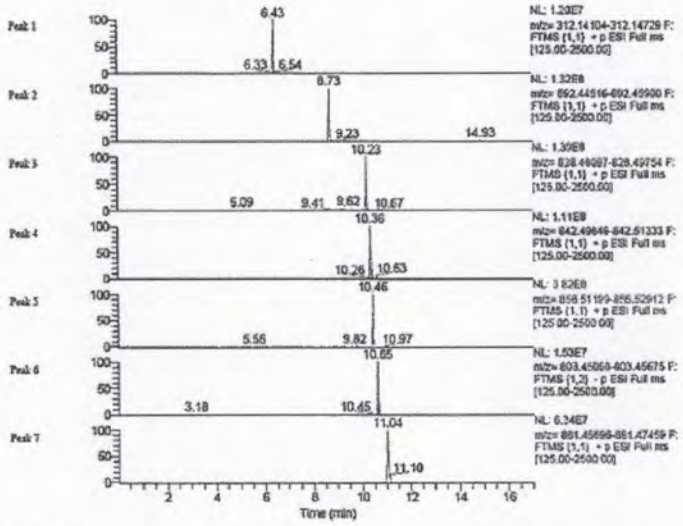
Table 1. Precision and recovery of extraction protocol

Sample	Target concentration (µg/g)	Mean recovery (%)	Standard Deviation (%)
1	0.48	83	9
2	0.24	83	9
3	0.12	84	10

LC-MS method

The high resolution mass spectrometry method was performed on a Thermo Exactive mass spectrometer with a resolving power of 100 000. To accommodate four of the analytes that required negative polarity ionization, alternating positive and negative polarity scans were acquired throughout the length of the chromatographic run. In order to maintain a sufficient number of data points across chromatographic peaks and reduce cycle times, data was acquired at a reduced resolution of 50,000 to allow for acquisitions at 2 Hz. Data was acquired in a non-targeted manner over a wide mass range, in contrast to conventional triple-quadrupole mass spectrometry methods where the analytes are specified in the acquisition method. Data is then processed extracting narrow mass windows (ie 5 ppm) centered around the masses from a specified target list. Finally, the non-targeted data acquired in this study will be archived and available for screening of additional toxins or contaminants upon request.

Shown in **Figure 1** is a typical LC-MS chromatogram for a mixture of toxin standards containing domoic acid, desmethylspirolide C, azaspiracid-1, azaspiracid-2, azaspiracid-3, okadaic acid and pectenotoxin-2. With the exception of okadaic acid that is detected in negative ion mode, all toxins were detected at 1-2 ppm mass accuracy. This method was run daily throughout this study as a quality control of instrument performance.



#	Comp. Index	Compound Name	Formula	Detected m/z	Delta (ppm)	Expected RT	Actual RT	Intensity	Adducts			Fragments		
									H+	NH4+	Na+	1	2	3
1	0	Domoic Acid	C15H21NO6	312.14365	-1.7	0.00	6.43	13021743	Y*	N	N	-	-	-
2	0	Desmethylspirolide C	C42H61NO7	692.45123	-1.2	0.00	8.73	132176160	Y*	N	N	-	-	-
3	0	Azaspiracid 3	C46H65NO12	828.48773	-1.8	0.00	10.23	130214806	Y*	N	N	-	-	-
4	0	Azaspiracid 1	C47H71NO12	842.50854	-1.6	0.00	10.36	110544712	Y*	Y	N	-	-	-
5	0	Azaspiracid 2	C48H73NO12	856.51898	-1.8	0.00	10.46	382147584	Y*	N	N	-	-	-
6	0	Okadaic Acid	C44H68O13	803.46191	4.0	0.00	10.65	15336107	Y*	N	N	-	-	-
7	0	Pectenotoxin 2	C47H70O14	881.46405	-2.0	0.00	11.04	63398068	N	Y	Y*	-	-	-

Figure 1. Typical LC-MS chromatograms for marine toxin standards generated by extracting narrow mass windows (5ppm) centered around the masses from a specified target list.

D. SCREENING RESULTS

Using the LC-MS methodology described above, none of the toxins listed in **Table 2** were detected in either the algal biomass nor the oil sample. When a chromatographic peak was observed in any of the extracted mass chromatograms, even at a retention time not corresponding to the appropriate toxin, data was inspected manually to determine the origin of the signal. For instance, **Figure 1A** displays an apparent signal in the extracted ion chromatograms for domoic acid at *m/z* 312.1447, although the retention time for the peak eluting at 5.3 min does not match the expected retention time for domoic acid. Upon inspection of the mass spectrum for this peak in **Figure 1B**, it is apparent that the signal observed at m/z 312.1447 is due to an isotope or noise generated from the peak at m/z 309.18121, and thus is not attributed to domoic acid. This manual inspection of the data was performed in all cases when a signal of any type was observed, and in all cases the signal could not be attributed to one of the target toxins.

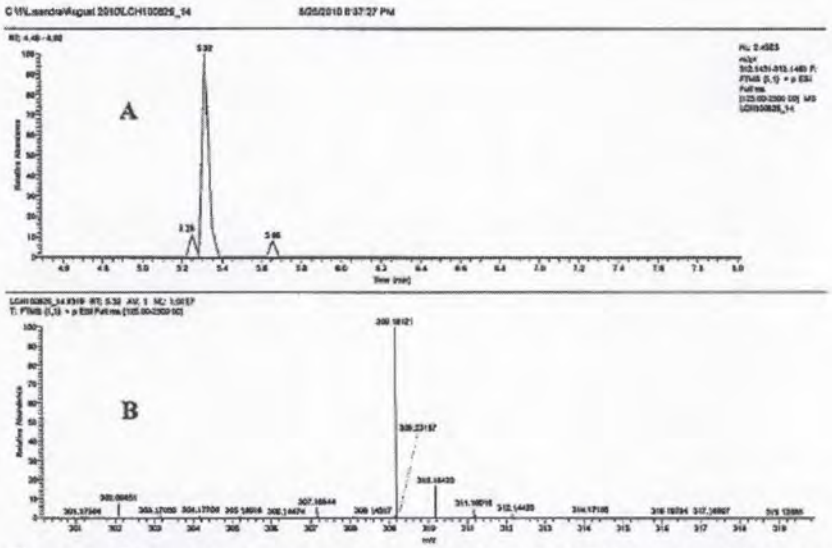


Figure 2. Extracted ion chromatogram for *m/z* 312.1447 (domoic acid) (**A**) and mass spectrum (**B**) for peak eluting at 5.3 minutes in dried biomass sample.

As no toxins were detected in either the algal biomass nor the oil sample, toxin levels were reported as less than the limits of detection (LOD) for each toxin, as listed in **Table 2**. Due to the widely varying ionization efficiencies for the toxins investigated, limits of detection vary by nearly two orders of magnitude, with those detected in negative mode (okadaic acid, Dinophysistoxin-1&2, and yessotoxin) having the largest LODs. To correct for loses and dilution during extraction, instrumental LODs were increased to account for the 80% recovery and the dilution factor of the extraction procedure. As standards are not available for the prymnesins, their LODs were estimated as the average LOD of all other toxins studied. This is a reasonable assumption given the fact that the prymnesins' structures contain a primary amine group that will enhance their ionization efficiencies in positive mode similar to the azaspiracids, while their larger structure would likely yield broader peaks that would lower sensitivity. Therefore, a moderate response factor would be anticipated for the prymnesins.

Table 2. List of toxins screened for this analysis, their monoisotopic masses and limits of detection corrected for recovery and dilution during extraction.

Toxin	Mass-to-charge (m/z)	Result (< LOD in all cases)
Domoic Acid	312.1447	< 27 ppb
Gymnodimine	508.3427	< 3.2 ppb
Desmethylspirolide C	692.4526	< 4.2 ppb
Azaspiracid-1	842.5055	< 4.1 ppb
Azaspiracid-2	856.5211	< 5.0 ppb
Azaspiracid-3	828.4898	< 4.8 ppb
Pectenotoxin-2	881.4663	< 7.9 ppb
Okadaic Acid	803.4582	< 220 ppb
Dinophysistoxin-1	817.4738	< 160 ppb
Dinophysistoxin-2	803.4582	< 120 ppb
Yessotoxin	1141.4706	< 400 ppb
Prymnesin-2	1968.8037	< 86 ppb*
Prymnesin-1	2262.8988	< 86 ppb*

* Standards not available for prymnesins, LOD based on average of all toxins.

APPENDIX B

Certificates of Analysis

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eurofins | WEJ Contaminants

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Person in charge Mrs C. Blaszk - 2912
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Report date 29.09.2016

Analytical report: AR-16-JC-110071-06

This report replaces report number: AR-16-JC-110071-05

Sample Code 706-2016-00520839

Reference	Refined Algal Oil 005-08346-0000506323
Client Sample Code	2
Purchase Order Code	4503288318
Lot-no.	16039.0
Number	2
Amount	2317 g
Reception temperature	room temperature
Ordered by	Ms. Lariza Beristain
Submitted by	Ms. Lariza Beristain
Sender	006-08346-0000102874
Reception date time	14.07.2016
Packaging	aluminium can with ring pull closure
Start/end of analyses	18.07.2016 / 29.07.2016

TEST RESULTS

Physical-chemical Analysis

JJ00V Density
Method: DGF C-IV 2d, mod., PV 01025, Densitometry
Subcontracted to a Eurofins laboratory accredited for this test.

Density	0.917	g/ml

J7035 Colour Lovibond 1"-cuvette
Method: ISO 15305, PV 00106, Visual examination
Subcontracted to a Eurofins laboratory accredited for this test.

Blue	0.0	
Yellow	10.0	
Neutral	0.0	
Red	0.3	

J7112 Moisture and volatile matter content
Method: ISO 662 (method B), mod., PV 00164, Gravimetry
Subcontracted to a Eurofins laboratory accredited for this test.

moisture and volatile matter content	<0.01	* %

J7087 Insoluble impurities content
Method: DIN EN ISO 663, mod., PV 00149, Gravimetry
Subcontracted to a Eurofins laboratory accredited for this test.

