SUMMARY OF DATA FOR A GRAS CONCLUSION

Algal Oil produced by *Schizochytrium* sp.

for use as a source of DHA and EPA in Food for Cats

17 March 2020

By:

Veramaris USA, LLC

Blair, Nebraska

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Parsippany, NJ

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Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

Veramaris is hereby confirming that this GRAS conclusion dossier has been produced in accordance with 21 CFR 570.225.

1.2 Name and Address of Notifier

Veramaris USA LLC
650 Industrial Road
Blair, NE 68008
USA

A joint venture of DSM Nutritional Products and Evonik Industries.

Person responsible for the dossier

James La-Marta
45 Waterview Boulevard
Parsippany, New Jersey 07054
Tel: 973-257-8325

1.3 Name and Address of Manufacturer

Veramaris USA LLC
650 Industrial Road
Blair, NE 68008
USA
(402) 533-1500

1.4 Name and Address of the Distributor

DSM Nutritional Products
45 Waterview Boulevard
Parsippany, NJ 07054
973-257-8500

1.5 Name of the Notified Substance

The common or usual name of the notified substance is Marine Micro-algae Oil, an oil from the marine micro algae, *Schizochytrium sp.* rich in the omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic (EPA) and identified in some reports as AOCED, algal oil containing EPA and DHA.

1.6 Intended Conditions of Use

AOCED from marine microalgae *Schizochytrium sp*. Is intended to be used as an ingredient in canned and dry/extruded cat foods for all life stages at a level not to exceed 1.5% wt/wt of the diet on a dry matter basis. The ratio of DHA to EPA is about 2.5:1 but higher ratios are possible. AOCED will be used to replace fish oil` commonly used in food for cats.

1.7 Statutory Basis for the GRAS Conclusion

Pursuant to 21 C.F.R. § 570.30(a)(1), a panel of independent experts assembled by DSM Nutritional Products on behalf of Veramaris, the GRAS Panel, has been asked to review the accumulated data regarding the safety of AOCED, containing high levels of the omega-3 fatty acids, DHA and EPA, in order to evaluate through scientific procedures, if AOCED would be safe for consumption by cats and therefore, to also conclude that AOCED would be Generally Recognized As Safe (GRAS) for use as an ingredient in food for cats specified above in Section 1.5.

1.8 Exemption from Premarket Approval Requirements

The notified substance is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on the conclusion by Veramaris USA LLC that the substance is GRAS under the conditions of its intended use.

1.9 Availability of Information

The data and information that are the basis for Veramaris' GRAS determination is available for the FDA's review and copying upon request during normal business hours at:

DSM Nutritional Products
45 Waterview Blvd
Parsippany, NJ 07054

We also agree to provide a complete digital copy of the data and information upon request.

1.10 Freedom of Information Act (FOIA) Exemptions

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

1.11 Certification

To the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes favorable information, as well as unfavorable information, known to Veramaris USA LLC and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

James La Marta, Ph.D., CFS
Sr. Manager Regulatory Affairs
Agent for Veramaris USA LLC

Date: 17 March 2020

2: Identity of the Notified Substance

2.1 Common or usual name of the notified substance

The common and usual name of the substance is: algal oil from marine microalgae *Schizochytrium* sp. AOCED, is the DSM Nutritional Products internal identification name for an oil obtained from marine microalgae (*Schizochytrium sp.)* rich in the omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic (EPA). This name is used throughout this dossier.

2.2 Characterization of the product

2.2.1 Chemical Identity

The commercial product is an oily material produced by the marine microalgae *Schizochytrium sp*. It is a source of the long chain polyunsaturated fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

Figure 1 Structure of Docosahexaenoic Acid (DHA, C22:6*n*-3)

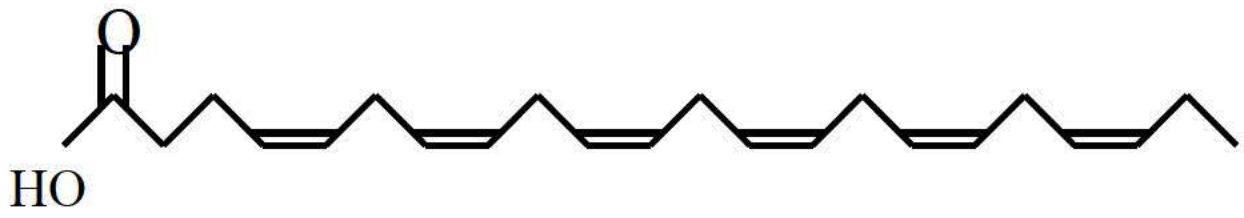
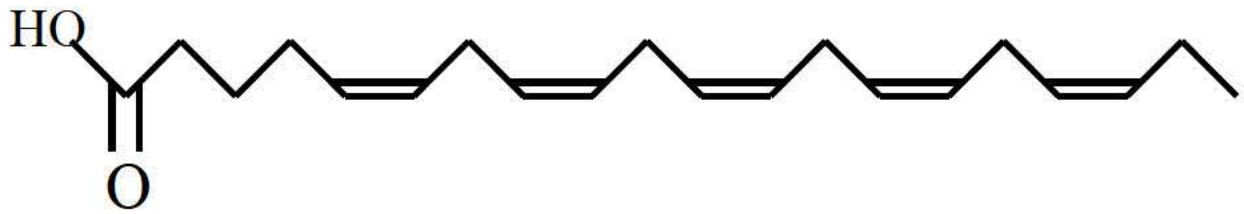


Figure 2 Structure of Eicosapentaenoic Acid (EPA, C20:5*n*-3)



2.2.2 Physical Properties

Appearance: Orange to golden brown oil
Solubility in water: Insoluble
Solubility in solvents: Partly miscible with acetone and methanol. Completely miscible with chloroform and hexane.

2.2.3 Composition of the commercial formulation

The commercial product is composed of the algal oil with added mixed tocopherols used as an antioxidant.

Table 1 Composition of the commercial product

Ingredient	Amount	Regulation
Algal Oil from *Schizochytrium sp.*	Min. 98.85%	Subject of this dossier
Tocopherols	Max. 0.15%/1500 ppm	21 CFR 582.3890

At the maximum recommended use level of 1.5% as noted in Section 1.5, the level of tocopherols contributed by algal oil will not exceed 22.5 ppm in the complete cat food.

2.2.4 Chemical Composition

Veramaris produced four non-consecutive pilot batches of the algal oil for analytical purposes. The results of analysis of these batches are summarized in Table 2. The analyses were conducted by (b) (4) using validated or verified test methods. Certificates of analysis are provided in Annex 1.

Table 2 Gross Composition (Crude Protein, Crude Fat, Total Fat, Crude Fiber, Ash, Moisture)

	Test Method	VY00014962	VY00015240	VY00015465	VY00015572
Crude Fat (%)	AOAC 954.02	(b) (4)			
Total Fat (%)	AOAC 996.06				
Free Fatty Acid as Oleic (%)	AOCS Ca 5a-40				
Insoluble Impurities (%)	AOCS Ca 3a-46				
Ash (g/100 g)	AOAC 923.03				
Moisture (%)	AOAC 930.15				
Moisture & Volatiles (%)	AOCS Ca 2c-25				
Protein (%)	AOAC 2001.11				
Total Dietary Fiber (%)	AOAC 991.43				

Table 3 Table Fatty Acid Profile (relative area % via AOCS Ce 1b-89)

	VY00014962	VY00015240	VY00015465	VY00015572
6:0 Caproic				(b) (4)
8:0 Caprylic				
10:0 Capric				
12:0 Lauric				
13:0 Tridecanoic				
14:0 Myristic				
14:1 Myristoleic				
15:0 Pentadecanoic				
15:1 Pentadecenoic				
16:0 Palmitic				
16:1 Palmitoleic				
16:2 Hexadecadienoic				
16:3 Hexadecatrienoic				
16:4 Hexadecatetraenoic				
17:0 Margaric				
17.1 Margaroleic				
18.0 Stearic				
18:1n-9 Oleic				
18:1n-7 Vaccenic				
18:2n-6 Linoleic				
20:0 Arachidic				
18:3n-6 Gamma-Linolenic				
20:1 Eicosenoic				
20:1n-9 Eicosenoic				
18:3n-3 alpha-linolenic				
21:0 Heneicosanoic				
18:4n-3 Stearidonic				
20:2n-6 Eicosadienoic				
22:0 Behenic				
22:1n-9 Erucic				
22:1n-11 Cetoleic				
20:3n-6 homo-gamma-Linolenic acid				
20:3n-3 Eicosatrienoic				
20:4n-5 Eicosatetraenoic				
20:4n-6 Arachidonic				
23:0 Tricosanoic				

	VY00014962	VY00015240	VY00015465	VY00015572
22:2n-6 Docosadienoic	(b) (4)			
20:4n-3 Eicosatetraenoic				
22:4n-3 Eicosatetraenoic				
24:0 Lignoceric				
20:5n-3 Eicosapentaenoic				
24:1 Nervonic				
21:5n-3 Heneicosapentaenoic				
22:4n-6 Docosatetraenoic				
22:5n-6 Docosapentaenoic				
22:5n-3 Docosapentaenoic				
22:6n-3 Docosahexaenoic				
Unknown				

2.3 Description of the production organism

2.3.1 Classification of the organism: *Schizochytrium sp.*

Below is an overview of the organism's taxonomy according to the Uniprot database (Uniprot, 2014).

Kingdom: Chromalveolata (recent refinement of Chromatista)
Division: Stramenophiles
Class: Labyrinthulomycetes
Order: *Saprolegniales
Family: Thraustochytriaceae
Genus: Schizochytrium
Species: sp. ATCC PTA-10208

*DSM believes *Thraustrochytriales* is more accurate but otherwise agrees with UniProt.

Schizochytrium sp. are thraustochytrids that belong to the Chromista kingdom (Bennett et al., 2017). The genus *Schizochytrium* is characterized by multiple binary cell divisions leading to clusters of sporangia. *Schizochytrium* spores are biflagellate at discharge and the number of spores discharged can range from two to one hundred.
Thraustochytrids are microscopic, unicellular algae. The earliest research of thraustochytrids placed them in the fungi because of their heterotrophic nature and superficial resemblance to chytrids (Sparrow, 1936). Current analysis using molecular biology techniques have demonstrated that thraustochytrids are not fungi, but are related to the heterokont algae (Cavalier-Smith, 2018) and as a result are now considered microalgae. Current phylogenic analysis indicates that the members of the Chromista are all photosynthetic, but some groups such as the Thraustochytridae have lost their chloroplast and the capability of photosynthesis and as a result, are exclusively heterotrophic (Tsui et al. 2009).

It should be noted that taxonomic categorization of microorganisms is in flux due to advances in molecular biology that result in slight shifts in the allocation of organisms to new groups and occasionally assignment of new names.

2.3.3 Parental strain

The microalgae parent strain was isolated from the intertidal coastline in 2007. Preliminary examination of the organism indicated that it is a Thraustochytrid. Subsequent detailed examination of the organism was conducted by Dr. Gunther Bahnweg, Head of Plant Toxicology and Mycology, Institute of Biochemical Plant Pathology, GSF National Research Centre for Environment and Health, Munich Germany. Dr. Bahnweg has studied thraustochytrids for over 30 years and published extensively on the organism (Bahnweg & Sparrow, 1974). His examination concluded that this microalga possessed the definitive characteristics of the genus Schizochytrium, and further concluded it was a previously unpublished species of that genus.(Bahnweg, 2009, 2010) Both DNA sequences and morphology characteristics were utilized in the identification of the microalgae and it has also been deposited with the ATCC as PTA-10208.

2.3.4 Production strain

Veramaris has developed an improved strain from the patented wild-type parent strain using a classical mutagenesis screening program. This program utilized well-accepted techniques commonly used in industrial strain improvement programs. No recombinant DNA technology was employed. The parent, ATCC PTA-10208, underwent three successive mutagenesis steps using a standard UV exposure procedure. The production strain was selected as a single colony out of the surviving population after screening for oil production ability and DHA to EPA ratio. The selected strain has consistent DHA and EPA content, and high biomass density under large scale fermentation conditions. Further manipulation of the fermentation media composition, pH and temperature led to an optimal oil production level without adversely affecting the DHA to EPA ratio.

2.3.5 Absence of Toxins

Schizochytrium sp. Is a thraustrochytrid and a member of the Chromista kingdom (Stramenopilia), which includes the golden algae, diatoms, yellow-green algae, haptophyte and cryptophyte algae, and oomycetes.

Within the microalgae in the Chromista (Stramenopilia), there are two toxins known to be produced, domoic acid and pymnesin. Domoic acid is a potent neurotoxin which causes amnesic shellfish poisoning in humans. It is a naturally occurring amino acid whose production appears to be limited to a few species of microalgae (diatoms) in the genus Pseudonitzschia (and possibly by one species of Chrysochromulina, a flagellated species of golden algae) (Villac et al., 1993). Species of the genus Pseudonitzschia are common members of marine phytoplankton throughout the world. Four of these diatom species have been identified as being able to produce domoic acid, and these species can be generally found in the colder waters of the Northern Hemisphere (Fritz et al., 1992; Garrison, et al., 1992; Lundholm et al., 1994).

In the phylum Heterokonta, the thrautochytrids are in a separate subphylum and class from the diatoms, so one would not expect to find domoic acid in *Schizochytrium sp.* Prior confirmatory testing of *Schizochytrium sp.* dried microalgae for domoic acid was performed using standard HPLC methods – ultraviolet (modified version of AOAC Official Method 991.26) and ELISA (Biosense).Both methods quantitatively detect domoic acid and these analyses showed no evidence of domoic acid present in *Schizochytrium sp.* dried microalgae.

The other toxins found in a member of the Chromista (Stramenopilia) are limited to two species of Prymnesium (*P. parvum* and *P. patelliferum*). These toxins (called prymnesins by some) exhibit a broad spectrum of activity including lethal effects on gill breathing animals, cytotoxic effects on erythrocytes, nucleated mammalian cells, protozoa, and bacteria. Prymnesin toxins are acidic polar phosphor proteolipids, which because of their chemical nature, form micelles in water. These toxins are not heat stable. See Annex 2 for the analysis of a related strain in the DSM collection used for algal oil production for consumption by humans.
There are no literature reports of this organism producing toxins or being pathogenic nor does the sponsor have any data indicating the initially isolated strains nor the daughter after over 30 years of strain improvement have produced toxins under the conditions of large-scale fermentation. Analysis of related and parent strains in the DSM collection used for the manufacture of algal oil for human consumption did not reveal the presence of domoic acid (Annex 3). Analysis of a pilot batch

of the production strain performed in 2019 also showed no production of domoic acid (Annex 13). Toxicology studies with the production strain confirmed the absence of toxins. Another strain in the DSM collection was approved for use in food for adult cats under a Food Additive Petition, 21 CFR 573.615. (Fed Reg 2018).

Algal oil is produced via a fermentation process using heterotropic *Schizochytrium sp*., a microscopic, primarily saprotrophic, unicellular microalgae which is usually found in estuarine and marine environments around the world. The scientific literature indicates that thraustochytrids, especially those of the genus Schizochytrium, are regulatory consumed as food by a wide range of filter feeding invertebrates (clams and mussels). The available published and unpublished scientific data show no evidence that these organisms produce toxins. Blue-green algae and photosynthetic species of Dinophyta produce most of the toxic compounds produced by microalgae, and the *Schizochytrium sp*. Is in a separate kingdom from both of these types of microalgae.

Due to the unavailability of authentic prymnesin standards, *Schizochytrium sp.* microalgae have not been directly analyzed for the presence of prymnesin toxins. However, a bioassay for prymnesin has been developed (Houdan et al., 2004; Graneli et al., 2003; Larsen et al., 1993; Vanhaecke et al., 1981) utilizing *Artemia nauplii* as the test organism. Results of this bioassay on *Schizochytrium sp*. fermentation broth from parent strains show no significant different in the mortality of Artemia culture from the control, indicating the absence of prymnesin toxin.

2.4 Manufacturing process for Oil from *Schizochytrium* sp. Marine Microalgae

2.4.1 Overview of Manufacturing Method

The manufacturing process for algal oil from *Schizochytrium* sp. marine microalgae consists of the following steps: fermentation, cell lysis, recovery of the oil phase, and quality control of the finished product. The entire process is performed in accordance with the Food Safety Modernization Act (FSMA) and its implementing regulations, including current Good Manufacturing Practices (cGMP). An overview of the process is provided in Figure 3.

Figure 3 Overview of the manufacturing process

2.4.2 Raw Materials

The raw materials used for the fermentation and recovery of the product are suitable for the intended use, leading to the required safety status of the product. The raw materials used for the media are of feed-grade quality or better and meet predefined quality standards that are strictly monitored and controlled by the Quality Assurance Department. Internal specifications for the raw materials are attached in Annex 4. The composition of the fermentation medium has been developed for optimum production of algal oil with the desired fatty acid profile.

Table 4 Raw materials used to manufacture Oil from *Schizochytrium sp.* Marine Microalgae

Ingredient	Approved Use	Ingredient	Approved Use
Ammonium Sulfate	21 CFR[1] 184.1143	Sodium Chloride	AAFCO OP[2] 57.31
Anhydrous Ammonia	AAFCO OP 87.11	Sodium hydroxide	21 CFR 582.1763
Calcium Chloride	AAFCO OP 57.51	Sodium Molybdate	AAFCO OP 57.145
Calcium Pantothenate	21 CFR 582.5212	Sodium Sulfate	AAFCO OP 57.109
Citric Acid	21 CFR 582.6033	Thiamine HCl	21 CFR 582.5875
D-Biotin	21 CFR 582.5159	Zinc Sulfate	AAFCO OP 57.118
Cupric Sulfate	AAFCO OP 57.69	Yeast Extract	AAFCO 96.11
Glucose syrup	AAFCO OP 87.35	Phosphoric Acid	21 CFR 582.1073

Ingredient	Approved Use	Ingredient	Approved Use
Water	Potable	Cyanocobalamin	AAFCO OP 90.11
Silicone Anti-Foam	21 CFR 582.1711	Nickel Sulfate	21 CFR 184.1537
Ferrous Sulfate	AAFCO OP 57.83	Potassium Chloride	AAFCO OP 57.102
Magnesium Sulfate	AAFCO OP 57.88	Monosodium glutamate	21 CFR 582.1
Manganese Chloride	AAFCO OP 57.91	Mono-Potassium phosphate	21 CFR 582.5628
Protease from fermentation of a nonpathogenic and nontoxigenic strain of Bacillus licheniformis	AAFCO OP 30.1	Tocopherols	21 CFR 582.3890

[1] The Code of Federal Regulations (CFR)

[2] The Association of American Feed Control Officials (AAFCO) is an association of state agencies who regulate the sale and distribution of animal feeds in the individual states. Every year AAFCO publishes the Official Publication (OP) which includes a list of feed ingredients approved for use in the United States.

2.4.3 Fermentation Process

Veramaris's algal oil ingredient is manufactured by fed-batch culture fermentation using the strain of *Schizochytrium* sp. described above. During each step of fermentation, physical and chemical control measures are incorporated.

Frozen seed of *Schizochytrium* sp. isolate is thawed and grown through a series of process steps, which include transfers from shake flasks to puntbus (inoculum vessel), and then to seed fermenters, and finally progressing to a large-scale production fermenter. Temperature, agitation, pressure, pH, and dissolved oxygen are controlled during the initial stages of fermentation.

Generally speaking, products formed by organisms are produced as a result of their response to environmental conditions such as nutrients and ions. Carbon compounds are major sources of cellular carbon and energy. Nitrogen is incorporated into cell mass in the form of proteins and nucleic acids. Oxygen is required as a terminal electron acceptor in aerobic metabolism. Phosphorus is present in nucleic acids and in the cell wall. Sulfur is present in proteins and some coenzymes. Sodium and potassium are required for energy metabolism and are important in the transport of charged species. Potassium and magnesium are cofactors for many enzymes. Trace metals are mainly cofactors and play a regulatory role in many enzyme reactions. Vitamins usually function as coenzymes to stimulate cell growth and synthesis of some metabolites.

Fermentation media is continuously sterilized via a heat exchanger and charged to pre-cleaned and pre-sterilized fermenters. While in the main fermenter, temperature, airflow, overpressure, agitation, dissolved oxygen and carbon dioxide, pH, ammonia feed, foaming, and glucose feed are all controlled to optimize growth of the organism, oil yield, and fatty acid profile of the oil. Various samples are taken during fermentation to control the process. These are summarized in Table 5. Samples are taken to determine fatty acid profile at the end of the seed and main fermentations. Glucose is sampled every 4 hours during the cell growth phase and every 6 hours during the oil production phase. Glucose feed rate is adjusted based on the measurement of glucose concentration. In the final seed fermenter and in the main fermenter, ammonia is sampled every 4

hours. Ammonium sulfate may be added to maintain the appropriate ammonia concentration. In the seed fermenter, the phosphate concentration is measured to confirm if the addition of monopotassium phosphate is needed to maintain cell growth. Wet fatty acid profile is used to measure DHA and EPA concentrations in the main fermenter every 12 hours. These samples are analyzed using the same test method as that for full fatty acid profile except the sample is undried and no internal standard is added. Indication of biomass concentration using infrared balance allows for determination of the amount of tocopherols to add before pasteurization. ABS is a measure of optical density at a wavelength of 465 nanometers; this number should be very low. If the starting media, also called mash, is contaminated then this value will increase. Samples are taken throughout the fermentation process, from the inoculum to the main fermenter, to confirm the absence of microbiological contamination. The fermentation broth is examined under a phase contrast microscope to look for any foreign cells. Also, fermentation broth is put into sterile Tryptone Soy Broth (TSB) tubes and the tubes are static incubated at 35°C ± 2°C for 48 hours, after which they are examined. In the event of contamination, the facility internal laboratory isolates the contaminant and utilizes a third-party laboratory for identification. The fermentation broth is discarded if the contaminant is identified as a pathogen.

Table 5 Fermentation Sample Profile

Analysis	Inoculum			Seed Fermenter and Main Fermenter				
	Starting Mash	Every 24 Hr (or more frequently)	Before Use	Starting Mash	Every 12 Hr (or more frequently)	Every 24 Hrs	Grow out	Feed
Fatty Acid Profile			X			X	X	
Glucose	X	X	X	X	X 4-6hrs		X	X
Ammonia			X	X	X 4 hrs			
Phosphate					X 4hrs until depletion			
Wet Fatty Acid Profile					X			
Biomass (infrared)			X			X	X	
ABS(465)	X			X				

Analysis	Inoculum			Seed Fermenter and Main Fermenter				
	Starting Mash	Every 24 Hr (or more frequently)	Before Use	Starting Mash	Every 12 Hr (or more frequently)	Every 24 Hrs	Grow out	Feed
Microbiological purity	X	X	X	X		X	X	

2.4.4 Recovery Process

Unpasteurized fermentation broth is heated to a minimum 60 °C and pasteurized for a minimum of 1 hour with agitation. This step kills the microorganism. Temperature is monitored throughout pasteurization. Mixed tocopherols are added to the pasteurized fermentation broth. The amount added depends on the total amount of biomass in the fermentation broth, but it is up to 1500 ppm. At 55-65 °C, the broth is pH adjusted with caustic and protease enzyme is added to lyse the cells. 0.3% enzyme is added based on the weight of the broth. The broth is then demulsified under the combined effects of pH and heat, which also deactivates the enzyme. Specifically, the pH of the broth is adjusted with 50% caustic and sodium chloride is added. The temperature of the broth is increased to 90-94 °C for 12-48 hours. The pH of the broth is monitored online during the demulsification process; offline pH is also checked to confirm the online reading. When demulsification is complete, the pH is adjusted to 7.5-9.0 with phosphoric acid or caustic.

A centrifuge is then used to separate the oil from the water and solids present in the demulsified broth. After 15 minutes of feeding material to the centrifuge, samples are collected. Thereafter, sampling is done each hour and the back pressures on the centrifuge are changed if necessary. The oil from centrifugation is collected in a holding tank. It is then dried with nitrogen sparge, heat (40-60 °C), and vacuum to a moisture of < 0.5%. The dried oil is then transferred to totes for storage.

2.4.5 Method to ensure stability of the production organism

DSM maintains a master cell bank (MCB) of cryovials in a liquid nitrogen storage freezer. The MCB is evaluated periodically to confirm that the production characteristics have not degraded. A working cell bank (WCB) is maintained at each production facility and is replenished from the master cell bank, as needed. WCB samples are checked for identity, viability, microbial purity, and media, by enrichment and viewing morphology (colony shape and microscopy). If all these parameters are correct, the strain is tested for production capacity in laboratory scale level fermenters. Only if the productivity and the product quality meet the required standards, is the new WCB accepted for production runs.
Each production run originates with a new WCB cryovial minimizing the chances of a strain becoming unstable and leading to poor oil quality.

2.5 Specifications

Representative samples from each production batch are subjected to evaluation by the quality control department to ensure conformance to the established specification, following the method

indicated for each material characteristic. The specifications for algal oil from marine microalgae *Schizochytrium sp.* . are shown below. These are the same lots notified to CVM in AGRN 36. The peroxide value noted in the Table 6 is for the product at manufacture.

Table 6 Product Specifications

Specification		Method	Lot Number			
			VY0001-4962	VY0001-5240	VY0001-5465	VY0001-5572
DHA + EPA content (mg/g)	Min. 500	AOCS Ce 1b-89	(b) (4)			
DHA content (mg/g)	Min. 250	AOCS Ce 1b-89				
EPA content (mg/g)	Min. 100	AOCS Ce 1b-89				
Free fatty acids (%)	Max. 5	AOCS Ca 5a-40				
Moisture (%)	Max. 0.75	AOAC 930.15				
Crude Fat (%)	Min. 92	AOAC 954.02				
Peroxide Value meq/kg	Max. 0.5	AOCS Ce 1b-89				

The range of DHA concentration is from 250 to 400 mg/g and the range of EPA concentration is from 100 to 250 mg/g when the minimum EPA + DHA concentration is 500 mg/g. A range of ratios is possible as are higher levels of total omega-3 fatty acids as evident in Table 6 above.

2.6 Comparison to Algal Oil used in Human Nutrition

The oil produced by the process described above yields a product similar to the algal oil notified in human GRAS, GRN 553 in which the use of the oil rich in Omega-3 fatty acids is in infant formula. The oil described for infant formula complies with the USP-NF specification for 'Schizochytrium Oil'.

Table 7 Comparison of algal oils for cat food and infant nutrition

Specification	Cats	Infants
DHA + EPA content (mg/g)	Min. 500	Min. 500
DHA content (mg/g)	Min. 250	Min. 350
EPA content (mg/g)	Min. 100	Max. 100
Free fatty acids (%)	Max. 5	Max. 4
Moisture (%)	Max. 0.75	Max. 0.2
Crude Fat (%)	Min. 92	NA
Peroxide Value meq/Kg	Max. 0.5	Max. 0.5

2.7 Analytic Methods

As noted in the table above, all the analytical methods employed to ensure that the product meets the specifications are readily available compendial methods.

2.8 Stability

A series of stability studies were performed with the anticipated commercial product, AOCED and with prototypes of cat food produced under manufacturing conditions typical of the industry and stored at 25°C and 40°C.