Insoluble impurities content	<0.01	* %

This report replaces report number: AR-16-JC-110071-05

JK04T	**Peroxide value**		
Method:	ISO 27107, PV 01148, Potentiometry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Peroxid value		1.0	meqO2/kg
JK073	**Sterol profile and content**		
Method:	Internal Method, PV 01376, LC-GC-FID		
Subcontracted to a Eurofins laboratory accredited for this test.			
Total sterol		900	mg/kg fat
Cholesterol (% total sterols)		21.7	%
Brassicasterol (% total sterols)		6.5	%
24-Methylene-cholesterol (% tot. sterol)		2.8	%
Campesterol (% total sterols)		1.5	%
Campestanol (% total sterols)		<0.1	* %
Stigmasterol (% total sterols)		22.5	%
delta-7-Campesterol (% total sterols)		<0.1	* %
Delta-5,23-stigmastadienol (% total ster.)		3.0	%
Clerosterol (% total sterols)		14.5	%
Beta-Sitosterol "real" (% total sterols)		14.8	%
Sitostanol (% total sterols)		<0.1	* %
Delta-5-avenasterol (% total sterols)		3.8	%
Delta-5,24-stigmastadienol (% total sterols)		4.1	%
Delta-7-stigmastenol (% total sterols)		<0.1	* %
Delta-7-avenasterol (% total sterols)		5.0	%
JK07L	**Erucic acid (% total fat)**		
Method:	ISO 12966-2 and ISO 5508, PV 00100, PV 01362, GC-FID		
Subcontracted to a Eurofins laboratory accredited for this test.			
Erucic acid C22:1n9		<0.1	* %
JK07G	**Unsaponifiable matter**		
Method:	ISO 18609, PV 01377, Gravimetry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Unsaponifiable matter		0.3	%
JJ0HV	**Free fatty acids (FFA)**		
Method:	DGF C-V 2, PV 01147, Titrimetry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Acid value (mg KOH/g)		Not analysable	mg KOH/g
J1001	**Sample preparation (#)**		
Method:	§64 LFGB L 00.00-19/1, CON-PV 00001, Digestion (microwave)		
J8306	**Lead (Pb) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Lead (Pb)		<0.05	* mg/kg
J8308	**Cadmium (Cd) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Cadmium (Cd)		<0.01	* mg/kg
JCHG2	**Mercury (Hg) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Mercury (Hg)		<0.005	* mg/kg
J8312	**Arsenic (As) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Arsenic (As)		<0.1	* mg/kg
JJW2B	**Copper (Cu) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Copper (Cu)		<0.1	* mg/kg

This report replaces report number: AR-16-JC-110071-05

JJ0CJ	**Iron (Fe) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Iron (Fe)		<0.5	* mg/kg
JJ0CG	**Chromium (Cr) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Chromium (Cr)		<0.05	* mg/kg
JJ0CM	**Nickel (Ni) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Nickel (Ni)		<0.1	* mg/kg
JJ0CI	**Manganese (Mn) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Manganese (Mn)		<0.1	* mg/kg
JJ0CW	**Phosphorus (P) (#)**		
Method:	DIN EN ISO 17294-2-E29, PV000857, ICP-MS		
Phosphorus		<3	* mg/kg
J1054	**Sulphur (S) (#)**		
Method:	DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES		
Sulphur total (S)		3.0	mg/kg
		± 2	mg/kg
J1056	**Silicon (Si) (#)**		
Method:	DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES		
Silicon (Si)		110	mg/kg
		± 23	mg/kg
J8318	**Molybdenum (Mo) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Molybdenum (Mo)		<0.1	* mg/kg
JKB7E	**Nutrient value in 100 ml**		
Method:	according to regulation 1169/2011, , Calculation		
Subcontracted to a Eurofins laboratory			
Energy		3393	kJ
Energy		825	kcal
Salt		0.00	g
of which sugars		0.0	g
Protein		0.0	g
Fat		91.7	g
Carbohydrate		0.0	g

Analytical report: AR-16-JC-110071-06
Sample Code 706-2016-00520839

This report replaces report number: AR-16-JC-110071-05

Microbiological Analysis

UM8TK Mould 25°C-Yeast 25°C E [aw ≤ 0.95] <1 >150 /ml
Method: ISO 21527-2, PV 0002, E-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Moulds 25°C	< 10	cfu/ml
Yeast 25°C	< 10	cfu/ml

UMFD3 Coagulase positive Staphylococcus 37°C E <10 >4500 /g (1) BP Agar-S ISO 6888-1-M
Method: ISO 6888-1-M, , Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Coagulase positive Staphylococcus 37°C	< 10	cfu/g

UM44H Coliforms 30°C E <10 >15000 /g (1-2) VRB Agar-P ISO 4832
Method: ISO 4832, , E-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Coliforms 30°C	< 10	cfu/g

UMH7G Escherichia coli 44°C E <10 >1500 /g (1) TBX Agar-P ISO 16649-2
Method: ISO 16649-2:, , E-Cultural technique (chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Escherichia coli 44°C	< 10	cfu/g

UMTK5 Salmonella D Abs Pres /25 g ISO 6579
Method: ISO 6579, , D-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Salmonella	Not detectable	/25 g

* = Below indicated quantification level
(#) = Eurofins WEJ Contaminants GmbH (Hamburg) is accredited for this test.
Result +/- expanded measurement uncertainty (95%; k=2)

Signature (b) (6)

Analytical Service Manager (Carolina Blaszk)

eurofins | Nutrition Analysis Center

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Eurofins Sample Code: 464-2016-08190198
Sample Description: Algal Oil
Client Sample Code: 16039.0
PO Number:
Client Code: QD0007275

Entry Date: 10/07/2016
Reporting Date: 10/07/2016

MARA RENEWABLES CORPORATION
attn: LARIZA BERIATAIN
101 RESEARCH DRIVE
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CANADA

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CANADA

CERTIFICATE OF ANALYSIS

AR-16-QD-123421-02

This analytical report supersedes AR-16-QD-123421-01.

Test	Result	
QD052 - Protein - Combustion		**Completed: 08/22/2016**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93		
Protein, Combustion	<0.10 %	
QD681 - Ash - low level		**Completed: 08/23/2016**
AOAC 942.05		
Ash	<0.05 %	
QD059 - Fat by Acid Hydrolysis		**Completed: 08/23/2016**
AOAC 954.02		
Crude Fat By Acid Hydrolysis	100.24 %	
QD005 - Acid Value		**Completed: 08/22/2016**
AOCS Cd 3d-63		
Acid value	0.05 mg KOH/g	

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing
Cert:3329:01

Chemical Testing
Cert:2927:01

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Eurofins Sample Code: 464-2016-08290202
Sample Description: Algal Oil
Client Sample Code: 16039
PO Number:
Client Code: QD0007275

Entry Date: 09/29/2016
Reporting Date: 09/29/2016

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CERTIFICATE OF ANALYSIS

AR-16-QD-128376-03

This analytical report supersedes AR-16-QD-128376-02.

Test	Result
QA934 - Trans Fatty Acids, relative area% (GC-FID)	**Completed: 09/07/2016**
AOCS 2a-94	
C 18:1 (trans) Elaidic acid	<0.01 %
C 18:2 (c9/t11)	0.08 %
C 18:3 (trans/cis/cis)	0.13 %
total trans fatty acids C18:1	<0.01 %
total trans fatty acids C18:2 (without CLA)	0.08 %
total trans fatty acids C18:3	0.13 %
Total Trans Fatty Acids	0.20 %

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

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Eurofins Sample Code: 464-2016-08290202
Sample Description: Algal Oil
Client Sample Code: 16039
PO Number:
Client Code: QD0007275

Entry Date: 09/29/2016
Reporting Date: 09/29/2016

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CERTIFICATE OF ANALYSIS

AR-16-QD-128376-04

This analytical report supersedes AR-16-QD-128376-03.

Test	Result
QD084 - Fatty Acid Profile, % Relative	**Completed: 09/29/2016**
AOCS Ce 2-66	
Fatty Acid Profile, % Relative	Area Percent
C08:0 Octanoic (Caprylic)	<0.10 %
C10:0 Decanoic (Capric)	<0.10 %
C11:0 Undecanoic (Hendecanoic)	<0.10 %
C12:0 Dodecanoic (Lauric)	0.92 %
C13:0 Tridecanoic	<0.10 %
C14:0 Tetradecanoic (Myristic)	12.30 %
C14:1 Tetradecenoic (Myristoleic)	<0.10 %
C15:0 Pentadecanoic	0.68 %
C15:1 Pentadecenoic	<0.10 %
C16:0 Hexadecanoic (Palmitic)	22.67 %
C16:1 Hexadecenoic (Palmitoleic)	6.16 %
C16:2 Hexadecadienoic	<0.10 %
C16:3 Hexadecatrienoic	<0.10 %
C16:4 Hexadecatetraenoic	<0.10 %
C17:0 Heptadecanoic (Margaric)	0.15 %
C17:1 Heptadecenoic Margaroleic	<0.10 %
C18:0 Octadecanoic (Stearic)	0.77 %
C18:1 Octadecenoic (Oleic)	7.49 %
C18:2 Octadecadienoic (Linoleic)	0.34 %
C18:3 Octadecatrienoic (Linolenic)	0.24 %
C18:4 Octadecatetraenoic	0.24 %
C20:0 Eicosanoic (Arachidic)	<0.10 %
C20:1 Eicosenoic (Gondoic)	<0.10 %
C20:2 Eicosadienoic	<0.10 %
C20:3 Eicosatrienoic	<0.10 %
C20:4 Eicosatetraenoic (Arachidonic)	0.65 %
C20:5 Eicosapentaenoic	1.08 %
C21:5 Heneicosapentaenoic	<0.10 %
C22:0 Docosanoic (Behenic)	<0.10 %
C22:1 Docosenoic (Erucic)	<0.10 %
C22:2 Docosadienoic	<0.10 %
C22:3 Docosatrienoic	<0.10 %

Eurofins Sample Code: 464-2016-08290202
Client Sample Code: 16039

Test	Result
QD084 - Fatty Acid Profile, % Relative (Cont.)	**Completed: 09/29/2016**
AOCS Ce 2-66	
C22:4 Docosatetraenoic	<0.10 %
C22:5 Docosapentaenoic	7.21 %
C22:6 Docosahexaenoic	37.10 %
C24:0 Tetracosanoic (Lignoceric)	<0.10 %
C24:1 Tetracosenoic (Nervonic)	0.41 %
Unknown Components	0.91 %

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing
Cert:3329:01

Chemical Testing
Cert:2927:01

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Eurofins Sample Code: 464-2016-08190199
Sample Description: Algal Oil
Client Sample Code: 16040
PO Number:
Client Code: QD0007275

Entry Date: 10/07/2016
Reporting Date: 10/07/2016

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CERTIFICATE OF ANALYSIS

AR-16-QD-123422-02

This analytical report supersedes AR-16-QD-123422-01.

Test	Result	
QD052 - Protein - Combustion		**Completed: 08/22/2016**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93		
Protein, Combustion	<0.10 %	
QD681 - Ash - low level		**Completed: 08/23/2016**
AOAC 942.05		
Ash	<0.05 %	
QD059 - Fat by Acid Hydrolysis		**Completed: 08/23/2016**
AOAC 954.02		
Crude Fat By Acid Hydrolysis	99.17 %	
QD005 - Acid Value		**Completed: 08/22/2016**
AOCS Cd 3d-63		
Acid value	0.06 mg KOH/g	

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing
Cert:3329:01

Chemical Testing
Cert:2927:01

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Eurofins Sample Code: 464-2016-08290203
Sample Description: Algal Oil
Client Sample Code: 16040
PO Number:
Client Code: QD0007275

Entry Date: 09/29/2016
Reporting Date: 09/29/2016

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CANADA

CERTIFICATE OF ANALYSIS

AR-16-QD-128377-03

This analytical report supersedes AR-16-QD-128377-02.

Test	Result
QA934 - Trans Fatty Acids, relative area% (GC-FID)	**Completed: 09/07/2016**
AOCS 2a-94	
C 18:1 (trans) Elaidic acid	<0.01 %
C 18:2 (c9/t11)	0.08 %
C 18:3 (cis/trans/cis)	0.15 %
total trans fatty acids C18:1	<0.01 %
total trans fatty acids C18:2 (without CLA)	0.08 %
total trans fatty acids C18:3	0.15 %
Total Trans Fatty Acids	0.22 %

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

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KANADA

Person in charge	Mrs C. Blaszk	- 2912
Client support	Mrs C. Blaszk	- 2912

Report date 29.09.2016

Analytical report: AR-16-JC-108561-07

This report replaces report number: AR-16-JC-108561-06

Sample Code 706-2016-00520840

Reference	Refined Algal Oil 005-08346-0000506324
Client Sample Code	3
Purchase Order Code	4503288318
Lot-no.	16040.0
Number	2
Amount	2310 g
Reception temperature	room temperature
Ordered by	Ms. Lariza Beristain
Submitted by	Ms. Lariza Beristain
Sender	006-08346-0000102874
Reception date time	14.07.2016
Packaging	aluminium can with ring pull closure
Start/end of analyses	18.07.2016 / 25.07.2016

TEST RESULTS

Physical-chemical Analysis

JJ00V Density
Method: DGF C-IV 2d, mod., PV 01025, Densitometry
Subcontracted to a Eurofins laboratory accredited for this test.