2.8.1 Stability of AOCED

The stability of AOCED was evaluated at four temperatures; -20°C, 5°C, 25°C and 40°C and in two packages, coated aluminum bottles and opaque, high density polyethylene (HDPE) screw cap bottles to simulate commercial packaging materials. At all conditions the AOCED was stable for 12 months, the anticipated typical commercial storage time for the oil, DHA and EPA levels remained above the minimum specification and the formation of free fatty acids was inhibited by the antioxidants. Annex 5 contains the analytical reports and the sampling protocol. Annex 6 contains the summary report for both packages at 25° C and 40°C. The table below (Table 8) presents the average values for the four lots used in the study.

Table 8 Stability of AOCED at 25° C and 40°C in Aluminum and HDPE Bottles

	Aluminum 25°C			HDPE 25°C			Aluminum 40°C			HDPE 40°C		
Months	**EPA mg/ g oil**	**DHA mg/g oil**	**Peroxide Value meq/kg**	**EPA mg/ g oil**	**DHA mg/g oil**	**Peroxide Value meq/kg**	**EPA mg/ g oil**	**DHA mg/g oil**	**Peroxide Value meq/kg**	**EPA mg/ g oil**	**DHA mg/g oil**	**Peroxide Value meq/kg**
0	122	430	0.3	123.25	430.5	0.4	123.25	430.5	0.4	123.25	430.5	0.4
1	122.25	430.75	0.525	120.75	420.75	0.975	123.25	431.75	0.4	119.25	416.5	1.65
2	120.75	425.75	0.675	121.25	425	1.6	122.5	426.75	0.2	122.25	425.25	2.55
3			1.067			2.1			< 0.1			2.825
4	121	430	2.55	122	430.25	2.975	122.5	431.5	< 0.1	121.25	425.75	2.7
5			2.35			3.6			< 0.1			2.5
6	121.25	428.75	2.75	122	427	5.025	122.5	429	< 0.1	121.25	424	2.475
9	121	431	6	121.5	422.5	5.725						
12	123.5	430.5	6.7	124	427.75	6.05						

A peroxide value >5 is considered to be an indication that polyunsaturated oils are going rancid. (Ismail et al., 2016) and a PV ≥10 has been considered an indicator of full rancidity (NSF/ANSI Standard 173 – 2013)

2.7.2 Stability of Algal Oil in Canned Cat Food

To measure the storage stability of AOCED in canned cat food, the food was formulated to be representative of typical commercial formulae (see Table 9) and to meet the requirements for adult maintenance. The ingredients and metal cans used in this study are in current commercial use by pet food companies.

The wet cat food was produced at (b) (4) . After grinding the chicken liver (b) (4) the ingredients were mixed with the algal oil (b) (4) and heated in a Groen steam jacketed kettle to reach 170° F. The mixture was filled in 5.5 oz metal cans and sealed before being retorted (SSA Surdry Stock America Sterilization Systems) at 250° F for about 45 minutes to reach sterilization ($F_o \geq 8$). Samples were taken after retort and stored at either 25° C and 60% relative humidity (RH) or 40° C and 75% RH for 24 months. After the initial timepoint, one can from each lot was analyzed every three months for DHA and EPA (AOAC 996.06), moisture (AOAC 925.09), and peroxide value (AOCS Cd 8-53). DHA and EPA concentrations in the samples were corrected for moisture and reported on a dry matter basis. Sample analysis was conducted at (b) (4)

Table 9 Ingredients Used in Canned Cat Food

Ingredients	%
Chicken, M. Deboned	65.00
Water	20.75
Meat By-Product (pork)	10.00
Chicken Liver	2.00
Brewer's Rice	1.00
AOCED	0.41
Vitamin and Mineral Premix	0.15
Guar Gum	0.14
Carrageenan	0.14
Potassium Chloride	0.14
Natural Flavors	0.10
Choline Chloride	0.07
Taurine	0.07
Salt	0.03
Total	**100.00**

[1] Three lots of AOCED were used in the stability study. The lot numbers are VY00014962, VY00015240, and VY00015572.

Table 10 Composition of Vitamin and Mineral Premix

Nutrients	Units	Qty/kg
VITAMIN A	IU	4,513,170.10
VITAMIN A	IU	865,125
VITAMIN D	IU	173,025
VITAMIN E	IU	23,966.10
MENADIONE	MG	1, 144.6
NIACIN	MG	801.2
NIACIN	MG	18,619.40
THIAMINE	MG	2,335. 3
RIBOFLAVIN	MG	1,905. 3
PYRIDOXINE	MG	1,661
VITAMIN B12	MG	7.9
PANTOTHENIC ACID	MG	3, 655.7
FOLIC ACID	MG	175.1
BIOTIN	MG	20.6
COPPER	MG	2, 700
IRON	MG	15,840
MANGANESE	MG	1,368
IODINE	MG	324
ZINC	MG	18,000
SELENIUM	MG	63
CALCIUM CARBONATE	MG	390, 000
MINERAL OIL	MG	10,000
PEA BRAN MEAL	MG	392, 664

Table 11 Nutritional Analysis of Canned Cat Food

Nutrients (calculated)	AS Fed	DM	AAFCO Cat Food Nutrient Profile for Adult Maintenance[1]
Moisture, %	75.7	24.3	N/A
Crude protein, %	11.11	34.611	26.0
Arginine, %	0.69	2.150	1.04
Histidine, %	0.27	0.841	0.31
Isoleucine, %	0.43	1.340	0.52
Leucine, %	0.78	2.430	1.24
Lysine, %	0.81	2.523	0.83
Methionine, %	0.26	0.810	0.20
Methionine-cystine, %	0.36	1.121	0.40
Phenylalanine, %	0.43	1.340	0.42
Phenylalanine-tyrosine, %	0.78	2.430	1.53

Nutrients (calculated)	AS Fed	DM	AAFCO Cat Food Nutrient Profile for Adult Maintenance[1]
Threonine, %	0.44	1.371	0.73
Tryptophan, %	0.13	0.405	0.16
Valine, %	0.51	1.589	0.62
Crude fat, %	9.59	29.875	9.0
Linoleic acid, %	1.54	4.798	0.6
Alpha-Linolenic acid, %	0.10	0.312	ND
Arachidonic acid, %	0.10	0.312	0.02
EPA+DHA, %	0.6	1.869	ND
Calcium, %	0.641	1.997	0.6
Phosphorus, %	0.397	1.237	0.5
Potassium, %	0.227	0.707	0.6
Sodium, %	0.09	0.280	0.2
Chloride, %	0.17	0.530	0.3
Magnesium, %	0.024	0.075	0.04
Iron, mg/kg	0.005	0.016	80
Copper, mg/kg	5.2	16.199	5.0
Manganese, mg/kg	3.0	9.346	7.6
Zinc, mg/kg	45	140.187	75
Iodine, mg/kg	1.48	4.611	0.6
Selenium, mg/kg	0.25	0.779	0.3
Vitamin A, IU/kg	2,220	6915.888	3,332-333,300
Vitamin D, IU/kg	24.4	76.012	280-30,080
Vitamin E, IU/kg	4.07	12.679	40
Vitamin K, mg/Kg	0.27	0.841	0.1
Thiamine, mg/kg	0.32	0.997	5.6
Riboflavin, mg.kg	0.532	1.657	4.0
Pantothenic acid, mg/kg	1.28	3.988	5.75
Niacin, mg/kg	6.35	19.782	60
Pyridoxine, mg/kg	0.362	1.128	4.0
Folic acid, mg/kg	0.0893	0.278	0.8
Biotin, mg/Kg	0.0141	0.044	0.07
Vitamin B12, mg/kg	4.76	14.829	0.020
Choline, mg/kg	87.1	271.340	2400
Taurine, %	0.11	0.343	0.20

[1] Based on Dry matter and Minimum unless otherwise stated

DHA and EPA concentration and their retentions as well as peroxide value of three lots of AOCED in the wet cat food are shown in Table 12 (25° C, 60% RH) and Table 13 (40° C and 75% RH). Overall, DHA and EPA were stable in the wet food at both conditions for 18 months. This study will continue for a total of 36 months.

Table 12 **Stability of AOCED in Canned Cat Food (25° C, 60% RH)**

			Time points, month								
Lots	Measures		Initial*	3	6	9	12	18	24	30	36
VY00014962	EPA	Concentration, %	0.20	0.21	0.17	0.21	0.21	0.21			
		Retention#, %		107	85	106	105	104			
	DHA	Concentration, %	0.68	0.72	0.67	0.67	0.66	0.66			
		Retention#, %		106	99	99	97	97			
	PV	meq/kg fat	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0			
VY00015240	EPA	Concentration, %	0.21	0.20	0.21	0.20	0.21	0.2			
		Retention#, %		98	101	99	102	100			
	DHA	Concentration, %	0.65	0.68	0.62	0.65	0.67	0.61			
		Retention#, %		105	95	100	103	94			
	PV	meq/kg fat	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0			
VY00015572	EPA	Concentration, %	0.20	0.20	0.20	0.21	0.20	0.2			
		Retention#, %		101	101	102	101	99			
	DHA	Concentration, %	0.63	0.66	0.61	0.62	0.65	0.64			
		Retention#, %		104	97	98	103	101			
	PV	meq/kg fat	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0			

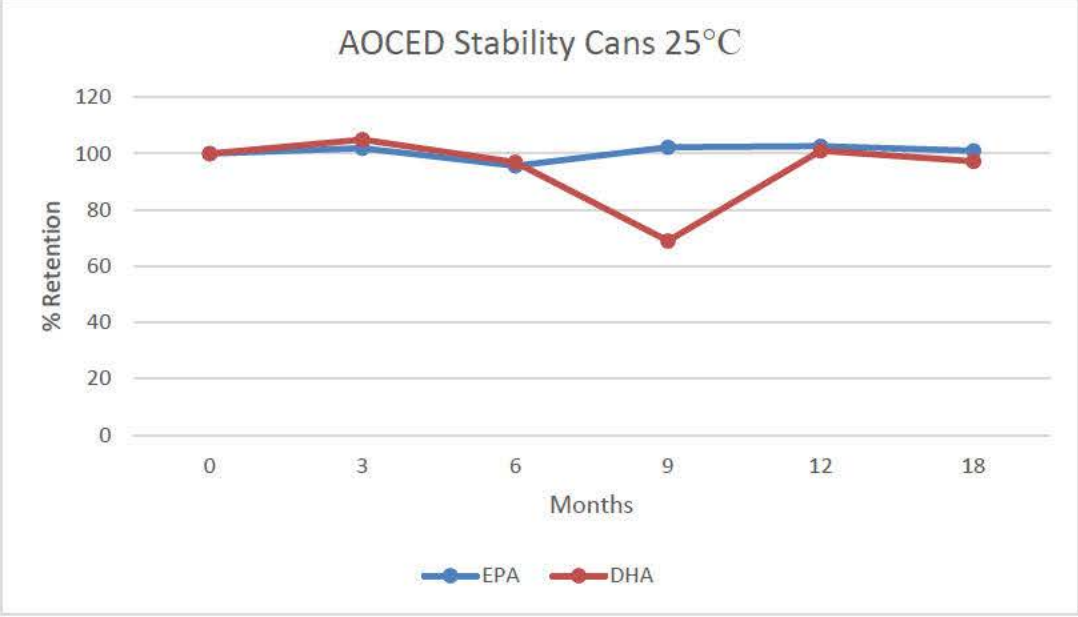


Figure 4 **Stability of ACED in canned cat food at 25° C**

Table 13 Stability of AOCED in Canned cat Food (40° C and 75% RH)

Lots	Measures		Initial*	3	6	9	12	18
VY00014962	EPA	Concentration, %	0.20	0.21	0.21	0.21	0.21	0.21
		Retention#, %		106	106	105	105	104
	DHA	Concentration, %	0.68	0.71	0.71	0.75	0.67	0.7
		Retention#, %		104	104	110	98	103
	PV	meq/kg fat	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015240	EPA	Concentration, %	0.21	0.20	0.21	0.21	0.20	0.2
		Retention#, %		98	101	102	97	98
	DHA	Concentration, %	0.65	0.60	0.66	0.7	0.67	0.64
		Retention#, %		93	101	108	103	98
	PV	meq/kg fat	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015572	EPA	Concentration, %	0.20	0.20	0.20	0.20	0.20	0.2
		Retention#, %		101	101	100	100	100
	DHA	Concentration, %	0.63	0.65	0.66	0.65	0.65	0.65
		Retention#, %		103	104	103	102	102
	PV	meq/kg fat	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

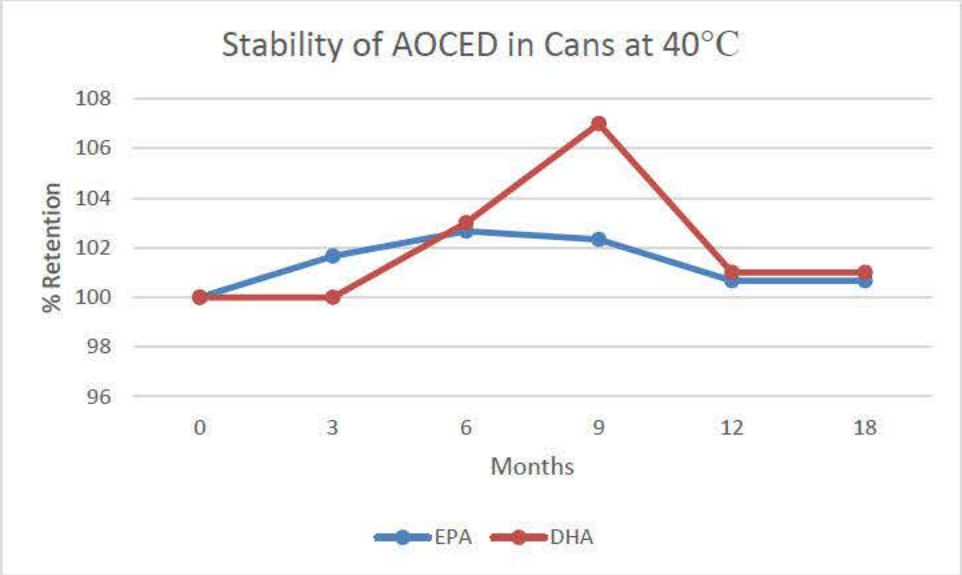


Figure 5 **Stability of AOCED in canned cat food at 40° C**

The results indicate that in a model matrix of canned cat food AOCED is stable for at least 18 months. Actual stability in commercial formulations will need to be confirmed by the pet food manufacturers using their food formulations and processing conditions. The variance in trend in the DHA concentration at both temperatures at 9 months was probably due to an error in sampling as the 12 and 18 month samples showed a more probable value.