Density	0.934	g/ml

J7035 Colour Lovibond 1"-cuvette
Method: ISO 15305, PV 00106, Visual examination
Subcontracted to a Eurofins laboratory accredited for this test.

Blue	0.0
Yellow	11.6
Neutral	0.3
Red	1.2

J7112 Moisture and volatile matter content
Method: ISO 662 (method B), mod., PV 00164, Gravimetry
Subcontracted to a Eurofins laboratory accredited for this test.

moisture and volatile matter content	<0.01	* %

J7087 Insoluble impurities content
Method: DIN EN ISO 663, mod., PV 00149, Gravimetry
Subcontracted to a Eurofins laboratory accredited for this test.

Insoluble impurities content	<0.01	* %

This report replaces report number: AR-16-JC-108561-06

JK04T	**Peroxide value**		
Method:	ISO 27107, PV 01148, Potentiometry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Peroxid value		1.0	meqO2/kg
JK073	**Sterol profile and content**		
Method:	Internal Method, PV 01376, LC-GC-FID		
Subcontracted to a Eurofins laboratory accredited for this test.			
Total sterol		1070	mg/kg fat
Cholesterol (% total sterols)		14.5	%
Brassicasterol (% total sterols)		4.6	%
24-Methylene-cholesterol (% tot. sterol)		2.3	%
Campesterol (% total sterols)		3.9	%
Campestanol (% total sterols)		<0.1	* %
Stigmasterol (% total sterols)		23.1	%
delta-7-Campesterol (% total sterols)		<0.1	* %
Delta-5,23-stigmastadienol (% total ster.)		3.8	%
Clerosterol (% total sterols)		19.3	%
Beta-Sitosterol "real" (% total sterols)		11.4	%
Sitostanol (% total sterols)		0.5	%
Delta-5-avenasterol (% total sterols)		4.7	%
Delta-5,24-stigmastadienol (% total sterols)		6.8	%
Delta-7-stigmastenol (% total sterols)		<0.1	* %
Delta-7-avenasterol (% total sterols)		5.1	%
JK07L	**Erucic acid (% total fat)**		
Method:	ISO 12966-2 and ISO 5508, PV 00100, PV 01362, GC-FID		
Subcontracted to a Eurofins laboratory accredited for this test.			
Erucic acid C22:1n9		<0.1	* %
JK07G	**Unsaponifiable matter**		
Method:	ISO 18609, PV 01377, Gravimetry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Unsaponifiable matter		0.4	%
JJ0HV	**Free fatty acids (FFA)**		
Method:	DGF C-V 2, PV 01147, Titrimetry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Acid value (mg KOH/g)		Not analysable	mg KOH/g
J1001	**Sample preparation (#)**		
Method:	§64 LFGB L 00.00-19/1, CON-PV 00001, Digestion (microwave)		
J8306	**Lead (Pb) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Lead (Pb)		<0.05	* mg/kg
J8308	**Cadmium (Cd) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Cadmium (Cd)		<0.01	* mg/kg
JCHG2	**Mercury (Hg) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Mercury (Hg)		<0.005	* mg/kg
J8312	**Arsenic (As) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Arsenic (As)		<0.1	* mg/kg
JJW2B	**Copper (Cu) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Copper (Cu)		<0.1	* mg/kg

This report replaces report number: AR-16-JC-108561-06

JJ0CJ	**Iron (Fe) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Iron (Fe)		<0.1	* mg/kg
JJ0CG	**Chromium (Cr) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Chromium (Cr)		<0.05	* mg/kg
JJ0CM	**Nickel (Ni) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Nickel (Ni)		<0.1	* mg/kg
JJ0CI	**Manganese (Mn) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Manganese (Mn)		<0.1	* mg/kg
JJ0CW	**Phosphorus (P) (#)**		
Method:	DIN EN ISO 17294-2-E29, PV000857, ICP-MS		
Phosphorus		<3	* mg/kg
J1054	**Sulphur (S) (#)**		
Method:	DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES		
Sulphur total (S)		<2	* mg/kg
J1056	**Silicon (Si) (#)**		
Method:	DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES		
Silicon (Si)		51	mg/kg
		± 10	mg/kg
J8318	**Molybdenum (Mo) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Molybdenum (Mo)		<0.1	* mg/kg
JKB7E	**Nutrient value in 100 ml**		
Method:	according to regulation 1169/2011, Calculation		
Subcontracted to a Eurofins laboratory			
Salt		0.00	g
Energy		3456	kJ
Energy		841	kcal
Protein		0.0	g
Carbohydrate		0.0	g
Fat		93.4	g
of which sugars		0.0	g

Analytical report: AR-16-JC-108561-07
Sample Code 706-2016-00520840

This report replaces report number: AR-16-JC-108561-06

Microbiological Analysis

UM8TK **Mould 25°C-Yeast 25°C E [aw ≤ 0.95] <1 >150 /ml**
Method: ISO 21527-2, PV 0002, E-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Moulds 25°C	< 10	cfu/ml
Yeast 25°C	< 10	cfu/ml

UMCMW **Coagulase positive Staphylococcus 37°C E <10 >1500 /ml (0) BP Agar-S ISO 6888-1**
Method: EN ISO 6888-1, , E-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Coagulase positive Staphylococcus 37°C	< 10	cfu/ml

UM44H **Coliforms 30°C E <10 >15000 /g (1-2) VRB Agar-P ISO 4832**
Method: ISO 4832, , E-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Coliforms 30°C	< 10	cfu/g

UMH7G **Escherichia coli 44°C E <10 >1500 /g (1) TBX Agar-P ISO 16649-2**
Method: ISO 16649-2:, , E-Cultural technique (chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Escherichia coli 44°C	< 10	cfu/g

UMTK5 **Salmonella D Abs Pres /25 g ISO 6579**
Method: ISO 6579, , D-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Salmonella	Not detectable	/25 g

* = Below indicated quantification level
(#) = Eurofins WEJ Contaminants GmbH (Hamburg) is accredited for this test.
Result +/- expanded measurement uncertainty (95%; k=2)

Signature (b) (6)

Analytical Service Manager (Carolina Blaszk)

eurofins | Nutrition Analysis Center

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Nutrition Analysis Center
2200 Rittenhouse Street, Suite 150
Des Moines, IA 50321
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Eurofins Sample Code: 464-2016-08290203
Sample Description: Algal Oil
Client Sample Code: 16040
PO Number:
Client Code: QD0007275

Entry Date: 09/29/2016
Reporting Date: 09/29/2016

MARA RENEWABLES CORPORATION
attn: LARIZA BERIATAIN
101 RESEARCH DRIVE
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CANADA

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CERTIFICATE OF ANALYSIS

AR-16-QD-128377-04

This analytical report supersedes AR-16-QD-128377-03.

Test	Result
QD084 - Fatty Acid Profile, % Relative	**Completed: 09/29/2016**
AOCS Ce 2-66	
Fatty Acid Profile, % Relative	Area Percent
C08:0 Octanoic (Caprylic)	<0.10 %
C10:0 Decanoic (Capric)	<0.10 %
C11:0 Undecanoic (Hendecanoic)	<0.10 %
C12:0 Dodecanoic (Lauric)	0.74 %
C13:0 Tridecanoic	<0.10 %
C14:0 Tetradecanoic (Myristic)	9.00 %
C14:1 Tetradecenoic (Myristoleic)	<0.10 %
C15:0 Pentadecanoic	0.45 %
C15:1 Pentadecenoic	<0.10 %
C16:0 Hexadecanoic (Palmitic)	21.46 %
C16:1 Hexadecenoic (Palmitoleic)	3.63 %
C16:2 Hexadecadienoic	<0.10 %
C16:3 Hexadecatrienoic	<0.10 %
C16:4 Hexadecatetraenoic	<0.10 %
C17:0 Heptadecanoic (Margaric)	0.12 %
C17:1 Heptadecenoic Margaroleic	<0.10 %
C18:0 Octadecanoic (Stearic)	0.83 %
C18:1 Octadecenoic (Oleic)	8.06 %
C18:2 Octadecadienoic (Linoleic)	0.78 %
C18:3 Octadecatrienoic (Linolenic)	0.42 %
C18:4 Octadecatetraenoic	0.32 %
C20:0 Eicosanoic (Arachidic)	<0.10 %
C20:1 Eicosenoic (Gondoic)	<0.10 %
C20:2 Eicosadienoic	<0.10 %
C20:3 Eicosatrienoic	<0.10 %
C20:4 Eicosatetraenoic (Arachidonic)	0.76 %
C20:5 Eicosapentaenoic	1.59 %
C21:5 Heneicosapentaenoic	<0.10 %
C22:0 Docosanoic (Behenic)	<0.10 %
C22:1 Docosenoic (Erucic)	<0.10 %
C22:2 Docosadienoic	<0.10 %
C22:3 Docosatrienoic	<0.10 %

Eurofins Sample Code: 464-2016-08290203
Client Sample Code: 16040

Test	Result
QD084 - Fatty Acid Profile, % Relative (Cont.)	**Completed: 09/29/2016**
AOCS Ce 2-66	
C22:4 Docosatetraenoic	<0.10 %
C22:5 Docosapentaenoic	7.65 %
C22:6 Docosahexaenoic	42.47 %
C24:0 Tetracosanoic (Lignoceric)	<0.10 %
C24:1 Tetracosenoic (Nervonic)	<0.10 %
Unknown Components	1.19 %

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing
Cert:3329:01

Chemical Testing
Cert:2927:01

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Mara Renewables Corporation
attn. Ms. Lariza Beristain
101 Research Drive
B2Y 4T6 Dartmouth, Nova Scotia
KANADA

Person in charge Mrs C. Blaszk - 2912
Client support Mrs C. Blaszk - 2912

Report date 29.09.2016

Analytical report: AR-16-JC-108560-08

This report replaces report number: AR-16-JC-108560-07

Sample Code 706-2016-00520841

Reference	Refined Algal Oil 005-08346-0000506325
Client Sample Code	4
Purchase Order Code	4503288318
Lot-no.	16041.0
Number	2
Amount	2311 g
Reception temperature	room temperature
Ordered by	Ms. Lariza Beristain
Submitted by	Ms. Lariza Beristain
Sender	006-08346-0000102874
Reception date time	14.07.2016
Packaging	aluminium can with ring pull closure
Start/end of analyses	18.07.2016 / 25.07.2016

TEST RESULTS

Physical-chemical Analysis

JJ00V Density
Method: DGF C-IV 2d, mod., PV 01025, Densitometry
Subcontracted to a Eurofins laboratory accredited for this test.

Density	0.934	g/ml

J7035 Colour Lovibond 1"-cuvette
Method: ISO 15305, PV 00106, Visual examination
Subcontracted to a Eurofins laboratory accredited for this test.

Blue	0.0
Yellow	12.1
Neutral	0.5
Red	1.1

J7112 Moisture and volatile matter content
Method: ISO 662 (method B), mod., PV 00164, Gravimetry
Subcontracted to a Eurofins laboratory accredited for this test.

moisture and volatile matter content	<0.01	* %

J7087 Insoluble impurities content
Method: DIN EN ISO 663, mod., PV 00149, Gravimetry
Subcontracted to a Eurofins laboratory accredited for this test.

Insoluble impurities content	<0.01	* %

This report replaces report number: AR-16-JC-108560-07

JK04T	**Peroxide value**		
Method:	ISO 27107, PV 01148, Potentiometry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Peroxid value		1.3	meqO2/kg
JK073	**Sterol profile and content**		
Method:	Internal Method, PV 01376, LC-GC-FID		
Subcontracted to a Eurofins laboratory accredited for this test.			
Total sterol		831	mg/kg fat
Cholesterol (% total sterols)		12.8	%
Brassicasterol (% total sterols)		6.3	%
24-Methylene-cholesterol (% tot. sterol)		3.3	%
Campesterol (% total sterols)		3.2	%
Campestanol (% total sterols)		<0.1	* %
Stigmasterol (% total sterols)		21.7	%
delta-7-Campesterol (% total sterols)		<0.1	* %
Delta-5,23-stigmastadienol (% total ster.)		<0.1	* %
Clerosterol (% total sterols)		17.9	%
Beta-Sitosterol "real" (% total sterols)		13.7	%
Sitostanol (% total sterols)		<0.1	* %
Delta-5-avenasterol (% total sterols)		5.7	%
Delta-5,24-stigmastadienol (% total sterols)		6.2	%
Delta-7-stigmastenol (% total sterols)		<0.1	* %
Delta-7-avenasterol (% total sterols)		9.1	%
JK07L	**Erucic acid (% total fat)**		
Method:	ISO 12966-2 and ISO 5508, PV 00100, PV 01362, GC-FID		
Subcontracted to a Eurofins laboratory accredited for this test.			
Erucic acid C22:1n9		<0.1	* %
JK07G	**Unsaponifiable matter**		
Method:	ISO 18609, PV 01377, Gravimetry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Unsaponifiable matter		0.3	%
JJ0HV	**Free fatty acids (FFA)**		
Method:	DGF C-V 2, PV 01147, Titrimetry		
Subcontracted to a Eurofins laboratory accredited for this test.			
Acid value (mg KOH/g)		Not analysable	mg KOH/g
J1001	**Sample preparation (#)**		
Method:	§64 LFGB L 00.00-19/1, CON-PV 00001, Digestion (microwave)		
J8306	**Lead (Pb) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Lead (Pb)		<0.05	* mg/kg
J8308	**Cadmium (Cd) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Cadmium (Cd)		<0.01	* mg/kg
JCHG2	**Mercury (Hg) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Mercury (Hg)		<0.005	* mg/kg
J8312	**Arsenic (As) (#)**		
Method:	EN 15763:2009, CON-PV 01274, ICP-MS		
Arsenic (As)		<0.1	* mg/kg
JJW2B	**Copper (Cu) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Copper (Cu)		<0.1	* mg/kg

This report replaces report number: AR-16-JC-108560-07

JJ0CJ	**Iron (Fe) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Iron (Fe)		<0.1	* mg/kg
JJ0CG	**Chromium (Cr) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Chromium (Cr)		<0.05	* mg/kg
JJ0CM	**Nickel (Ni) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Nickel (Ni)		<0.1	* mg/kg
JJ0CI	**Manganese (Mn) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Manganese (Mn)		<0.1	* mg/kg
JJ0CW	**Phosphorus (P) (#)**		
Method:	DIN EN ISO 17294-2-E29, PV000857, ICP-MS		
Phosphorus		<3	* mg/kg
J1054	**Sulphur (S) (#)**		
Method:	DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES		
Sulphur total (S)		<2	* mg/kg
J1056	**Silicon (Si) (#)**		
Method:	DIN EN ISO 11885, mod., CON-PV 00006, ICP-OES		
Silicon (Si)		67	mg/kg
		± 13	mg/kg
J8318	**Molybdenum (Mo) (#)**		
Method:	DIN EN ISO 17294-2-E29, CON-PV 00857, ICP-MS		
Molybdenum (Mo)		<0.1	* mg/kg
JKB7E	**Nutrient value in 100 ml**		
Method:	according to regulation 1169/2011, Calculation		
Subcontracted to a Eurofins laboratory			
Salt		0.00	g
Energy		3456	kJ
Energy		841	kcal
Protein		0.0	g
Carbohydrate		0.0	g
Fat		93.4	g
of which sugars		0.0	g

Analytical report: AR-16-JC-108560-08
Sample Code 706-2016-00520841

This report replaces report number: AR-16-JC-108560-07

Microbiological Analysis

UM8TK Mould 25°C-Yeast 25°C E [aw ≤ 0.95] <1 >150 /ml
Method: ISO 21527-2, PV 0002, E-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Moulds 25°C	< 10	cfu/ml
Yeast 25°C	< 10	cfu/ml

UMCMW Coagulase positive Staphylococcus 37°C E <10 >1500 /ml (0) BP Agar-S ISO 6888-1
Method: EN ISO 6888-1, , E-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Coagulase positive Staphylococcus 37°C	< 10	cfu/ml

UMG2T Coliforms 30°C E <1 >1500 /ml (0-1) VRB Agar-P ISO 4832
Method: ISO 4832, PV0003, E-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Coliforms 30°C	< 1	cfu/ml

UMIKP Escherichia coli 44°C E <1 >150 /ml (0) TBX Agar-P ISO 16649-2
Method: ISO 16649-2:, , E-Cultural technique (chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Escherichia coli 44°C	< 1	cfu/ml

UMTK5 Salmonella D Abs Pres /25 g ISO 6579
Method: ISO 6579, , D-Cultural technique (non-chromogenic media)
Subcontracted to a Eurofins laboratory accredited for this test.

Salmonella	Not detectable	/25 g

* = Below indicated quantification level
(#) = Eurofins WEJ Contaminants GmbH (Hamburg) is accredited for this test.
Result +/- expanded measurement uncertainty (95%; k=2)

Signature (b) (6)

Analytical Service Manager (Carolina Blaszk)

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Eurofins Sample Code: 464-2016-08290204
Sample Description: Algal Oil
Client Sample Code: 16041
PO Number:
Client Code: QD0007275

Entry Date: 09/29/2016
Reporting Date: 09/29/2016

MARA RENEWABLES CORPORATION
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CERTIFICATE OF ANALYSIS

AR-16-QD-128378-03

This analytical report supersedes AR-16-QD-128378-02.

Test	Result
QA934 - Trans Fatty Acids, relative area% (GC-FID)	**Completed: 09/08/2016**
AOCS 2a-94	
C 18:1 (trans) Elaidic acid	<0.01 %
C 18:2 (c9/t11)	0.08 %
C 18:3 (cis/trans/cis)	0.15 %
total trans fatty acids C18:1	<0.01 %
total trans fatty acids C18:2 (without CLA)	0.08 %
total trans fatty acids C18:3	0.15 %
Total Trans Fatty Acids	0.23 %

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

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Eurofins Sample Code: 464-2016-08190200
Sample Description: Algal Oil
Client Sample Code: 16041
PO Number:
Client Code: QD0007275

Entry Date: 10/07/2016
Reporting Date: 10/07/2016

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CERTIFICATE OF ANALYSIS

AR-16-QD-123423-02

This analytical report supersedes AR-16-QD-123423-01.

Test	Result	
QD052 - Protein - Combustion		**Completed: 08/22/2016**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93		
Protein, Combustion	<0.10 %	
QD681 - Ash - low level		**Completed: 08/23/2016**
AOAC 942.05		
Ash	<0.05 %	
QD059 - Fat by Acid Hydrolysis		**Completed: 08/23/2016**
AOAC 954.02		
Crude Fat By Acid Hydrolysis	99.86 %	
QD005 - Acid Value		**Completed: 08/22/2016**
AOCS Cd 3d-63		
Acid value	0.05 mg KOH/g	

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing
Cert:3329:01

Chemical Testing
Cert:2927:01

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Eurofins Sample Code: 464-2016-08290204
Sample Description: Algal Oil
Client Sample Code: 16041
PO Number:
Client Code: QD0007275

Entry Date: 09/29/2016
Reporting Date: 09/29/2016

MARA RENEWABLES CORPORATION
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CERTIFICATE OF ANALYSIS

AR-16-QD-128378-04

This analytical report supersedes AR-16-QD-128378-03.

Test	Result
QD084 - Fatty Acid Profile, % Relative	**Completed: 09/29/2016**
AOCS Ce 2-66	
Fatty Acid Profile, % Relative	Area Percent
C08:0 Octanoic (Caprylic)	<0.10 %
C10:0 Decanoic (Capric)	<0.10 %
C11:0 Undecanoic (Hendecanoic)	<0.10 %
C12:0 Dodecanoic (Lauric)	0.79 %
C13:0 Tridecanoic	<0.10 %
C14:0 Tetradecanoic (Myristic)	9.50 %
C14:1 Tetradecenoic (Myristoleic)	<0.10 %
C15:0 Pentadecanoic	0.56 %
C15:1 Pentadecenoic	<0.10 %
C16:0 Hexadecanoic (Palmitic)	21.76 %
C16:1 Hexadecenoic (Palmitoleic)	4.21 %
C16:2 Hexadecadienoic	<0.10 %
C16:3 Hexadecatrienoic	<0.10 %
C16:4 Hexadecatetraenoic	<0.10 %
C17:0 Heptadecanoic (Margaric)	0.14 %
C17:1 Heptadecenoic Margaroleic	<0.10 %
C18:0 Octadecanoic (Stearic)	0.78 %
C18:1 Octadecenoic (Oleic)	7.26 %
C18:2 Octadecadienoic (Linoleic)	0.56 %
C18:3 Octadecatrienoic (Linolenic)	0.33 %
C18:4 Octadecatetraenoic	0.30 %
C20:0 Eicosanoic (Arachidic)	<0.10 %
C20:1 Eicosenoic (Gondoic)	<0.10 %
C20:2 Eicosadienoic	<0.10 %
C20:3 Eicosatrienoic	<0.10 %
C20:4 Eicosatetraenoic (Arachidonic)	0.75 %
C20:5 Eicosapentaenoic	1.49 %
C21:5 Heneicosapentaenoic	<0.10 %
C22:0 Docosanoic (Behenic)	<0.10 %
C22:1 Docosenoic (Erucic)	<0.10 %
C22:2 Docosadienoic	<0.10 %
C22:3 Docosatrienoic	<0.10 %

Eurofins Sample Code: 464-2016-08290204
Client Sample Code: 16041

Test	Result
QD084 - Fatty Acid Profile, % Relative (Cont.)	**Completed: 09/29/2016**
AOCS Ce 2-66	
C22:4 Docosatetraenoic	<0.10 %
C22:5 Docosapentaenoic	8.12 %
C22:6 Docosahexaenoic	41.98 %
C24:0 Tetracosanoic (Lignoceric)	<0.10 %
C24:1 Tetracosenoic (Nervonic)	<0.10 %
Unknown Components	0.95 %

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing
Cert:3329:01

Chemical Testing
Cert:2927:01

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Eurofins Sample Code: 464-2015-08180580
Sample Description: Algal Oil ss=5g
Client Sample Code: N-2-006C
PO Number: 4502810122

Entry Date: 08/18/2015
Reporting Date: 09/06/2016

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

AR-15-QD-105556-11

This analytical report supersedes AR-15-QD-105556-10.