2.7.3 Storage Stability of AOCED in Dry Cat Food

In order to measure the stability of AOCED in dry cat food, extruded kibble, over time, two different applications were studied: addition of AOCED pre-extrusion and addition of AOCED post-extrusion. Three lots of AOCED were used. The lot numbers were: VY00014962, VY00015240, and VY00015572. Each lot was applied at 1.45%. The dry cat food was formulated to be representative of commercial dry cat food (see Table 14 for composition) and produced at the (b) (4) . The same lots of oil were used to manufacture the dog food that was reported in AGRN 36.

Table 14 **Ingredient Composition of Kibble used in Shelf Life Study**

Ingredient Name	%
Chicken By Product Meal	23.3
Corn Gluten Meal	21.1
Dried Eggs	15.1
Brewers Rice	10.8
Whole Corn	9.72
Chicken Fat	4.55
Wheat	4.3
Flavor	3.0

Ingredient Name	%
Sorghum	2.0
Beet Pulp	1.9
Algal Oil	1.45
Potassium chloride	0.75
Salt	0.70
Dicalcium Phosphate	0.5
Vitamin Premix	0.30
Taurine	0.21
Trace Mineral	0.14
Verdilox GT Dry	0.10
Choline Chloride	0.08

Table 15 Composition of the Vitamin Premix

Nutrients	Amount/kg
Vitamin A (IU)	7,785,137
Vitamin D3 (IU)	417,312
Vitamin E (IU)	36,237
Vitamin B12 (mg)	10
Riboflavin (mg)	2,141
D-Pantothenic Acid (mg)	5,528
Niacin (mg)	29,364
Folic Acid (mg)	327
Vitamin B6 (mg)	2,512
Thiamine (mg)	6,465
Biotin (mg)	32

Table 16 Composition of the Mineral Premix

Nutrients	Amount/kg
Iodine (ppm)	1500
Cobalt (ppm)	1000
Selenium (ppm)	200
Zinc (%)	20
Iron (%)	10
Manganese (%)	5.5
Copper (%)	1.5

Table 17 Nutritional Analysis of Dry Cat Food used in the stability study

Nutrients (calculated)	AS Fed	DM	AAFCO Cat Food Nutrient Profile for Adult Maintenance[1]
Moisture, %	6.0		N/A
Crude protein, %	40.97	43.59	26.0
Arginine, %	2.14	2.28	1.04
Histidine, %	0.88	0.94	0.31
Isoleucine, %	1.74	1.85	0.52
Leucine, %	4.21	4.48	1.24
Lysine, %	2.11	2.24	0.83
Methionine, %	0.98	1.04	0.20
Methionine-cystine, %	1.59	1.69	0.40
Phenylalanine, %	2.0	2.13	0.42
Phenylalanine-tyrosine, %	3.36	3.57	1.53
Threonine, %	1.61	1.71	0.73
Tryptophan, %	0.43	0.46	0.16
Valine, %	2.12	2.26	0.62
Crude fat, %	16.81	17.88	9.0
Linoleic acid, %	3.05	3.24	0.6
Alpha-Linolenic acid, %	0.15	0.16	ND
Arachidonic acid, %	0.03	0.03	0.02
EPA+DHA, %	0.77	0.82	ND
Calcium, %	1.09	1.16	0.6
Phosphorus, %	1.11	1.18	0.5
Potassium, %	0.84	0.89	0.6
Sodium, %	0.671	0.71	0.2
Chloride, %	1.14	1.21	0.3
Magnesium, %	0.10	0.11	0.04
Iron, %	0.028	0.03	80
Copper, mg/kg	22.0	23.40	5.0
Manganese, mg/kg	87.0	92.55	7.6
Zinc, mg/kg	347.0	369.15	75
Iodine, mg/kg	3.22	3.43	0.6
Selenium, mg/kg	1.08	1.15	0.3
Vitamin A, IU/kg	28,800	30638.30	3,332-333,300
Vitamin D, IU/kg	2,370	2521.28	280-30,080
Vitamin E, IU/kg	375.0	398.94	40
Thiamine, mg/kg	25.4	27.02	0.1
Riboflavin, mg/kg	22.0	23.40	5.6
Pantothenic acid, mg/kg	37.0	39.36	4.0
Niacin, mg/kg	258.0	274.47	5.75
Pyridoxine, mg/kg	22.3	23.72	60

Nutrients (calculated)	AS Fed	DM	AAFCO Cat Food Nutrient Profile for Adult Maintenance[1]
Folic acid, mg/kg	3.66	3.89	0.8
Biotin, mg/Kg	0.62	0.66	0.07
Vitamin B12, mg/kg	114.0	121.28	0.020
Choline, mg/kg	3150	3351.06	2400
Taurine, %	0.31	0.33	0.20

In the pre-extrusion application, AOCED was injected into the preconditioner of a Wenger X20 extruder during the dry kibble production. The major extrusion parameters were: feed rate, 172 kg/hour; preconditioner discharge temperature, 98° C; die temperature, 176° C; and cone head pressure, 185 psig. The extruded kibbles which already contained algal oil were dried in a Wenger dryer at 127° C for 23 minutes and cooled for 7 minutes before coating with chicken fat and flavors. In the post-extrusion application, AOCED was mixed with the chicken fat before coating the dry kibbles previously extruded without AOCED.

Samples for the storage stability tests were obtained from the finished dry cat food after both applications, put into metalized 5 mil KPET/VMPET/PE zip bags (Uline S-20713GLD, Uline) that were heat sealed, and stored at either 25 °C and 60% RH or 40 °C and 75% RH for 24 months. After the initial analysis, a bag from each batch was analyzed every three months for DHA and EPA (AOAC 996.06), moisture (AOAC 925.09), and peroxide value (AOCS Cd 8-53). DHA and EPA concentrations were corrected for moisture and reported on a dry matter basis. The sample analysis was conducted at (b) (4)
The statistical analysis report and analytical data for the stability of AOCED in extruded cat food post extrusion application can be found in Annex 7.

Table 18 Stability of DHA and EPA in dry cat food – Post-extrusion application (25 °C, 60% RH)

			Time points, month						
Lots	Measures		Initial*	3	6	9	12	18	24
VY00014962	EPA	Concentration, %	0.17	0.17	0.17	0.17	0.17	0.17	0.17
		Retention#, %		101	101	100	101	101	101
	DHA	Concentration, %	0.65	0.65	0.64	0.64	0.67	0.64	0.60
		Retention#, %		100	99	100	103	99	99
	PV	meq/kg fat		3.2	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015240	EPA	Concentration, %	0.18	0.17	0.18	0.18	0.17	0.17	0.17
		Retention#, %		96	102	102	96	97	96
	DHA	Concentration, %	0.62	0.59	0.63	0.61	0.61	0.62	0.57

Lots	Measures		Time points, month						
			Initial*	3	6	9	12	18	24
		Retention#, %		96	101	98	98	100	98
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015572	EPA	Concentration, %	0.17	0.17	0.18	0.17	0.18	0.17	0.17
		Retention#, %		100	106	99	105	100	100
	DHA	Concentration, %	0.59	0.60	0.62	0.57	0.59	0.61	0.60
		Retention#, %		101	104	95	99	103	101
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

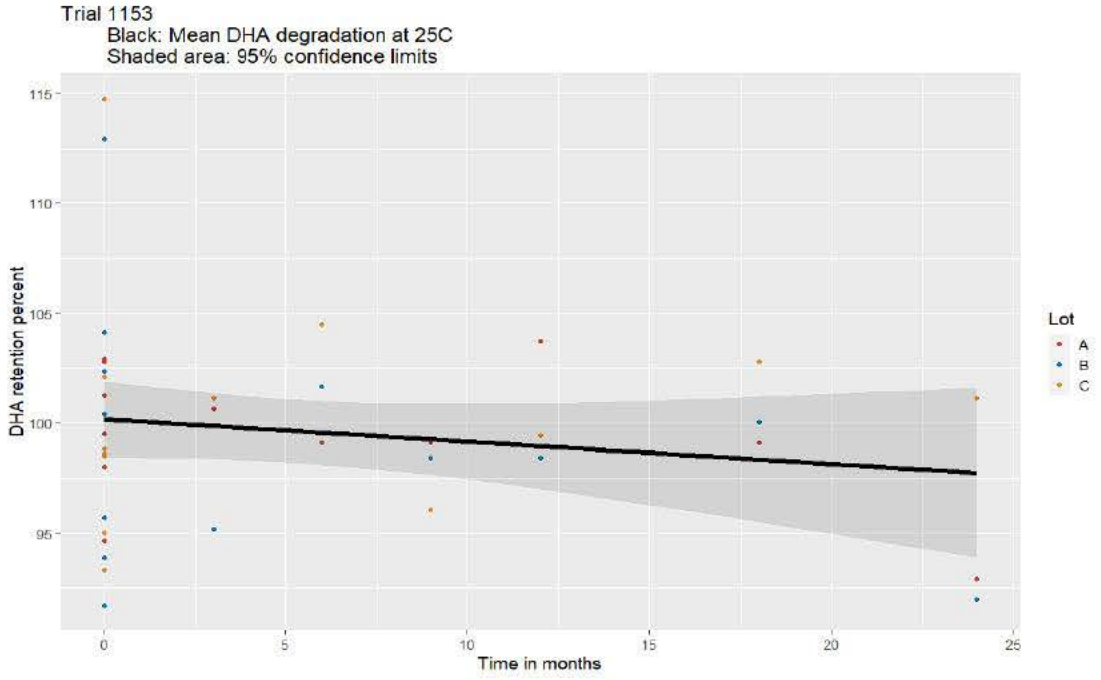


Figure 6 **Stability of DHA in Extruded Cat Food @ 25°C, Post Extrusion Application**

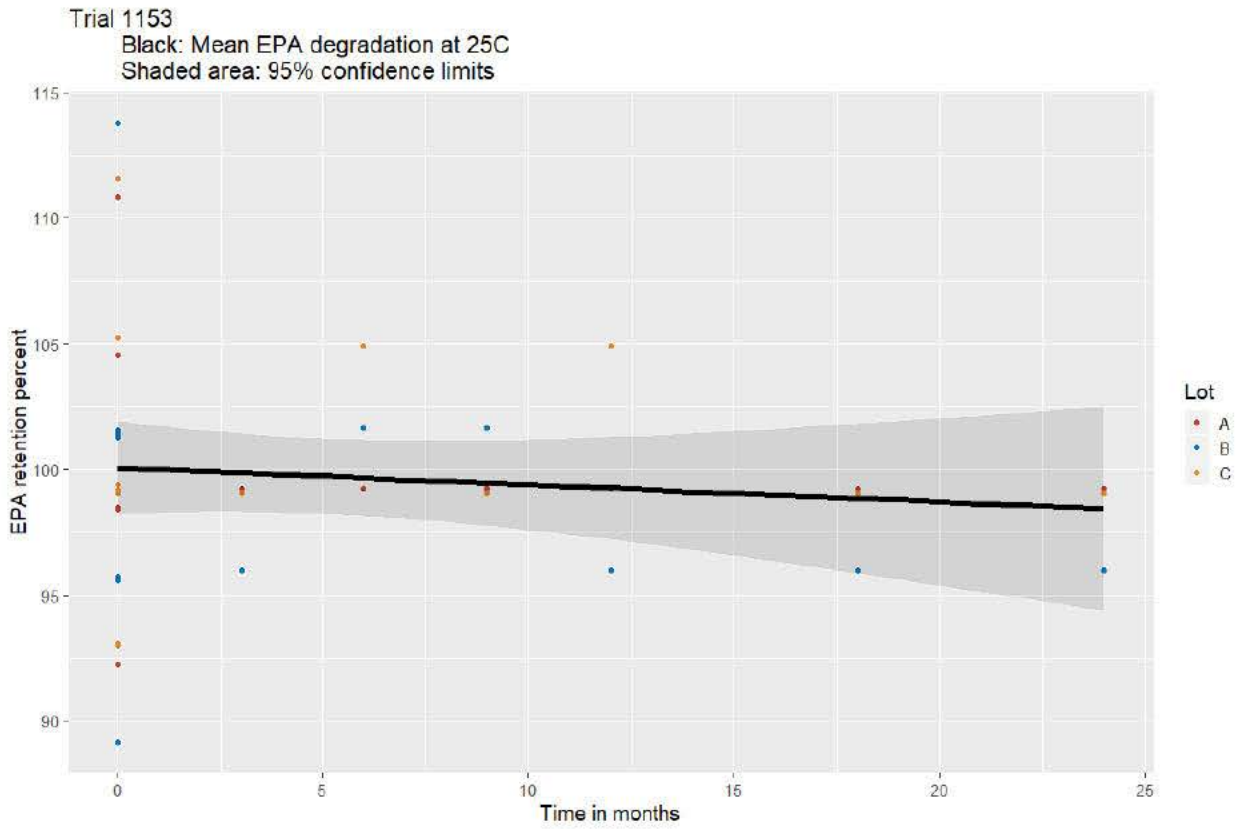


Figure 7 **Stability of EPA in Extruded Cat Food @ 25°C, Post Extrusion Application**

Table 19 **Stability of DHA and EPA in dry cat food @ 40 °C – Post-extrusion application**

			Time points, month						
Lots	Measures		Initial*	3	6	9	12	18	24
VY00014962	EPA	Concentration, %	0.17	0.16	0.17	0.16	0.17	0.17	0.17
		Retention#, %		95	102	97	101	98	98
	DHA	Concentration, %	0.65	0.62	0.64	0.61	0.67	0.64	0.62
		Retention#, %		96	100	94	103	99	96
	PV	meq/kg fat		2.7	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015240	EPA	Concentration, %	0.16	0.17	0.18	0.17	0.17	0.18	0.17
		Retention#, %		97	103	98	99	100	93
	DHA	Concentration, %	0.58	0.59	0.64	0.60	0.61	0.63	0.56

Lots	Measures		Time points, month						
			Initial*	3	6	9	12	18	24
		Retention#, %		95	104	96	99	101	91
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015572	EPA	Concentration, %	0.17	0.17	0.18	0.16	0.17	0.18	0.18
		Retention#, %		101	107	95	102	103	103
	DHA	Concentration, %	0.59	0.60	0.63	0.58	0.60	0.62	0.61
		Retention#, %		102	105	97	101	104	102
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

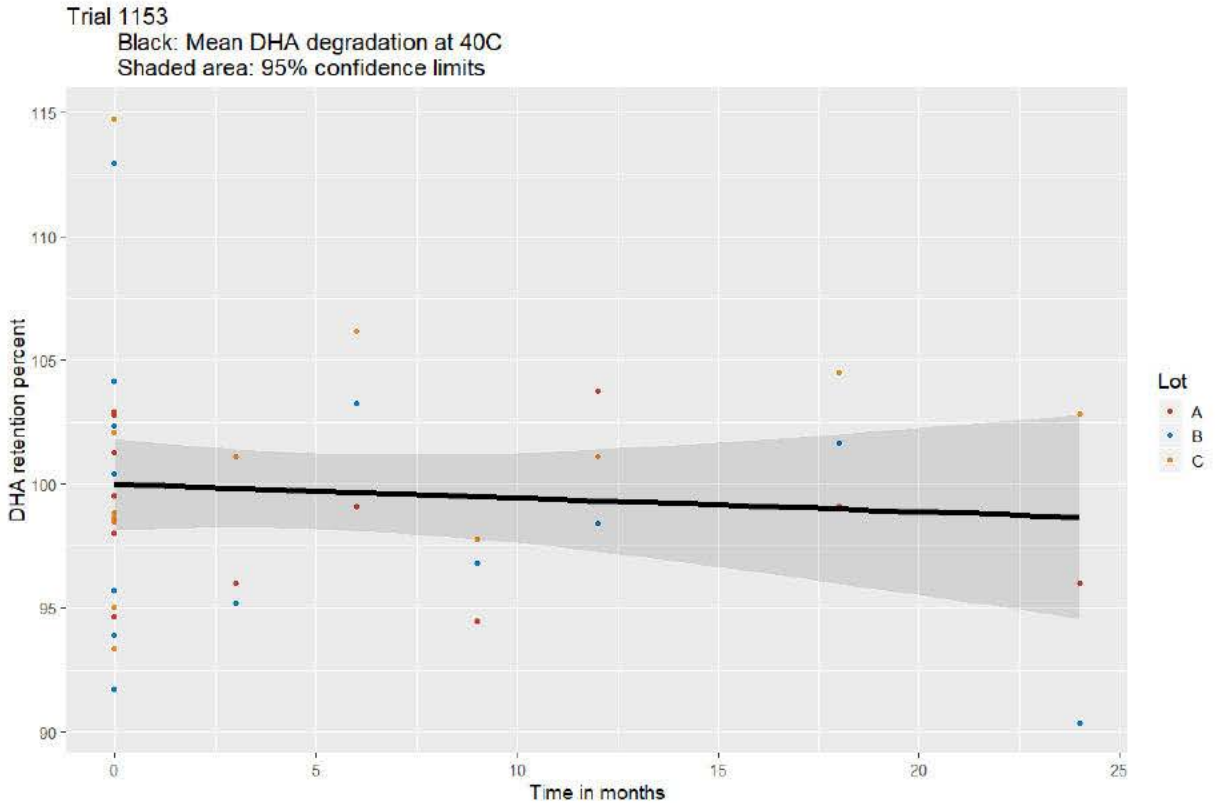


Figure 8 **Stability of DHA in Extruded Cat Food @ 40°C, Post Extrusion Application**

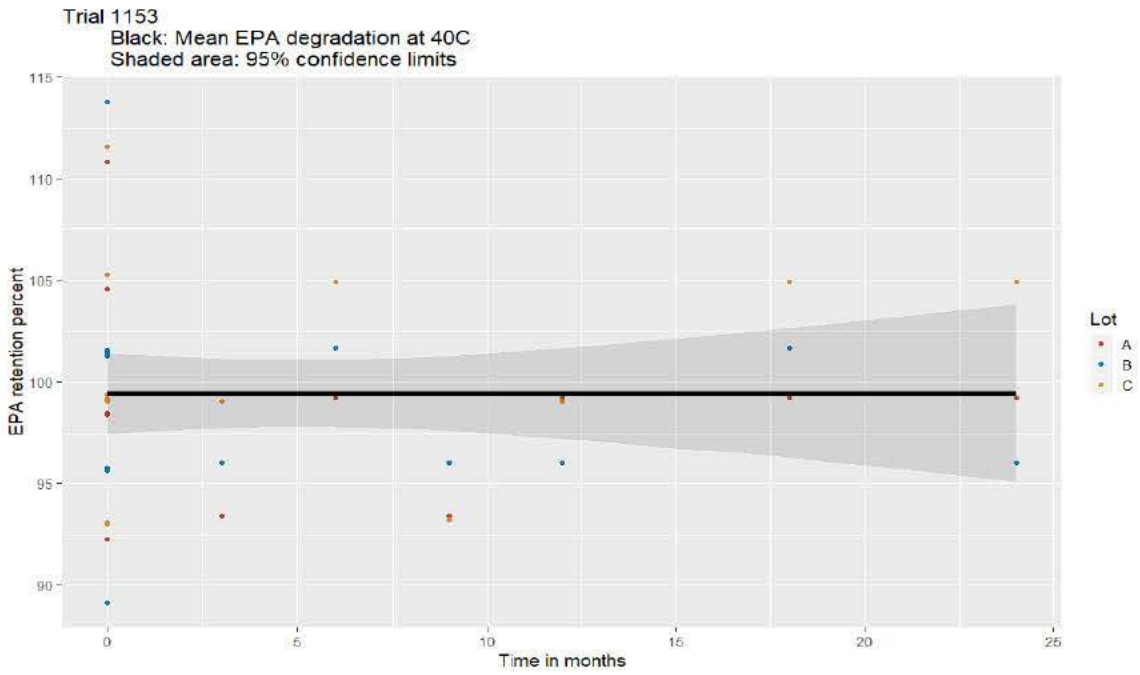


Figure 9 **Stability of EPA in Extruded Cat Food @ 25°C, Post Extrusion Application**

Pre-extrusion application

The statistical analysis report and analytical data for the stability of AOCED in extruded cat food pre-extrusion application can be found in Annex 8.

Table 20 **Stability of DHA and EPA in dry cat food @25°C – Pre-extrusion application**

			Time points, month						
Lots	Measures		Initial*	3	6	9	12	18	24
VY00014962	EPA	Concentration, %	0.16	0.17	0.17	0.16	0.16	0.17	0.17
		Retention#, %		105	105	98	98	99	105
	DHA	Concentration, %	0.63	0.65	0.65	0.62	0.62	0.61	0.64
		Retention#, %		103	103	98	98	97	102
	PV	meq/kg fat		14	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015240	EPA	Concentration, %	0.16	0.16	0.17	0.16	0.16	0.16	0.17
		Retention#, %		101	107	100	100	101	107
	DHA	Concentration, %	0.58	0.56	0.59	0.55	0.58	0.57	0.59

Lots	Measures		Time points, month						
			Initial*	3	6	9	12	18	24
		Retention#, %		96	102	95	99	98	102
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015572	EPA	Concentration, %	0.17	0.17	0.17	0.17	0.17	0.16	0.17
		Retention#, %		101	101	101	101	95	101
	DHA	Concentration, %	0.58	0.58	0.59	0.58	0.59	0.59	0.58
		Retention#, %		100	102	99	102	102	100
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

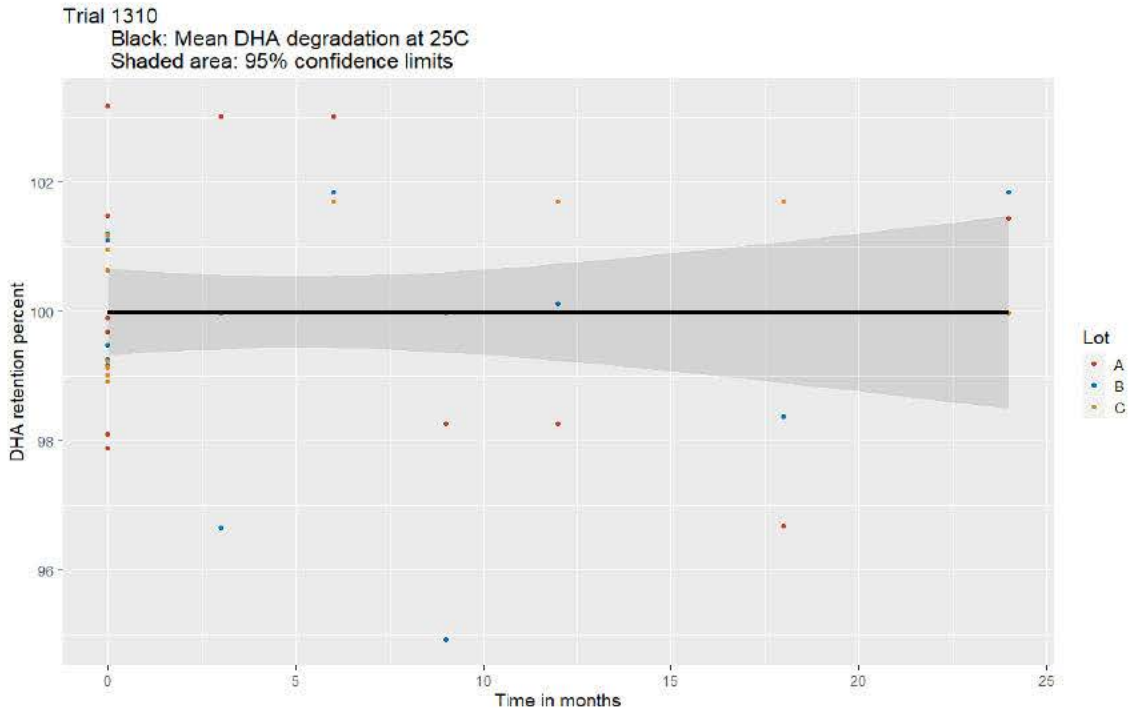


Figure 10 **Stability of DHA in Extruded Cat Food @ 25°C, Pre-Extrusion Application**

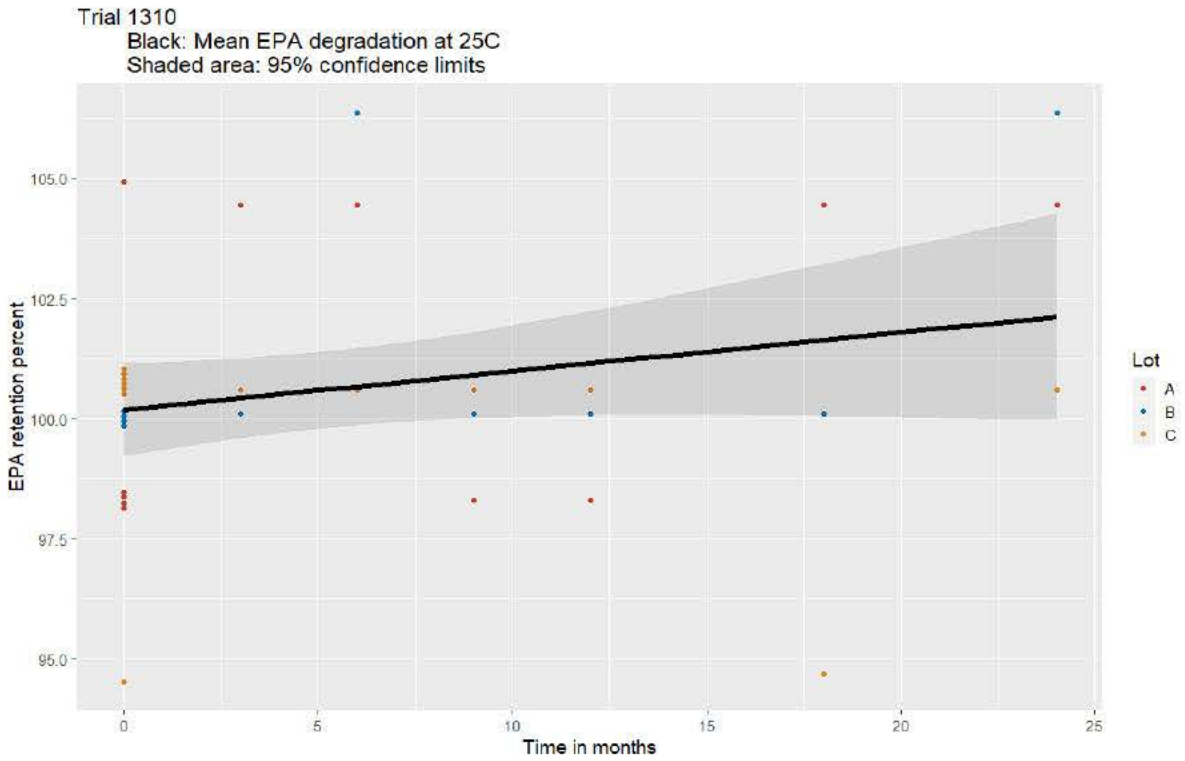


Figure 11 Stability of EPA in Extruded Cat Food @ 25°C, Pre-Extrusion Application