Test	Result	Theoretical Level
QD058 - Copper by ICP		**Completed: 09/06/2016**
AOAC 965.17 / 985.01 mod. * Copper	<1 ppm	
QD174 - Phosphorus In Vegetable Oil		**Completed: 09/06/2016**
AOCS Ca 12-55 Phosphorus In Vegetable Oil	< 2 ppm	
QA395 - Nickel (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Nickel (Ni)	0.3 mg/kg	
QA375 - Molybdenum (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Molybdenum (Mo)	<0.05 mg/kg	
QA373 - Manganese (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Manganese (Mn)	<0.01 mg/kg	
QA278 - Iron (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Iron (Fe)	<0.020 mg/kg	
QD06T - Cadmium (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Cadmium (Cd)	<0.010 mg/kg	
QD06S - Lead (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Lead (Pb)	<0.010 mg/kg	
QD06R - Mercury (Mwd-ICP-MS, Most Matrices)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Mercury (Hg)	<0.010 mg/kg	
QD06Q - Arsenic (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Arsenic (As)	<0.010 mg/kg	
QA867 - Silicon (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Silicon (Si)	79 mg/kg	

Eurofins Sample Code: 464-2015-08180580
Client Sample Code: N-2-006C

Test	Result	Theoretical Level
QA849 - Sulfur (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01		
Sulfur	1.6 mg/kg	
QA227 - Chromium (SolD-ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01		
Chromium (Cr)	<0.1 mg/kg	
QD094 - Free Fatty Acids (FFA)		**Completed: 09/06/2016**
AOCS Ca 5a-40		
* FFA (Free Fatty Acids)	0.03 %	
QD005 - Acid Value		**Completed: 09/06/2016**
AOCS Cd 3d-63		
* Acid value	0.06 mg KOH/g	
QD103 - Peroxide Value (PV)		**Completed: 09/06/2016**
AOCS Cd 8-53		
* Peroxide Value - Initial	6.0 meq/kg	
QA967 - Unsaponifiable Matter (Ethyl ether ext)		**Completed: 09/06/2016**
AOCS Ca 6b-53		
Unsaponifiable matter	2.97 %	
QD084 - Fatty Acid Profile, % Relative		**Completed: 09/06/2016**
AOCS Ce 2-66		
* Fatty Acid Profile, % Relative	Area Percent	
* C08:0 Octanoic (Caprylic)	<0.10 %	
* C10:0 Decanoic (Capric)	<0.10 %	
* C11:0 Undecanoic (Hendecanoic)	<0.10 %	
* C12:0 Dodecanoic (Lauric)	0.97 %	
* C13:0 Tridecanoic	<0.10 %	
* C14:0 Tetradecanoic (Myristic)	13.12 %	
* C14:1 Tetradecenoic (Myristoleic)	<0.10 %	
* C15:0 Pentadecanoic	0.42 %	
* C15:1 Pentadecenoic	<0.10 %	
* C16:0 Hexadecanoic (Palmitic)	27.87 %	
* C16:1 Hexadecenoic (Palmitoleic)	2.09 %	
* C16:2 Hexadecadienoic	<0.10 %	
* C16:3 Hexadecatrienoic	<0.10 %	
* C16:4 Hexadecatetraenoic	<0.10 %	
* C17:0 Heptadecanoic (Margaric)	<0.10 %	
* C17:1 Heptadecenoic Margaroleic	<0.10 %	
* C18:0 Octadecanoic (Stearic)	0.84 %	
* C18:1 Octadecenoic (Oleic)	2.17 %	
* C18:2 Octadecadienoic (Linoleic)	<0.10 %	
* C18:3 Octadecatrienoic (Linolenic)	0.15 %	
* C18:4 Octadecatetraenoic	0.23 %	
* C20:0 Eicosanoic (Arachidic)	<0.10 %	
* C20:1 Eicosenoic (Gondoic)	<0.10 %	
* C20:2 Eicosadienoic	<0.10 %	
* C20:3 Eicosatrienoic	0.15 %	
* C20:4 Eicosatetraenoic (Arachidonic)	0.74 %	
* C20:5 Eicosapentaenoic	1.12 %	
* C21:5 Heneicosapentaenoic	<0.10 %	
* C22:0 Docosanoic (Behenic)	<0.10 %	
* C22:1 Docosenoic (Erucic)	<0.10 %	
* C22:2 Docosadienoic	<0.10 %	
* C22:3 Docosatrienoic	<0.10 %	

Eurofins Sample Code: 464-2015-08180580
Client Sample Code: N-2-006C

Test	Result	Theoretical Level
QD084 - Fatty Acid Profile, % Relative (Cont.)		**Completed: 09/06/2016**
AOCS Ce 2-66		
* C22:4 Docosatetraenoic	<0.10 %	
* C22:5 Docosapentaenoic	8.38 %	
* C22:6 Docosahexaenoic	40.54 %	
* C24:0 Tetracosanoic (Lignoceric)	<0.10 %	
* C24:1 Tetracosenoic (Nervonic)	<0.10 %	
* Unknown Components	0.77 %	
QA934 - Trans Fatty Acids, relative area% (GC-FID)		**Completed: 09/06/2016**
AOCS 2a-94		
C 18:1 (trans) Elaidic acid	<0.01 %	
C 18:2 (c9/t11)	<0.01 %	
C 18:2 (trans/cis)	<0.01 %	
C 18:2 (trans/trans)	<0.01 %	
C 18:3 (cis/cis/trans)	<0.01 %	
C 18:3 (cis/trans/cis)	<0.01 %	
C 18:3 (trans/cis/cis)	<0.01 %	
C 18:3 (trans/cis/trans)	<0.01 %	
total trans fatty acids C18:1	<0.01 %	
total trans fatty acids C18:2 (without CLA)	<0.02 %	
total trans fatty acids C18:3	<0.02 %	
Total Trans Fatty Acids	<0.05 %	
UM4BV - Moulds - BAM Chapter 18		**Completed: 09/06/2016**
FDA BAM Chapter 18		
* Mold	< 10 (est) cfu/g	
* Yeast	< 10 (est) cfu/g	
UM5DP - Coliforms - AOAC 991.14		**Completed: 09/06/2016**
AOAC 991.14		
* Total Coliforms	< 10 (est) cfu/g	
* E. coli	< 10 (est) cfu/g	
UMAEK - Salmonella - AOAC 2003.09		**Completed: 09/06/2016**
AOAC 2003.09		
* Salmonella spp.	Negative /25 g	
UMDE0 - Aerobic Plate Count - AOAC 990.12		**Completed: 09/06/2016**
AOAC 990.12		
* Aerobic Plate Count	< 10 (est) cfu/g	
UM70B - Coagulase positive staphylococcus - BAM Chapter 12		**Completed: 09/06/2016**
BAM Chapter 12		
Coagulase positive staphylococcus	< 10 cfu/g	

**The test result is covered by our current A2LA accreditation.*

Interpretation:

< - Concentration below the indicated limit of quantification (LOQ)
ND - not determined since none of the corresponding congeners was above the LOQ

Eurofins Sample Code: 464-2015-08180580
Client Sample Code: N-2-006C

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing Cert:3329:01

Chemical Testing Cert:2927:01

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Eurofins Sample Code: 464-2015-10020415
Sample Description: Algal Oil ss=5g
Client Sample Code: N-2-006C
PO Number: 4502798817
Client Code: [redacted]

Entry Date: 10/02/2015
Reporting Date: 10/15/2015

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

AR-15-QD-127183-04

This analytical report supersedes AR-15-QD-127183-03.

Test	Result	
QD146 - Moisture - Forced Draft Oven		**Completed: 10/14/2015**
AOAC 930.15		
* Moisture by Forced Draft Oven	<0.05 %	
QD052 - Protein - Combustion		**Completed: 10/15/2015**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93		
* Protein, Combustion	<0.15 %	
QD025 - Ash		**Completed: 10/14/2015**
AOAC 942.05		
* Ash	<0.1 %	
QD143 - Moisture & Volatiles By Air Oven		**Completed: 10/05/2015**
AOCS Ca 2c-25		
Moisture & Volatiles By Air Oven	0.10 %	
QD059 - Fat by Acid Hydrolysis		**Completed: 10/06/2015**
AOAC 954.02		
* Crude Fat By Acid Hydrolysis	101.44 %	
QD114 - Lovibond Color - AOCS Scale		**Completed: 10/05/2015**
AOCS Cc 13b-45		
Lovibond Color - AOCS Scale	0.8R. 70.0Y	
JK073 - Sterol profile and content		**Completed: 10/13/2015**
Internal Method		
Total sterol	2,310 mg/kg fat	
Cholesterol (% total sterols)	24.3 %	
Brassicasterol (% total sterols)	< 0.1 %	
24-Methylene-cholesterol (% tot. sterol)	3.9 %	
Campesterol (% total sterols)	1.2 %	
Campestanol (% total sterols)	< 0.1 %	
Stigmasterol (% total sterols)	< 0.1 %	
delta-7-Campesterol (% total sterols)	3.4 %	
Delta-5,23-stigmastadienol (% total ster.)	6.9 %	
Clerosterol (% total sterols)	8.8 %	
Beta-Sitosterol "real" (% total sterols)	13.4 %	
Sitostanol (% total sterols)	< 0.1 %	
Delta-5-avenasterol (% total sterols)	1.4 %	
Delta-5,24-stigmastadienol (% total sterols)	7.0 %	
Delta-7-stigmastenol (% total sterols)	26.1 %	

eurofins

Eurofins Sample Code: 464-2015-10020415
Client Sample Code: N-2-006C

Test	Result	
JK073 - Sterol profile and content (Cont.)		**Completed: 10/13/2015**
Internal Method		
Delta-7-avenasterol (% total sterols)	3.6 %	
QA230 - Copper (ICP-AES)		**Completed: 10/09/2015**
AOCS Ca 17-01		
Copper (Cu)	0.08 mg/kg	

**The test result is covered by our current A2LA accreditation.*

Interpretation:

JUDGEMENT
For this matrix sufficient validation data for each test are not available.

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

David Gross
Support Services Manager

Biological Testing Cert:3329:01
Chemical Testing Cert:2927:01

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Udaya Wanasundara

Project No: 15-536
Report Date: 10/16/2015
Lab Group ID: 151016007
Lab Number: AA65954

ANALYTICAL REPORT

Sample Description: RBD Mara Algal Oil Lot# N-2-006-C

PO#:

Analysis Results:

Analyte	Result	Units	Analysis Reference
Peroxide Value	1.06	meq/kg	AOCS Cd 8b-90

This is a final report of analysis performed by POS Bio-Sciences.

These results have been approved for release by

Angie Johnson
Analytical Services
POS Bio-Sciences

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Eurofins Sample Code: 464-2015-08180581
Sample Description: Algal Oil ss=5g
Client Sample Code: N-2-008C
PO Number: 4502810122

[redacted]

[redacted]

Entry Date: 08/18/2015
Reporting Date: 09/06/2016

CERTIFICATE OF ANALYSIS

AR-15-QD-105557-10

This analytical report supersedes AR-15-QD-105557-09.