Table 21 Stability of DHA and EPA in dry cat food @40°C – Pre-extrusion application

			Time points, month						
Lots	Measures		Initial*	3	6	9	12	18	24
VY00014962	EPA	Concentration, %	0.16	0.16	0.17	0.17	0.16	0.16	0.17
		Retention#, %		99	105	107	100	101	102
	DHA	Concentration, %	0.63	0.61	0.65	0.64	0.62	0.61	0.63
		Retention#, %		97	104	101	98	97	99
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015240	EPA	Concentration, %	0.16	0.16	0.17	0.16	0.16	0.15	0.17
		Retention#, %		101	108	102	103	96	103
	DHA	Concentration, %	0.58	0.58	0.59	0.56	0.59	0.57	0.57
		Retention#, %		100	102	97	102	99	99

Lots	Measures		Time points, month						
			Initial*	3	6	9	12	18	24
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
VY00015572	EPA	Concentration, %	0.17	0.16	0.17	0.17	0.17	0.17	0.17
		Retention#, %		96	102	102	103	98	98
	DHA	Concentration, %	0.58	0.58	0.61	0.60	0.59	0.58	0.58
		Retention#, %		101	104	103	101	101	101
	PV	meq/kg fat		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

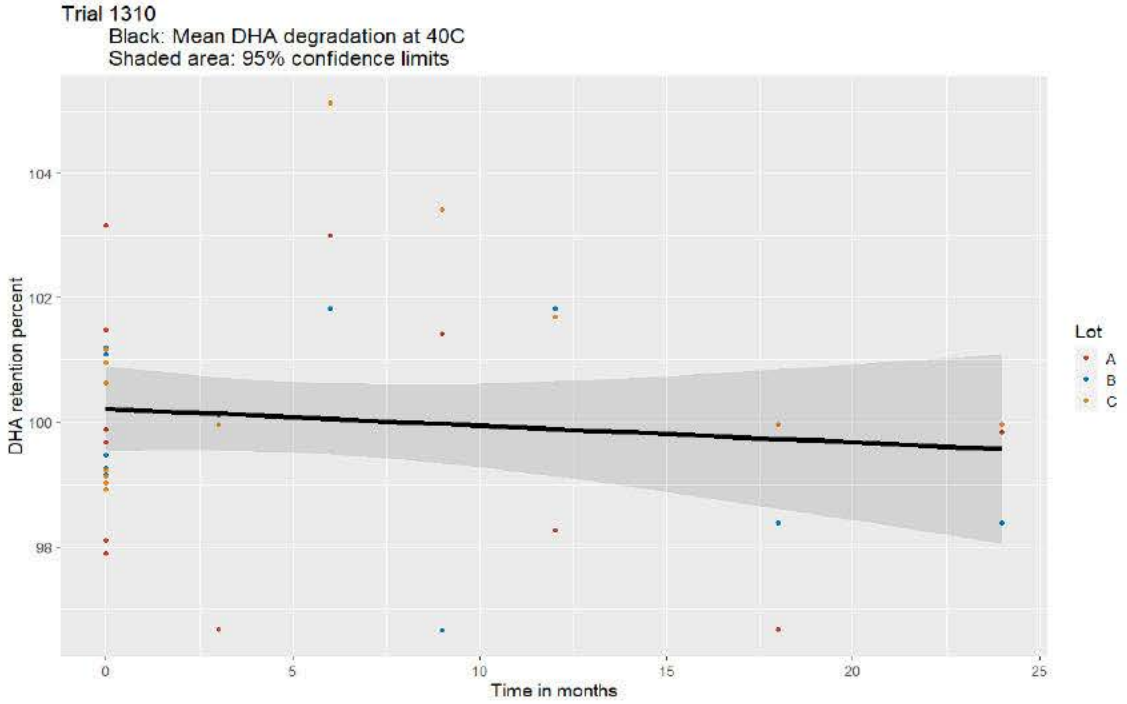


Figure 12 **Stability of DHA in Extruded Cat Food @ 40°C, Pre-Extrusion Application**

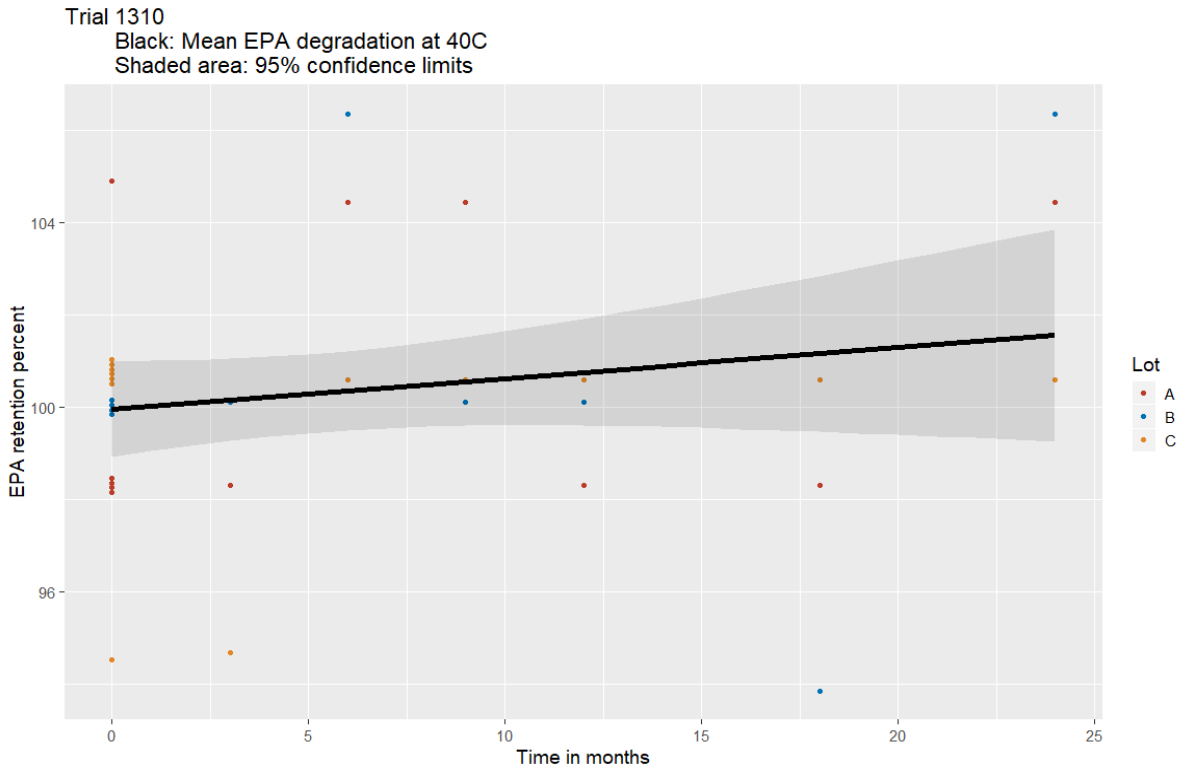


Figure 13 Stability of EPA in Extruded Cat Food @ 40°C, Pre-Extrusion Application

The results indicate that in a model matrix of extruded cat food, that AOCED is stable for at least 24 months. Actual stability in commercial formulations will need to be confirmed by the pet food manufacturers using their food formulations and processing conditions.

2.7.4 Homogeneity

In order to measure the ability of AOCED to be homogenously added to dry cat food, two different applications were studied: addition of AOCED pre-extrusion and addition of AOCED post-extrusion. Three lots of AOCED were used. The lot numbers are: VY00014962, VY00015240, and VY00015572. Each lot was present in the dry cat food formula at 1.45%. The dry cat food was produced at the [redacted] (b) (4) The same lots were used to evaluated homogeneity in dog food for AGRN 36.

In the post-extrusion application, AOCED was mixed with the chicken fat before coating the dry kibbles previously extruded without AOCED. In both applications, finished dry cat food of each coating batch (200 lbs) was packaged in 8 bags with equal amount and a sample was taken from each bag. Samples were analyzed for DHA and EPA (AOAC 996.06), as well as moisture (AOAC 925.09). DHA and EPA concentrations in the samples were corrected for moisture and reported on a dry matter basis. The coefficient of variation (CV) was calculated for DHA and EPA of 8 samples. Sample analysis was conducted at [redacted] (b) (4)
DHA and EPA concentrations and the CV for three lots of AOCED in the dry cat food are shown in

Table 22 (post-extrusion) and Table 23 (pre-extrusion). Overall, DHA and EPA were distributed well in the dry cat food and indicated by the low CV of 8 samples.

Table 22 Homogeneity of DHA and EPA in dry cat food – Post-extrusion application

Lots	VY00014962		VY00015240		VY00015572	
Fatty acids	**EPA, %**	**DHA, %**	**EPA, %**	**DHA, %**	**EPA, %**	**DHA, %**
Sample 1						(b) (4)
Sample 2						
Sample 3						
Sample 4						
Sample 5						
Sample 6						
Sample 7						
Sample 8						
Mean						
SD						
CV, %						

Table 23 Homogeneity of DHA and EPA in dry cat food – Pre-extrusion application

Lots	VY00014962		VY00015240		VY00015572	
Fatty acids	**EPA, %**	**DHA, %**	**EPA, %**	**DHA, %**	**EPA, %**	**DHA, %**
Sample 1						(b) (4)
Sample 2						
Sample 3						
Sample 4						
Sample 5						
Sample 6						
Sample 7						
Sample 8						
Mean						
SD						
CV, %						

Part 3: Dietary Exposure

3.1 Dietary Exposure to the notified substance

Algal oil from marine microalgae *Schizochytrium* sp. (AOCED) is intended to be a replacement for or alternative to fish oil. It is not intended to be added to diets of cats in excess of current levels of fish oil. Therefore, an increase in the current exposure to omega-3 fatty acids is not expected.

3.1.1 Target Animal

AOCED is meant for consumption by cats via the intentional addition to cat food to replace fish oil. Using the target animal study as an example of exposure, the mid-dose, the highest anticipated commercial use level resulted in an exposure of between 132.4 to 3905 mg/kg BW/day as illustrated in the table below. It should be noted that the high exposure happened in the first few weeks of post-weaning phase for the kittens. When this phase is excluded the range is 132.4 to 1122.4 mg/kg BW/day.

Table 24 Achieved AOCED intake

Dietary group	Queens Breeding (Wks 3-8)	Queens Pregnant/Lactation (Wks 9-19))	Kittens Combined Post-weaning Weeks 1-7	Kittens Combined Post-weaning Weeks 8-26
Low dose AOCED	132.4	197.0	818.2	264.1
Mid dose AOCED	250.8	434.1	1730.2	584.2
High dose AOCED	504.7	925.6	3905.0	1122.4

Values represent average daily AOCED intake over a period (mg/kg body weight/day).

3.2 Dietary Exposure to any other substance that is expected to be formed in or on food

AOCED is a mixture of fatty acids high in omega-3 fatty acids and like any material with unsaturated fat it is prone to oxidation through thermal abuse and/or exposure to light or oxygen. The typical degradation products from the oxidation of fatty acids are short chain ketones and aldehydes. The stability study performed with the AOCED product in dry and canned cat food indicate that the formation of the degradation products is minimal as represented by the peroxide values, consistently <2.0. The product itself has a stability of less than 9 months when stored at 25 °C.

3.3 Dietary Exposure to any other substance that is present with the notified substance either naturally or due to its manufacture (e.g.., contaminants or by-products)

Four lots of AOCED were tested for the presence of heavy metals as noted in the table below. The report from the Third-party lab is in Annex 9.

Table 25 Heavy Metals

	Test Method	VY00014962	VY00015240	VY00015465	VY00015572
Arsenic (ppm)	AOAC 2013.06	< 0.02	< 0.02	0.03	< 0.02
Cadmium (ppm)	AOAC 2013.06	< 0.01	< 0.01	< 0.01	< 0.01
Lead (ppm)	AOCS Ca 17-01	< 0.05	< 0.05	< 0.05	< 0.05
Mercury (ppm)	AOAC 2013.06	< 0.010	< 0.05	< 0.01	< 0.01

The same four lots of algal oil (AOCED) were tested for the presence of an extensive group of pesticides, including organochlorine, pyrethroid, and organophosphorus. No concentrations of concern were identified in the four lots of AOCED. See Annex 10.

Part 4: Self-limiting levels of use

It is expected that, as with other sources of omega-3 fatty acids, such as fish oil, that the use levels of AOCED from marine microalgae *Schizochytrium sp.* are self-limiting due to palatability and probable difficulties for kibble production.

Part 5 Common use in food prior to 1958

Veramaris is unaware of any documented use of the AOCED or other algal oils being used in any animal food application before 1958. Production of AOCED for use as a source of DHA and EPA did not commence on an industrial scale until the 1990's with the introduction of an AOCED for use in aquaculture by the Omega Tech company. A parent of the production strain was derived from the Omega Tech culture bank.

Part 6 Narrative of the basis for the GRAS conclusion

6.1 History of the Use of Algal Products in Animal Food

Dried marine microalgae *Schizochytrium* sp. is used as an animal feed ingredient for several species around the world. That material is approximately 50% oil. The material that is the subject of this GRAS is the oil produced by the same genus and species of algae.

Since 1995, a spray-dried form of *Schizochytrium* sp. has been used world-wide as a feed ingredient in aquaculture (Barclay and Zeller, 1996). The corresponding product HUFA2000 originally developed by OmegaTech has been successfully utilized as a DHA source for shrimp larva culture and finfish (red seabream, Japanese flounder) with no reports of adverse effects (OmegaTech, 1998).