Test	Result	Theoretical Level
QD058 - Copper by ICP		**Completed: 09/06/2016**
AOAC 965.17 / 985.01 mod. * Copper	<1 ppm	
QD174 - Phosphorus In Vegetable Oil		**Completed: 09/06/2016**
AOCS Ca 12-55 Phosphorus In Vegetable Oil	< 2 ppm	
QA395 - Nickel (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Nickel (Ni)	0.3 mg/kg	
QA375 - Molybdenum (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Molybdenum (Mo)	<0.05 mg/kg	
QA373 - Manganese (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Manganese (Mn)	<0.01 mg/kg	
QA278 - Iron (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Iron (Fe)	<0.020 mg/kg	
QD06T - Cadmium (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Cadmium (Cd)	<0.010 mg/kg	
QD06S - Lead (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Lead (Pb)	<0.010 mg/kg	
QD06R - Mercury (Mwd-ICP-MS, Most Matrices)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Mercury (Hg)	<0.010 mg/kg	
QD06Q - Arsenic (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Arsenic (As)	<0.010 mg/kg	
QA867 - Silicon (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Silicon (Si)	80 mg/kg	

Eurofins Sample Code: 464-2015-08180581
Client Sample Code: N-2-008C

Test	Result	Theoretical Level
QA849 - Sulfur (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01		
Sulfur	<1.0 mg/kg	
QA227 - Chromium (SolD-ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01		
Chromium (Cr)	<0.1 mg/kg	
QD094 - Free Fatty Acids (FFA)		**Completed: 09/06/2016**
AOCS Ca 5a-40		
* FFA (Free Fatty Acids)	0.03 %	
QD005 - Acid Value		**Completed: 09/06/2016**
AOCS Cd 3d-63		
* Acid value	0.06 mg KOH/g	
QA967 - Unsaponifiable Matter (Ethyl ether ext)		**Completed: 09/06/2016**
AOCS Ca 6b-53		
Unsaponifiable matter	2.43 %	
QD084 - Fatty Acid Profile, % Relative		**Completed: 09/06/2016**
AOCS Ce 2-66		
* Fatty Acid Profile, % Relative	Area Percent	
* C08:0 Octanoic (Caprylic)	<0.10 %	
* C10:0 Decanoic (Capric)	<0.10 %	
* C11:0 Undecanoic (Hendecanoic)	<0.10 %	
* C12:0 Dodecanoic (Lauric)	1.01 %	
* C13:0 Tridecanoic	<0.10 %	
* C14:0 Tetradecanoic (Myristic)	13.63 %	
* C14:1 Tetradecenoic (Myristoleic)	<0.10 %	
* C15:0 Pentadecanoic	0.51 %	
* C15:1 Pentadecenoic	<0.10 %	
* C16:0 Hexadecanoic (Palmitic)	29.45 %	
* C16:1 Hexadecenoic (Palmitoleic)	2.17 %	
* C16:2 Hexadecadienoic	<0.10 %	
* C16:3 Hexadecatrienoic	<0.10 %	
* C16:4 Hexadecatetraenoic	<0.10 %	
* C17:0 Heptadecanoic (Margaric)	<0.10 %	
* C17:1 Heptadecenoic Margaroleic	<0.10 %	
* C18:0 Octadecanoic (Stearic)	0.85 %	
* C18:1 Octadecenoic (Oleic)	1.81 %	
* C18:2 Octadecadienoic (Linoleic)	<0.10 %	
* C18:3 Octadecatrienoic (Linolenic)	0.13 %	
* C18:4 Octadecatetraenoic	0.20 %	
* C20:0 Eicosanoic (Arachidic)	<0.10 %	
* C20:1 Eicosenoic (Gondoic)	<0.10 %	
* C20:2 Eicosadienoic	<0.10 %	
* C20:3 Eicosatrienoic	<0.10 %	
* C20:4 Eicosatetraenoic (Arachidonic)	0.64 %	
* C20:5 Eicosapentaenoic	0.90 %	
* C21:5 Heneicosapentaenoic	<0.10 %	
* C22:0 Docosanoic (Behenic)	<0.10 %	
* C22:1 Docosenoic (Erucic)	<0.10 %	
* C22:2 Docosadienoic	<0.10 %	
* C22:3 Docosatrienoic	<0.10 %	
* C22:4 Docosatetraenoic	<0.10 %	
* C22:5 Docosapentaenoic	7.73 %	
* C22:6 Docosahexaenoic	39.64 %	

Eurofins Sample Code: 464-2015-08180581
Client Sample Code: N-2-008C

Test	Result	Theoretical Level
QD084 - Fatty Acid Profile, % Relative (Cont.)		**Completed: 09/06/2016**
AOCS Ce 2-66		
* C24:0 Tetracosanoic (Lignoceric)	<0.10 %	
* C24:1 Tetracosenoic (Nervonic)	<0.10 %	
* Unknown Components	0.76 %	
QA934 - Trans Fatty Acids, relative area% (GC-FID)		**Completed: 09/06/2016**
AOCS 2a-94		
C 18:1 (trans) Elaidic acid	<0.01 %	
C 18:2 (c9/t11)	<0.01 %	
C 18:2 (trans/cis)	<0.01 %	
C 18:2 (trans/trans)	<0.01 %	
C 18:3 (cis/cis/trans)	<0.01 %	
C 18:3 (cis/trans/cis)	<0.01 %	
C 18:3 (trans/cis/cis)	<0.01 %	
C 18:3 (trans/cis/trans)	<0.01 %	
total trans fatty acids C18:1	<0.01 %	
total trans fatty acids C18:2 (without CLA)	<0.02 %	
total trans fatty acids C18:3	<0.02 %	
Total Trans Fatty Acids	<0.05 %	
UM4BV - Moulds - BAM Chapter 18		**Completed: 09/06/2016**
FDA BAM Chapter 18		
* Mold	< 10 (est) cfu/g	
* Yeast	< 10 (est) cfu/g	
UM5DP - Coliforms - AOAC 991.14		**Completed: 09/06/2016**
AOAC 991.14		
* Total Coliforms	< 10 (est) cfu/g	
* E. coli	< 10 (est) cfu/g	
UMAEK - Salmonella - AOAC 2003.09		**Completed: 09/06/2016**
AOAC 2003.09		
* Salmonella spp.	Negative /25 g	
UMDE0 - Aerobic Plate Count - AOAC 990.12		**Completed: 09/06/2016**
AOAC 990.12		
* Aerobic Plate Count	20 (est) cfu/g	
UM70B - Coagulase positive staphylococcus - BAM Chapter 12		**Completed: 09/06/2016**
BAM Chapter 12		
Coagulase positive staphylococcus	< 10 cfu/g	

**The test result is covered by our current A2LA accreditation.*

Interpretation:

< - Concentration below the indicated limit of quantification (LOQ)
ND - not determined since none of the corresponding congeners was above the LOQ

Eurofins Sample Code: 464-2015-08180581
Client Sample Code: N-2-008C

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing Cert:3329:01
Chemical Testing Cert:2927:01

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Fax:+1 515 266 5453

Eurofins Sample Code: 464-2015-10020416
Sample Description: Algal Oil ss=5g
Client Sample Code: N-2-008C
PO Number: 4502798817
Client Code: [redacted]

Entry Date: 10/02/2015
Reporting Date: 10/15/2015

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

AR-15-QD-127223-04

This analytical report supersedes AR-15-QD-127223-03.

Test	Result	
QD146 - Moisture - Forced Draft Oven		**Completed: 10/14/2015**
AOAC 930.15		
* Moisture by Forced Draft Oven	<0.05 %	
QD052 - Protein - Combustion		**Completed: 10/15/2015**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93		
* Protein, Combustion	<0.15 %	
QD025 - Ash		**Completed: 10/14/2015**
AOAC 942.05		
* Ash	<0.1 %	
QD143 - Moisture & Volatiles By Air Oven		**Completed: 10/05/2015**
AOCS Ca 2c-25		
Moisture & Volatiles By Air Oven	0.11 %	
QD059 - Fat by Acid Hydrolysis		**Completed: 10/06/2015**
AOAC 954.02		
* Crude Fat By Acid Hydrolysis	100.31 %	
QD114 - Lovibond Color - AOCS Scale		**Completed: 10/05/2015**
AOCS Cc 13b-45		
Lovibond Color - AOCS Scale	0.3R. 19.0Y	
JK073 - Sterol profile and content		**Completed: 10/13/2015**
Internal Method		
Total sterol	1,900 mg/kg fat	
Cholesterol (% total sterols)	32.9 %	
Brassicasterol (% total sterols)	< 0.1 %	
24-Methylene-cholesterol (% tot. sterol)	7.1 %	
Campesterol (% total sterols)	1.4 %	
Campestanol (% total sterols)	< 0.1 %	
Stigmasterol (% total sterols)	7.2 %	
delta-7-Campesterol (% total sterols)	7.0 %	
Delta-5,23-stigmastadienol (% total ster.)	6.2 %	
Clerosterol (% total sterols)	8.2 %	
Beta-Sitosterol "real" (% total sterols)	9.4 %	
Sitostanol (% total sterols)	< 0.1 %	
Delta-5-avenasterol (% total sterols)	1.2 %	
Delta-5,24-stigmastadienol (% total sterols)	3.9 %	
Delta-7-stigmastenol (% total sterols)	14.0 %	

Eurofins Sample Code: 464-2015-10020416
Client Sample Code: N-2-008C

Test	Result	
JK073 - Sterol profile and content (Cont.)		**Completed: 10/13/2015**
Internal Method		
Delta-7-avenasterol (% total sterols)	1.5 %	
QA230 - Copper (ICP-AES)		**Completed: 10/09/2015**
AOCS Ca 17-01		
Copper (Cu)	0.02 mg/kg	

**The test result is covered by our current A2LA accreditation.*

Interpretation:

JUDGEMENT
For this matrix sufficient validation data for each test are not available.

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

David Gross
Support Services Manager

Biological Testing Cert:3329:01

Chemical Testing Cert:2927:01

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Saskatoon, SK
S7N 2R4
Udaya Wanasundara

Project No: 15-536
Report Date: 10/16/2015
Lab Group ID: 151016007
Lab Number: AA65955

ANALYTICAL REPORT

Sample Description: RBD Mara Algal Oil Lot# N-2-008-C

PO#:

Analysis Results:

Analyte	Result	Units	Analysis Reference
Peroxide Value	<0.10	meq/kg	AOCS Cd 8b-90

This is a final report of analysis performed by POS Bio-Sciences.

These results have been approved for release by

Angie Johnson
Analytical Services
POS Bio-Sciences

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Eurofins Sample Code: 464-2015-08180582
Sample Description: Algal Oil ss=5g
Client Sample Code: N-2-010C
PO Number: 4502810122

Entry Date: 08/18/2015
Reporting Date: 09/06/2016

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

AR-15-QD-105558-10

This analytical report supersedes AR-15-QD-105558-09.

Test	Result	Theoretical Level
QD058 - Copper by ICP		**Completed: 09/06/2016**
AOAC 965.17 / 985.01 mod. * Copper	<1 ppm	
QD174 - Phosphorus In Vegetable Oil		**Completed: 09/06/2016**
AOCS Ca 12-55 Phosphorus In Vegetable Oil	< 2 ppm	
QA395 - Nickel (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Nickel (Ni)	0.3 mg/kg	
QA375 - Molybdenum (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Molybdenum (Mo)	<0.05 mg/kg	
QA373 - Manganese (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Manganese (Mn)	<0.01 mg/kg	
QA278 - Iron (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Iron (Fe)	<0.020 mg/kg	
QD06T - Cadmium (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Cadmium (Cd)	<0.010 mg/kg	
QD06S - Lead (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Lead (Pb)	<0.010 mg/kg	
QD06R - Mercury (Mwd-ICP-MS, Most Matrices)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Mercury (Hg)	<0.010 mg/kg	
QD06Q - Arsenic (Mwd-ICP-MS)		**Completed: 09/06/2016**
J. AOAC vol. 90 (2007) 844-856 (Mod) * Arsenic (As)	<0.010 mg/kg	
QA867 - Silicon (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01 Silicon (Si)	75 mg/kg	

Eurofins Sample Code: 464-2015-08180582
Client Sample Code: N-2-010C

Test	Result	Theoretical Level
QA849 - Sulfur (ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01		
Sulfur	<1.0 mg/kg	
QA227 - Chromium (SolD-ICP-AES)		**Completed: 09/06/2016**
AOCS Ca 17-01		
Chromium (Cr)	<0.1 mg/kg	
QD094 - Free Fatty Acids (FFA)		**Completed: 09/06/2016**
AOCS Ca 5a-40		
* FFA (Free Fatty Acids)	0.03 %	
QD005 - Acid Value		**Completed: 09/06/2016**
AOCS Cd 3d-63		
* Acid value	0.06 mg KOH/g	
QA967 - Unsaponifiable Matter (Ethyl ether ext)		**Completed: 09/06/2016**
AOCS Ca 6b-53		
Unsaponifiable matter	2.50 %	
QD084 - Fatty Acid Profile, % Relative		**Completed: 09/06/2016**
AOCS Ce 2-66		
* Fatty Acid Profile, % Relative	Area Percent	
* C08:0 Octanoic (Caprylic)	<0.10 %	
* C10:0 Decanoic (Capric)	<0.10 %	
* C11:0 Undecanoic (Hendecanoic)	<0.10 %	
* C12:0 Dodecanoic (Lauric)	1.01 %	
* C13:0 Tridecanoic	<0.10 %	
* C14:0 Tetradecanoic (Myristic)	13.65 %	
* C14:1 Tetradecenoic (Myristoleic)	<0.10 %	
* C15:0 Pentadecanoic	0.52 %	
* C15:1 Pentadecenoic	<0.10 %	
* C16:0 Hexadecanoic (Palmitic)	29.39 %	
* C16:1 Hexadecenoic (Palmitoleic)	2.20 %	
* C16:2 Hexadecadienoic	<0.10 %	
* C16:3 Hexadecatrienoic	<0.10 %	
* C16:4 Hexadecatetraenoic	<0.10 %	
* C17:0 Heptadecanoic (Margaric)	0.10 %	
* C17:1 Heptadecenoic Margaroleic	<0.10 %	
* C18:0 Octadecanoic (Stearic)	0.85 %	
* C18:1 Octadecenoic (Oleic)	1.85 %	
* C18:2 Octadecadienoic (Linoleic)	<0.10 %	
* C18:3 Octadecatrienoic (Linolenic)	0.14 %	
* C18:4 Octadecatetraenoic	0.21 %	
* C20:0 Eicosanoic (Arachidic)	<0.10 %	
* C20:1 Eicosenoic (Gondoic)	<0.10 %	
* C20:2 Eicosadienoic	<0.10 %	
* C20:3 Eicosatrienoic	<0.10 %	
* C20:4 Eicosatetraenoic (Arachidonic)	0.63 %	
* C20:5 Eicosapentaenoic	0.90 %	
* C21:5 Heneicosapentaenoic	<0.10 %	
* C22:0 Docosanoic (Behenic)	<0.10 %	
* C22:1 Docosenoic (Erucic)	<0.10 %	
* C22:2 Docosadienoic	<0.10 %	
* C22:3 Docosatrienoic	<0.10 %	
* C22:4 Docosatetraenoic	<0.10 %	
* C22:5 Docosapentaenoic	7.78 %	
* C22:6 Docosahexaenoic	39.60 %	

Eurofins Sample Code: 464-2015-08180582
Client Sample Code: N-2-010C

Test	Result	Theoretical Level
QD084 - Fatty Acid Profile, % Relative (Cont.)		**Completed: 09/06/2016**
AOCS Ce 2-66		
* C24:0 Tetracosanoic (Lignoceric)	<0.10 %	
* C24:1 Tetracosenoic (Nervonic)	<0.10 %	
* Unknown Components	0.75 %	
QA934 - Trans Fatty Acids, relative area% (GC-FID)		**Completed: 09/06/2016**
AOCS 2a-94		
C 18:1 (trans) Elaidic acid	<0.01 %	
C 18:2 (c9/t11)	<0.01 %	
C 18:2 (trans/cis)	<0.01 %	
C 18:2 (trans/trans)	<0.01 %	
C 18:3 (cis/cis/trans)	<0.01 %	
C 18:3 (cis/trans/cis)	<0.01 %	
C 18:3 (trans/cis/cis)	<0.01 %	
C 18:3 (trans/cis/trans)	<0.01 %	
total trans fatty acids C18:1	<0.01 %	
total trans fatty acids C18:2 (without CLA)	<0.02 %	
total trans fatty acids C18:3	<0.02 %	
Total Trans Fatty Acids	<0.05 %	
UM4BV - Moulds - BAM Chapter 18		**Completed: 09/06/2016**
FDA BAM Chapter 18		
* Mold	< 10 (est) cfu/g	
* Yeast	< 10 (est) cfu/g	
UM5DP - Coliforms - AOAC 991.14		**Completed: 09/06/2016**
AOAC 991.14		
* Total Coliforms	< 10 (est) cfu/g	
* E. coli	< 10 (est) cfu/g	
UMAEK - Salmonella - AOAC 2003.09		**Completed: 09/06/2016**
AOAC 2003.09		
* Salmonella spp.	Negative /25 g	
UMDE0 - Aerobic Plate Count - AOAC 990.12		**Completed: 09/06/2016**
AOAC 990.12		
* Aerobic Plate Count	< 10 (est) cfu/g	
UM70B - Coagulase positive staphylococcus - BAM Chapter 12		**Completed: 09/06/2016**
BAM Chapter 12		
Coagulase positive staphylococcus	< 10 cfu/g	

**The test result is covered by our current A2LA accreditation.*

Interpretation:

< - Concentration below the indicated limit of quantification (LOQ)
ND - not determined since none of the corresponding congeners was above the LOQ

Eurofins Sample Code: 464-2015-08180582
Client Sample Code: N-2-010C

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Jacob Cross
Project Manager

Biological Testing Cert:3329:01
Chemical Testing Cert:2927:01

Results shown in this report relate solely to the item submitted for analysis.
All results are reported on an "As Received" basis unless otherwise stated.
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Eurofins Scientific, Inc. Measurement of Uncertainty can be obtained upon request.

eurofins | Nutrition Analysis Center

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Nutrition Analysis Center
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Eurofins Sample Code: 464-2015-10020417
Sample Description: Algal Oil ss=5g
Client Sample Code: N-2-010C
PO Number: 4502798817
Client Code: [redacted]

Entry Date: 10/02/2015
Reporting Date: 10/15/2015

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

AR-15-QD-127224-04

This analytical report supersedes AR-15-QD-127224-03.

Test	Result	
QD146 - Moisture - Forced Draft Oven		**Completed: 10/14/2015**
AOAC 930.15		
* Moisture by Forced Draft Oven	<0.05 %	
QD052 - Protein - Combustion		**Completed: 10/15/2015**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93		
* Protein, Combustion	<0.15 %	
QD025 - Ash		**Completed: 10/14/2015**
AOAC 942.05		
* Ash	<0.1 %	
QD143 - Moisture & Volatiles By Air Oven		**Completed: 10/05/2015**
AOCS Ca 2c-25		
Moisture & Volatiles By Air Oven	0.05 %	
QD059 - Fat by Acid Hydrolysis		**Completed: 10/06/2015**
AOAC 954.02		
* Crude Fat By Acid Hydrolysis	100.49 %	
QD114 - Lovibond Color - AOCS Scale		**Completed: 10/05/2015**
AOCS Cc 13b-45		
Lovibond Color - AOCS Scale	0.3R. 17.0Y	
JK073 - Sterol profile and content		**Completed: 10/13/2015**
Internal Method		
Total sterol	1,990 mg/kg fat	
Cholesterol (% total sterols)	32.2 %	
Brassicasterol (% total sterols)	< 0.1 %	
24-Methylene-cholesterol (% tot. sterol)	6.1 %	
Campesterol (% total sterols)	2.7 %	
Campestanol (% total sterols)	< 0.1 %	
Stigmasterol (% total sterols)	6.9 %	
delta-7-Campesterol (% total sterols)	6.7 %	
Delta-5,23-stigmastadienol (% total ster.)	7.7 %	
Clerosterol (% total sterols)	6.3 %	
Beta-Sitosterol "real" (% total sterols)	11.5 %	
Sitostanol (% total sterols)	< 0.1 %	
Delta-5-avenasterol (% total sterols)	1.3 %	
Delta-5,24-stigmastadienol (% total sterols)	6.1 %	
Delta-7-stigmastenol (% total sterols)	11.0 %	

Eurofins Sample Code: 464-2015-10020417
Client Sample Code: N-2-010C

Test	Result	
JK073 - Sterol profile and content (Cont.)		**Completed: 10/13/2015**
Internal Method		
Delta-7-avenasterol (% total sterols)	1.4 %	
QA230 - Copper (ICP-AES)		**Completed: 10/09/2015**
AOCS Ca 17-01		
Copper (Cu)	0.03 mg/kg	

**The test result is covered by our current A2LA accreditation.*

Interpretation:

JUDGEMENT
For this matrix sufficient validation data for each test are not available.

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

David Gross
Support Services Manager

Biological Testing Cert:3329:01

Chemical Testing Cert:2927:01

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POS BIO-SCIENCES

OUR PEOPLE.
YOUR OPPORTUNITIES.
BRILLIANT SOLUTIONS.

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To: POS Bio-Sciences
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S7N 2R4
Udaya Wanasundara

Project No: 15-536
Report Date: 10/16/2015
Lab Group ID: 151016007
Lab Number: AA65956

ANALYTICAL REPORT

Sample Description: RBD Mara Algal Oil Lot# N-2-010-C

PO#:

Analysis Results:

Analyte	Result	Units	Analysis Reference
Peroxide Value	<0.10	meq/kg	AOCS Cd 8b-90

This is a final report of analysis performed by POS Bio-Sciences.

These results have been approved for release by

Angie Johnson
Analytical Services
POS Bio-Sciences

EXHIBIT I

Report of the Expert Panel

OPINION OF AN EXPERT PANEL ON THE SAFETY AND GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF DOCOSAHEXAENOIC ACID (DHA) ALGAL OIL FOR USE IN INFANT FORMULA

Introduction

An independent panel of experts (Expert Panel), qualified by scientific training and experience to evaluate the safety of food and food ingredients, was requested by Mara Renewables Corporation (Mara) to determine the safety and Generally Recognized as Safe (GRAS) status of the use of docosahexaenoic acid (DHA) algal oil in infant formula. DHA and DHA algal oils are currently marketed for use in food, infant formula, and dietary supplements for human consumption. DHA algal oil is intended for use as a direct ingredient in exempt (pre-term) and non-exempt (term) infant formula (ages from birth to 12 months), and in combination with a source of arachidonic acid (ARA). The ratio of DHA to ARA would range from 1:1 to 1:2. The intended use level is similar to all other approved uses for incorporation of DHA in infant formula. The DHA algal oil is manufactured in accordance with Hazard Analysis Critical Control Point (HACCP) and current Good Manufacturing Practices (cGMP), including quality control (QC) checks at every stage of the production process. The DHA algal oil product meets the proposed specifications.