In addition, dried *Schizochytrium* sp. microalgae has been used as a poultry feed ingredient since 1996. This feed ingredient is used in Europe at an average rate of 1.5% (by weight) of feed providing approximately 165 mg DHA/hen/day. The DHA concentrations in the resulting eggs range from 120-150 mg/egg. These eggs have been commercialized in Europe since 1996 and in the U.S. since 1998 under the Gold Circle Farms® brand. Dried golden algae is now commercially marketed in Mexico, China, Spain, Portugal, Benelux countries, Italy, Norway, Israel and Australia (Fabricant, 2000).

The dried marine microalgae was determined to be GRAS (Generally Recognized As Safe) as a source of DHA when incorporated into the feed rations of laying hens and broiler chickens at up to 4.3% or 2.8%, respectively. The corresponding product DHAgold™ was commercialized by Omega Tech, Inc. in 1998 (Abril et al., 2000).

In 2014, DSM Nutritional Products filed a food additive petition (FAP 2288, Fed. Reg. 2014) for the use of *Schizochytrium* sp. dried algae (DHAgold™) as a source of docosahexaenoic acid (DHA) for use in standard pelleted foods for adult dogs. Recently, FDA published the final rule to DSM's FAP with the conclusion that the dried whole cells of nonviable, nontoxigenic, nonpathogenic marine microalgae *Schizochytrium* sp. may be safely used as a source of DHA and other omega-3 fatty acids as complete, dry adult maintenance food for dogs in accordance with specifically defined conditions (Fed. Reg. 2018).

In 2017 DSM Nutritional Products, with the aid of external experts concluded that the use of the DHAgold™ was generally recognized As Safe (GRAS) for use in food for ornamental birds and small mammals.

Today, different microalgae forms (algae, dried algae, algae meal, algal oil and algae extracts) are accepted as feedstuffs within the EU. The EU catalogue has listed algae meal as a product of algae oil manufacture, obtained by extraction of algae which has been inactivated (EU Regulations 2017;). More specifically, Germany has even listed a species of *Schizochytrium* (*S. limacinum*) in the positive list for straight feedstuffs since 2015 (Positive List for Straight Feeding Stuffs, 12th Edition, August 2017). Accordingly, algae meal such as dried microalgae

Schizochytrium sp. is considered safe and is recognized for use in different applications in animal feed rations in accordance with the EU catalogue and German positive list for feed materials. Table 21 shows an overview of globally utilized dried-whole cell *Schizochytrium* sp. microalgae products used in animal feed.

Table 26 Overview of *Schizochytrium* sp. applications in animal feed

Year	Company	Country	Application
1995	OmegaTech	worldwide	HUFA2000, a spray-dried form for aquaculture (shrimp, larva culture, and finfish)
1996	OmegaTech	EU	Drum-dried form for poultry
1998	OmegaTech	USA, Australia, Mexico, Germany, Spain, Portugal, Benelux countries, Italy, Norway, Israel	DHA GOLD™ for broiler chickens and laying hen feed
2002	Martek Biosciences	USA	DHA GOLD™ for swine
2013	Martek Biosciences/DSM	China	DHA GOLD™ for swine
2013	Martek Biosciences/DSM	China	DHA GOLD™ for aquaculture
2017	DSM	USA	DHA GOLD™ for ornamental birds and small mammals
2018	DSM	USA	DHA GOLD™ for dogs
2018	DSM	China	DHA GOLD™ for layers, broilers finisher swine, dogs, cats, aquaculture
2020	Veramaris	USA	Algal Oil for dogs

Additionally, Mordenti et al. evaluated the efficacy of DHAgold™ in rabbit feed fed to breeding does and their progeny at 2g/kg of feed. No adverse effects were noted except in the early growth phase where there appeared to be a reduction in feed intake by does consuming the algal treatment. There was a significant difference in fatty acid profile in the loin and thigh muscles of both the does and their progeny in the algal treatment compared to control. (Mordenti et al. 2009)

In 2015 DSM Nutritional Products and EVONIK Industries entered into a partnership to develop a new strain of *Schizochytrium sp*. that would produce an oil with a DHA to EPA ratio similar to fish oil. The new production strain was developed from the culture collection of DSM Nutritional Products using a strain lineage that has been proven to be safe for producing an oil rich in polyunsaturated fatty acids for consumption by both animals and humans, including infants. The table above supports the internationally recognized safety of the algae.

As noted in Section 2 of this dossier, an oil high in DHA and EPA can be produced consistently from the new strain of *Schizochytrium sp*. algae. Stability studies with AOCED alone and in typical cat food products, dry kibble and canned indicate that the material itself is stable for at least one year when stored according to the instructions on the label and that commercial cat food products containing AOCED will be stable for at least one year.

Exposure calculations based upon typical use levels of fish oil, the material that AOCED will replace do not indicate that the consuming animals could be easily exposed to an excessive amount of the material. Safety studies with AOCED detailed in Section 7.4 support the safety of the material at the maximum expected use level.

Veramaris also concluded in 2018 that Condensed Algal Residue Solubles, the fermentation solids left from the extraction of the oil from the algae, is generally recognized as safe (GRAS) for use as an ingredient in food for beef cattle based on scientific procedures and the review of an external panel.

6.2 Literature

Veramaris performed a search of the literature in October 2019 to determine if any there were any reported adverse effects in animals related to the consumption of *Schizochytrium* algae, product produced by Schizochytrium or any other algae products produced by other closely related species of algae. The Embase, Web of science, VetMed, CAB Abstracts and PubMed databases were searched and then parsed for peer-reviewed, controlled studies conducted in healthy felines of any age, orally administered a source of EPA/DHA ≥ 7 days, and reported a safety or bioavailability outcome of interest (see Annex 12 for inclusion/exclusion criteria). Studies were excluded for any of the following reasons: studies conducted in wild or diseased animals; administration of n-3 fatty acids other than EPA/DHA or the combination of these with other active ingredients; intravenous or topical fatty acid administration; no control group; acute feeding study (e.g. less than 7 days or one-time bolus administration); narrative review, case report, case study, abstract, letter, or other non-peer reviewed publications. Studies that could not be excluded or met all inclusion criteria were retrieved for full-text review. Reasons for exclusion at full-text screening included insufficient information to calculate the dose of n-3 LCPUFA administered, outcomes of interest weren't reported, or DHA/EPA was provided with other active ingredients. Data from studies included at full-text review were extracted into an Excel spreadsheet to facilitate qualitative synthesis. Results of the screened literature review are provided in section 7.1 of this dossier.

6.3 Safety of the proposed use of AOCED

Veramaris performed a complex *in vivo* study with cats, the intended species that will consume the AOCED, to determine if the material was safe for the intended use. The FDA Center for Veterinary Medicine reviewed the protocol for a gestation, lactation and growth study wherein female felines were fed AOCED from conception through the weaning of the kittens and then those kittens were tracked through the growth phase of development, 32 weeks.

The results of that study confirmed that the intended use was safe within the concentrations anticipated to be employed in food for cats; more details are provided in section 7.4 of this dossier and in Annex 11.

6.4 Efficacy of the proposed use of AOCED

During the Gestation/Lactation/Growth (GLG) study (Annex 11) and Vuorinen et al. 2020, the plasma DHA and EPA levels were measured in both the queens and the kittens to confirm that the addition of increasing levels of AOCED resulted in increased levels of DHA and EPA in the plasma of the animals as shown in the figures below. A more detailed discussion is in section 7.4.8 of this dossier.

Table 27 Blood Plasma Levels of DHA and EPA in Queens Fed AOCED

	Description	EPA Mean Value µg/mL	DHA Mean Value µg/mL
Group 1	Control	18.75	24.12
Group 2	Low dose	51.33	82.55
Group 3	Mid dose	76.17	99.45
Group 4	High dose	142.48	165.66

Table 28 Blood Plasma Level of EPA in Kittens Fed AOCED

EPA	Description	Week 8 µg/mL	Week 24 µg/mL	Week 32 µg/mL
Group 1	Control	4.33	6.80	6.43
Group 2	Low dose	25.94	45.54	48.19
Group 3	Mid dose	58.27	77.70	79.39
Group 4	High dose	96.11	148.99	139.41

Table 29 Blood Plasma Levels of DHA in Kittens Fed AOCED

DHA	Description	Week 8 µg/mL	Week 24 µg/mL	Week 32 µg/mL
Group 1	Control	37.71	26.64	25.12
Group 2	Low dose	131.37	92.85	78.29
Group 3	Mid dose	134.77	120.32	106.21
Group 4	High dose	170.89	134.33	116.88

Part 7 Supporting data and information

7.1 General function of polyunsaturated fatty acids (PUFA) in cats

Conversion of the n-3 essential fatty acid, α-linolenic acid (ALA, 18:3n-3), to its longer chain derivatives (n-3 LCPUFA), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6n-3), involves a sequence of desaturation and elongation steps. Conversion of the n-6 essential fatty acid, linoleic acid (LA; 18:2 n-6), to its longer chain derivatives (n-6 LCPUFA) relies on a similar process resulting in competition for the rate-limiting enzyme in the conversion pathway, Δ-6 desaturase. Evidence suggests that Δ-6 desaturase activity is exceptionally low in felines (Morris et al., 2004; Angell et al., 2012), limiting endogenous production and ultimately compromising tissue levels of both n-3 (e.g. EPA and DHA) and n-6 LCPUFA, namely arachidonic acid (ARA; 20:4 n-6). These findings of Pawlosky et al.(1994) with felines suggest that production of DPA n-3 from ALA may serve as an important circulating reservoir for tissue DHA synthesis with transport of DPA n-3 via plasma lipids to neural tissues. Neurologic tissue including that of the brain and retina are particular enriched with n-3 and n-6 LCPUFA with the greatest accumulation occurring during the perinatal period and directly influenced by maternal fatty acid status (Lauritzen et al., 2001; McCann and Ames, 2005). The 20-carbon members of the n-3 and n-6 fatty acid families (i.e. EPA and ARA) serve as precursors to eicosanoids, signaling molecules which include prostaglandins, thromboxanes, and leukotrienes. Eicosanoids derived from EPA are generally considered less biopotent than those derived from ARA, this difference in biopotency being offered as an explanation for differences in inflammatory and immune outcomes commonly noted in response to n-3 LCPUFA supplementation (Lauritzen et al., 2001). Maintaining a balance between n-6 and n-3 derived eicosanoids is particularly important during early life, with n-6 derived eicosanoids contributing to optimal growth (Lauritzen et al., 2001) and both n-3 and n-6 LCPUFA contributing to brain and eye development (McCann and Ames, 2005).

Feline maternal diets containing preformed DHA and EPA have resulted in higher levels of DHA in kitten brain and retinal tissue compared to diets providing only ALA (Morris et al., 2004). Similarly, feline maternal diets containing preformed ARA have been shown to support optimal reproduction (Morris et al., 2004). Felines are unable to efficiently convert long chain fatty acids into physiologically important LCPUFAs due to a low level of Δ-6 desaturase activity. Consequently omega-6 and omega-3 fatty acids may be conditionally essential for felines.(Bauer, 2006). Fish oil, as a source of preformed n-3 LCPUFA and n-6 LCPUFA, has been found to significantly increase plasma phospholipid levels of DHA and EPA in adult cats, while supporting ARA levels (Angell et al., 2012). Owing to the limited ability of cats to produce LCPUFA from n-6 and n-3 essential fatty acid precursors, the National Research Council (NRC) recommends intake of both ARA and EPA to support growth (0.05 and 0.025 g/1000 kcal, respectively) and maintain health (0.015 and 0.025 g/1000 kcal, respectively) in adult cats (NRC, 2006). Excess saturated and unsaturated fats in the diet of cats does not appear to contribute to hypercholesterolemia or hypertriglyceridemia in cats, (Butterworth et al. 2012).

7.1.1 Skin Health and Function

Omega-3 and 6 fatty acids play key roles in the structural integrity of cell membranes and thus

contribute to the normal function and maintenance of skin health (Kiezel-Tsugunova et al., 2018). Evidence from an uncontrolled supplementation study (Harvey, 1991) in a small number of cats (n=10-14) suggests a role for the combination of n-6 PUFA (evening primrose oil) and n-3 LCPUFA (fish oil), for the support of skin health in cats with miliary dermatitis. Specifically, cats fed a dietary supplement containing 80% evening primrose oil (linoleic acid and gamma-linolenic acid [GLA; 18:3n-6]) and 20% fish oil for 6 weeks, exhibited greater improvement (86% response rate) in clinical symptoms associated with feline crusting dermatosis when compared to cats fed either fish oil alone (0% response rate) or evening primrose oil alone (50% response rate). Analysis of serum fatty acids found 1.8 times higher EPA levels by the end of supplementation with the combined oil supplement. Removal of the supplement combination resulted in recurrence of symptoms and reinstitution of the supplement resolved symptoms. A somewhat larger study (n=28 cats) of a commercial product containing EPA, GLA, and DHA found nearly 60% of cats with allergic and inflammatory dermatoses, miliary dermatitis, or non-lesional dermatitis exhibited an "excellent" (complete resolution of symptoms) or "good" owner reported response after 14 days of supplementation (1.0 ml/9.1 kg BW/d) (Miller et al., 1993). Blood levels of n-3 LCPUFA were not reported. In responsive cats, owners reported a return of symptoms after supplement discontinuation, with resolution after reinstitution of the supplement (Miller et al., 1993). The authors reported "no clinical side effects".