A detailed review based on the existing scientific literature (through August 2016) on the safety of DHA and DHA algal oils was conducted by ToxStrategies, Inc. (ToxStrategies) and is summarized in the attached dossier. The Expert Panel members reviewed the dossier prepared by ToxStrategies and other pertinent information and convened on September 13, 2016 via teleconference. Based on an independent, critical evaluation of all of the available information and discussions during the September 13, 2016 teleconference, the Expert Panel unanimously concluded that the intended uses described herein for Mara's DHA algal oil, meeting appropriate food-grade specifications as described in the supporting dossier (GRAS Determination of DHA Algal Oil for Use in Infant Formula) and manufactured according to cGMP, are safe, suitable, and GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

Summary and Basis for GRAS Determination

Description

The DHA product that is the subject of this GRAS determination is a yellow to orange-colored semi-solid to liquid oil that is extracted and refined from the wild-type heterotrophic microalgae *Schizochytrium* sp. ONC-T18 (hereinafter referred to as T18). It is a mixture of triglycerides containing mostly polyunsaturated fatty acids (PUFA) in which the predominant fatty acid (>35%) is DHA.

Manufacturing Process

The DHA algal oil, which is rich in polyunsaturated fatty acids (PUFA), is produced by a heterotrophic fermentation process with a single cell marine microalgae of the genus *Schizochytrium*, in particular T18. The fermentation process uses a medium containing carbon and nitrogen sources, bulk and trace mineral nutrients, and vitamins. Once fermentation is complete (i.e., as determined by carbon usage, cell growth, oil synthesis activity, and oil fatty acid profile), the crude oil that accumulates intracellularly is recovered from the fermentation broth via an aqueous extraction process. To release the oil from the cells, the cell wall requires disruption. In the cell wall disruption process, the fermentation broth is pH-adjusted with sodium hydroxide and hydrolyzed enzymatically. As a result, no intact algae remain in the oil. Following recovery of the crude oil, the following refining steps are completed: fractionation/winterization (optional), degumming (optional), bleaching, and deodorization.

Analytical (chemical and microbiological) results for Mara's DHA algal oil product confirm that the finished product meets the proposed specifications as demonstrated by the consistency of production, the lack of impurities/contaminants (e.g., heavy metals, microbiological toxins), and its stability over a 12-month period.

All of the fatty acids detected in the DHA algal oil are well-known components of the human diet and found in both animal and vegetable food sources. The major fatty acids are DHA, myristic acid, palmitic acid, docosapentaenoic acid, and cis-vaccenic acid. Literature searches did not identify safety/toxicity concerns related to any individual fatty acids or their ratios in the proposed DHA algal oil, and the proposed DHA oil is similar to other commercially available edible oils. Similarly, the detected sterols and stanols are also present in the human diet from vegetable and animal food sources such as common edible oils. Cholesterol levels as a percentage of total sterols were higher than comparable algal oils. However, total sterol content (and thus absolute cholesterol content) was much lower in Mara 's DHA algal oil than in comparable algal oils. The total sterol intake from the DHA algal oil would be minimal. Additionally, the sterol profile of the proposed DHA algal oil is similar to that found in other algal oils and fish oils that are currently used in food, including infant formula.

History of Use

DHA-rich oils from numerous sources including microalgae are considered GRAS for use in food for human consumption, including infant formula. Global infant formula standards in the Food Chemicals Codex, as well as those in the EU, China, and Australia, allow the addition of DHA to infant formula as an optional ingredient. 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. In addition, FDA has approved other sources of DHA for use in human food and/or infant formula, such as menhaden and fish oils.

DHA, produced via fermentation employing various microalgae, has previously been approved and sold for incorporation in infant formula. This includes approval of algal oil from *Schizochytrium* sp. The approvals authorized the addition of DHA at levels up to 0.5% of the total fatty acids in both exempt (pre-term) and non-exempt (term) formulas. Most recently, DSM received a positive opinion letter from the UK Advisory Committee on novel Foods and Processes for the use of DHASCO-B from *Schizochytrium* sp. in infant and follow-on formula.

Intended Use and Intake Assessment

DHA algal oil is intended for use as a direct ingredient in exempt (pre-term) and non-exempt (term) infant formula (ages from birth to 12 months), in accordance with current good manufacturing practices (cGMP), and in combination with a source of arachidonic acid (ARA). The ratio of DHA to ARA would range from 1:1 to 1:2. The intended use level is similar to all other approved uses for incorporation of DHA in infant formula.

As presented and discussed in previous GRAS submissions for the use of DHA in infant formula, it is assumed that infants consume about 100-120 kcal/kg bw/day, of which fat constitutes approximately 50% of calories, or approximately 5.5–6.7 g fat/kg bw/day (1 g of fat is equivalent to 9 kcal). Assuming incorporation of the proposed DHA ingredient at a maximum use level of 0.5% of fatty acids, the intake of DHA would be 27–33 mg/kg bw/day.

Safety Data

DHA is an important component of most cell membranes and tissues. DHA and DHA algal oils are currently marketed for use in food, infant formula, and dietary supplements for human consumption. The oil from *Schizochytrium* sp. T18 has a similar lipid (fatty acid and sterol) profile to that of currently approved/marketed DHA oils from *Schizochytrium* sp. The safety of the fatty acid and sterol profiles have been confirmed through the numerous studies conducted on DHA sources, including DHA algal oils and the proposed DHA-rich algal oil from T18. Regulatory authorities have reviewed the safety of DHA and DHA algal oils and found their use to be safe for use in human food including infant formula. Numerous have been conducted and published in support of the safety of DHA and DHA algal oils, including *in vitro* studies, *in vivo* animal studies, and clinical studies in humans including infants. The most relevant studies on DHA acute and subchronic toxicity, reproductive and developmental toxicity, mutagenicity and genotoxicity, chronic toxicity, carcinogenicity, and irritation/sensitization, along with clinical and epidemiological studies, have been summarized and reviewed in the attached GRAS dossier. The published data, as well as reviews conducted by regulatory authorities, support the conclusion that Mara's DHA algal oil is safe for use as an ingredient in exempt (pre-term) and non-exempt (term) infant formula at the proposed levels of use.

General Recognition of the Safety of DHA Algal Oil

The intended use of DHA algal oil in infant formula has been determined to be safe through scientific procedures as set forth in 21 CFR§170.3(b), thus satisfying the so-called "technical" element of the GRAS determination and is based on the following:

- The DHA product that is the subject of this GRAS determination is extracted and refined oil from the wild-type heterotrophic microalgae *Schizochytrium* sp. T18. It is a mixture of triglycerides containing mostly PUFA in which the predominant fatty acid (>35%) is DHA. The DHA manufacturing process starts with fermentation followed by refining of the crude DHA algal oil isolated from the fermentation process. The DHA algal oil product is manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes.

- The possible presence of microalgae toxins has been previously addressed as part of a substantial equivalence submission (ONC, 2011) and in GRN 553 (FDA, 2014a). Toxin production is unlikely since there are no known reports of toxin production by thraustochytrids, of which *Schizochytrium* is a member (ONC, 2011; Hammond et al., 2002). In addition, T18 oil and algal biomass were screened for the presence of toxins including domoic acid, gymnodimine, desmethyl spirolide C, azaspiracid-1, azaspiracid-2, azaspiracid-3, pectenotoxin-2, okadaic acid, dinophysistoxin-1, dinophysistoxin-2, yessotoxin, prymnesin-1, and prymnesin-2, and none were detected (ONC, 2011).

- There is common knowledge of a long history of human consumption of DHA from food and foods containing added DHA such as infant formula, and other products such as dietary supplements. It will be added to infant formula in order to supplement the dietary intake of the omega-3 fatty acid DHA.

- Numerous algal and marine sources of DHA have been evaluated by the FDA and other global regulatory agencies over the past 15 years for proposed incorporation in food for human consumption including infant formula. Relevant US GRAS notifications include GRN 41, GRN 94, GRN 379, and GRN 553 (FDA, 2000; 2001; 2011; 2014). All of the GRAS notices provided information/clinical study data that supported the safety of the proposed DHA ingredients for use in infant formula. In all of the studies summarized in these notifications, there were no significant adverse effects/events or tolerance issues in infants attributable to DHA supplemented formulas when compared to control group infant formulas. The studies supported the safe use of DHA in infant formula up to 1% of total fatty acids.

- Literature searches did not identify safety/toxicity concerns related to any individual fatty acid or their ratios in the proposed DHA algal oil. The proposed DHA oil is similar to other commercially available edible oils incorporated in

infant formulas. While the fatty acid profile for the proposed DHA algal oil has higher myristic, palmitic, and cis-vaccenic acid concentrations when compared to other algal oil products, it is similar to that found in other algal oils and fish oils (e.g., krill oil) that are currently used in food and/or infant formula.

- The proposed uses of the DHA algal oil from *Schizochytrium* sp. T18 are identical to the approved uses for other GRAS DHA (and/or in combination with ARA) products incorporated in exempt (pre-term) and non-exempt (term) infant formulas.

- 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). 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 S2532Furthermore, other sources of DHA such as tuna/fish oil is approved by the FDA for addition to human food and infant formula.

- Toxicity testing has been conducted with the proposed DHA-rich algal oil product from *Schizochytrium* sp. T18 and includes acute and subchronic toxicity studies, a battery of genotoxicity studies, and developmental and reproductive toxicity studies. In all of the studies, no evidence of toxicity was noted at the highest dose levels tested.

- The publicly available scientific literature on the consumption and safety of DHA and DHA algal oil ingredients, in clinical studies in infants and adult humans as well as animals, is extensive and sufficient to support the safety and GRAS status of the proposed DHA algal oil product.

Conclusions of the Expert Panel

We, the undersigned members of the Expert Panel, have individually and collectively critically reviewed the published and ancillary information pertinent to the identification, use, and safety of Mara's DHA algal oil product. We conclude that the DHA algal oil produced by Mara under the conditions described in the attached dossier and meeting Mara specifications is safe.

We further unanimously conclude that the intended use of the DHA algal oil in infant formula, meeting the specifications described above, is Generally Recognized as Safe (GRAS) based on scientific procedures and that other experts qualified to assess the safety of foods and food additives, and critically evaluating the same information, would concur with these conclusions.

_____ _____

Michael Carakostas, DVM, PhD Date
Consultant
MC Scientific Consulting LLC

_____ _____

Lewis P. Rubin, MD Date
Professor of Pediatrics and Biomedical Science
Texas Tech University Health Sciences Center El Paso

_____ _____

I. Glenn Sipes, PhD, Fellow AAAS and ATS Date
Consultant

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(b) (6)

9/15/16

Michael Carakostas, DVM, PhD
Consultant
MC Scientific Consulting LLC

Date

Lewis P. Rubin, MD
Professor of Pediatrics and Biomedical Science
Texas Tech University Health Sciences Center El Paso

Date

I. Glenn Sipes, PhD, Fellow AAAS and ATS
Consultant

Date

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Michael Carakostas, DVM, PhD — Date
Consultant
MC Scientific Consulting LLC

(b) (6)

Lewis P. Rubin, MD — September 13, 2016 — Date
Professor of Pediatrics and Biomedical Science
Texas Tech University Health Sciences Center El Paso

I. Glenn Sipes, PhD, Fellow AAAS and ATS — Date
Consultant

Conclusions of the Expert Panel

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Michael Carakostas, DVM, PhD — Date
Consultant
MC Scientific Consulting LLC

Lewis P. Rubin, MD — Date
Professor of Pediatrics and Biomedical Science
Texas Tech University Health Sciences Center El Paso

(b) (6)

I. Glenn Sipes, PhD, Fellow AAAS and ATS — 20 Sept 2016 — Date
Consultant

SUBMISSION END