In a series of experiments, Park et al. (2011) investigated skin inflammatory responses in healthy young (~ 20 months old) cats (n=14/trt) fed diets containing either menhaden fish oil or flaxseed oil versus a poultry fat control diet for 12 weeks. In Experiment 1, the test diets provided 22% total dietary lipid (2.4% poultry fat; 1.9% fish oil; 1.1% flaxseed oil) and in Experiment 2, 14% lipid (1.3% poultry fat; 1.3% fish oil; 0.8% flaxseed oil). Fish oil diets in both experiments significantly ($P<0.05$) increased plasma EPA up to 45 times higher and DHA levels up to 5 times higher compared to control and flaxseed oil fed cats. Similarly, skin EPA levels were up to 12.5 times higher and DHA levels nearly 3 times higher in response to fish oil as compared to control and flaxseed oil fed cats. Fish oil had no effect on either plasma or skin levels of ARA. In both experiments, fish and flaxseed oil diets significantly ($P<0.01$) decreased skin inflammatory response to histamine (up to 50%) and significantly ($P<0.05$) lowered maximum skin thickness response up to 30%. Dietary fish oil significantly increased skin leukotriene (LT) B_5 concentrations, with no significant effect on LTB_4, regardless of dietary fat content. Leukotriene B_4 is recognized as "…one of the most potent chemotactic agents for recruiting neutrophils and macrophages and creating inflammatory conditions in a tissue" (Lands, 2012). In contrast, LTB_5 is estimated to be 100 times less potent in the support of inflammatory responses (Lands, 2012). The finding by Park et al. (2011) that LTB_4 levels remained unchanged, despite an increase in LTB_5 is surprising as typically dietary EPA competitively blocks ARA-derived eicosanoid generation (Lands, 2012). It is possible that either the level of EPA or the duration of supplementation was insufficient to observe decreases in LTB_4, or that the ARA content of fish oil was sufficient to maintain LTB_4 levels throughout the course of study.

Collectively, this evidence suggests a contribution of n-3 and n-6 LCPUFA, alone or in combination, to the normal structure and function of the feline epidermis.

7.1.2 Immune Health and Function

Park et al (2011) investigated the effect of feline diets enriched with n-3 PUFA on a variety of immune markers in healthy young (~ 20 months old) cats (n=14/trt). Lower fat diets (14% lipid) containing n-3 PUFA from either flaxseed oil or menhaden fish oil, resulted in significantly lower

CD5+ total T cells and CD4+ T helper cells compared to the control diet. CD21+ B cell populations were significantly lower in cats fed a higher fat diet (22% lipid) enriched with n-3 PUFA from either source. N-3 fatty acids, regardless of source, had no effect on a variety of additional immune parameters including delayed-type hypersensitivity responses, interleukin 2 (IL-2) production, or lymphocyte proliferation.

Rutherfurd-Markwick et al (2013) randomized 8-9 cats/trt to a low-protein diet (22.7% dry matter) with or without salmon oil (30 g/kg diet) for 5 weeks to determine the effect of n-3 LCPUFA supplementation on T-cell function. Diet had no effect on proliferative responses in Con A stimulated peripheral blood leucocytes but significantly increased lymphocyte proliferative responses to phytohaemagglutanin (PHA) with significant (P = 0.0001) time dependent increases after 14 and 35 days of feeding. These results are in contrast to those reported by Park et al. (2011) who reported no change in response to PHA stimulation, but the current trial was shorter, and fed a different level of n-3 LCPUFA. Rutherfurd-Markwick (2013) also reported significantly increased phagocytic activity of blood leucocytes after 14 days of feeding salmon oil, with a further increase after 35 days of feeding. The authors did not report blood or tissue LCPUFA levels post-supplementation. The authors concluded that "the enhanced phagocyte function and T-cell function seen here may result in an increased resistance to invading pathogens and disease and therefore benefit the animals' overall health status."

7.1.3 Asthma

Leemans and coworkers (2010) supplemented healthy cats (n=8) with tablets containing a combination of marine fatty acids from New Zealand Green Lipped Mussel (NZ GLM; 20 mg/d) and the flavonoid luteolin for 4 weeks, then determined airway responsiveness to an allergen challenge designed to induce an asthmatic response. Total erythrocyte n-3 PUFA levels were significantly increased at the end of the supplementation period, confirming supplement compliance and bioavailability. The authors reported no changes in hematologic values or adverse events following supplementation. Airway responsiveness was significantly reduced and lipoxin A_4 levels in bronchoalveolar lavage fluid significantly increased following supplementation. Lipoxins are eicosanoids responsible for the resolution of inflammatory responses but are derived from the n-6 LCPUFA, ARA. While the authors do not report individual n-6 fatty acid levels, total n-6 PUFA levels were significantly increased at the end of the supplementation period. The predominant PUFA in GLM are n-3 LCPUFA, however, small amounts of ARA are also present (Miller et al., 2014). The authors do not describe a dietary wash-out prior to supplementation and the fatty acid profile of the background diet is not reported. It is possible that the level of ARA in the GLM supplement may have been higher than the baseline/background diet, resulting in the observed lipoxin A_4 increase. This evidence suggests a role of both n-3 and n-6 LCPUFA in maintenance of normal lung function in healthy cats.

In summary, limited evidence suggests that n-3 and n-6 LCPUFA play a supportive role in the normal immune response of cats.

7.1.4 Maintenance of Normal Body Weight and Glucose Levels

Evidence from a study conducted by Wilkins et al. (2004) suggests a diet containing n-3 LCPUFA (3.91% EPA + 4.72% DHA) reduces plasma insulin and improves long-term glucose control in obese cats. Twenty-eight neutered adult cats were acclimatized to a low n-3 LCPUFA control diet for > 6 months after which cats were randomized to a high n-3 LCPUFA or high saturated fat (SFA) diet for 10 weeks of controlled feeding (lean period) followed by 21 weeks of *ad libitum* intake (obese period). *Ad libitum* food intake resulted in increased body weight and percent body fat in both groups. Plasma cholesterol, triglyceride, and non-esterified fatty acid concentrations were unaffected by diet, plasma n-3 LCPUFA levels were not reported. Areas under the insulin response curve were lower in obese cats fed the n-3 LCPUFA diet, compared with obese cats fed the SFA control (Wilkins et al, 2004). Obese cats fed the n-3 LCPUFA diet also exhibited lower glycosylated hemoglobin concentrations than those fed the SFA control diet. The authors reported no differences in body weight or condition in response to diets but suggest that decreasing insulin with n-3 LC PUFA may support maintenance of normal glucose control in cats.

A direct association was found between circulating levels of EPA and adiponectin, insulin and triglyceride concentrations in non-obese cats (n=34), (Mazaki-Tovi et al., 2011). However there was no relationship found for short chain n-3 PUFAs, such as ALA. Specifically, for every 1 mg EPA increase/100 mg serum fatty acids, adiponectin was 7.5 fold higher (P<.045) and insulin 70% lower (P<.0.035). Serum DHA, but not ALA, was also directly associated with adiponectin. Low adiponectin levels have been linked with an increased risk of obesity in humans. These findings suggest a role of n-3 LCPUFA in the maintenance of normal body weight in cats. In a follow-up experiment Mazaki-Tovi et al. (2019) examined the influence of fatty acids (ARA and EPA) on adiponectin, interleukin-6 (IL6) and tumor necrosis factor-α (TNFα) secretion in a cell-culture study of subcutaneous and visceral adipose tissue from healthy cats. ARA stimulated IL6 secretion and EPA stimulated TNFα secretion, but neither resulted in a change in adiponectin levels.
Collectively, this evidence suggests that dietary n-3 LCPUFA many contribute to maintenance of normal body weight and blood glucose levels in cats.

7.1.5 Joint Health and Function

Corbee and co-workers (2013) investigated the effects of n-3 LCPUFA supplementation on owner perceived activity levels and locomotion outcomes in older cats (≥ 8 years) with radiographically confirmed osteoarthritis (OA). Cats (n=16) participated in a 10-week randomized controlled cross-over study of 1.53 g EPA plus 0.31 g DHA/1000 kcal from fish oil vs a corn oil control diet. Oils were added by owners to meals via syringe. Compliance with supplements was confirmed by significant (P=0.041) increases (concentrations not reported) for EPA and DHA in plasma cholesterol esters. Supplementation with n-3 LCPUFA significantly improved owner's perception of activity level, ability to climb up and down stairs, stiff gait, owner interaction, and jump height.
Lascelles et al. (2010) investigated the role of n-3 LCPUFA from GLM, combined with glucosamine/chondroitin sulfate, on both owner-perceived, and veterinarian assessed OA symptoms and related behaviors. Cats (n=40) were enrolled following radiographic confirmation of OA in at least one joint. Similar to Corbee et al. (2013), diets were supplemented with 1.88 g EPA+DHA/1000 kcal (anchovy oil), versus a poultry fat-based control, fed for 9 weeks. Activity levels

were monitored using a collar-installed accelerometer. Activity levels were significantly greater and plasma phospholipid EPA levels nearly 6-fold higher in response to the test diets. However, due to the combination with other bioactives, the independent effect of the n-3 LCPUFA is not known. The authors also reported "clinically significant" decreases in ALT and increases in lipase in response to supplementation but provide no additional discussion on the consequence of these observations. The authors speculate that while n-3 LCPUFA may have decreased inflammation, OA in cats is not considered a significant inflammatory process (Lascelles et al., 2010).

In summary, evidence from two studies suggests a role of n-3 LCPUFA, alone or in combination with other bioactives on mobility in cats with OA, however, the mechanism behind this observation has not been determined.

7.1.6 Renal Health and Function

Plantinga et al. (2005) conducted a retrospective study on survival times of n=321 cats fed a variety therapeutic diets (n=7) compared to standard cat foods. Median survival time with therapeutic foods was 16 months compared to 7 months for the standard diets. The highest median survival time was 23 months and was associated with a wet diet ("Diet 3") containing 0.47 g EPA/MJ consumed by 24 cats. The remaining 6 diets were either devoid of EPA (n=3) or ranged between 0.02-0.05 g EPA/MJ. Importantly, however, "Diet 3" was also low in phosphorus and high in potassium leading the authors to conclude that all three dietary modifications were likely responsible for the observed benefits, but that future diet formulations should pay particular attention to providing a higher EPA content in renal therapy diets (Plantinga et al, 2005).

It has been reported that healthy cats with unobstructed kidney stones live, on average, 3 years less than healthy cats without kidney stones (Hall et al., 2017). Given prior observations of renal health benefits of n-3 LCPUFA-rich diets in cats (Plantinga et al., 2005) and reports of decreased stone formation in humans with high n-3 LCPUFA diets (Kerr et al., 2013), Hall et al (2017) conducted an randomized controlled trial of diets enriched with 0.09% EPA and 0.18% DHA from fish oil in a 56-day cross-over in healthy cats (n=12; ave. 5.6 years of age). Compliance with test diets and bioavailability of n-3 LCPUFA was evident from significantly (all $P<0.001$) increased serum concentrations of EPA (173%), DHA (61%), and ARA (35%) compared to cats fed the control diet. The authors found statistically significant ($P<0.02$) reductions in urine specific gravity and relative super saturation for magnesium ammonium phosphate stones ($P<0.03$; aka struvite crystals) in response to fish oil. Urinary calcium concentration was 21% lower ($P<0.06$) and oxalate crystal formation was 43% lower ($P<0.06$) in cats fed n-3 LCPUFA diets. Collectively, these results suggest that dietary n-3 LCPUFA helps reduce the risk of struvite and oxalate stones in healthy cats. The authors hypothesized that protection from kidney stones by dietary n-3 LCPUFA may be mediated through changes in prostaglandin metabolism and membrane fatty acid composition, resulting in changes in calcium excretion and oxalate transport.

Based on limited, but promising evidence regarding survival times of cats with chronic kidney disease (CKD), Cline (2016) recently concluded that a standard dosage of 40 mg/kg EPA plus 25 mg/kg DHA daily should be recommended for adult cats with CKD, which is approximately 1.16 to 1.18 g of EPA plus DHA/1000 kcal of diet for a cat consuming 1.2 times its resting energy requirement.

7.1.7 Cognitive Function

Pan et al. (2013) investigated a feline diet containing a blend of fish oil (0.27% DHA; 0.28% EPA), arginine, B vitamins, and antioxidants (Vitamins E and C, and selenium) on cognitive function in

middle-aged (ave. 6.6 years old) cats (n=16/trt) during a 345 day non-randomized, controlled trial. Cognitive function was assessed using a variety of tests including: delayed non-matching-to-position task for learning and short-term memory; landmark discrimination learning test for visual-spatial learning; reversal learning as a measure of executive function; and a size discrimination and reversal learning task. At the end of study, erythrocyte levels of EPA were 8.5-fold higher and DHA 2 -old higher than baseline in cats fed the test diet, confirming bioavailability and compliance. No differences in vitamin B12, homocysteine or total antioxidant status were reported for supplemented cats. On all but the landmark discrimination test, cats assigned to the fish oil blend showed significantly better performance than the controls. The authors concluded that a blend of bioactives, including EPA and DHA, "…can significantly improve cognitive function and may retard age-related decline in cognitive function in normal middle-aged and old cats."(Pan et al., 2013) These results are consistent with cognitive benefits observed in middle-aged humans supplemented with DHA alone or DHA and EPA combined (Yurko-Mauro et al., 2010; Yurko-Mauro et al., 2015).

Summary

Studies of n-3 LCPUFA in cats have generally focused on cutaneous inflammation, renal function, and joint-related mobility using diets with up to 8.5% of total fat as EPA+DHA, with higher levels primarily utilized for therapeutic indications. Many studies have examined a combination of n-3 LCPUFA with other bioactives, making it difficult to discern the independent effects of EPA and/or DHA on feline health but, alone or in combination with other ingredients, dietary n-3 LCPUFA appear safe and well tolerated by healthy cats throughout the life cycle. No significant adverse effects have been reported in response to n-3 LCPUFA, although the long-term safety of n-3 LCPUFA in feline diets has not been determined, nor has a safe upper limit been set by the NRC (Bauer et al., 2011). It has been suggested that up to 75 mg EPA+DHA/kg $^{0.67}$/d can be considered as a safe level of n-3 LCPUFA supplementation until further evaluations of long-term safety are available (Bauer et al., 2011).

7.2 Review of the Safety of *Schizochytrium sp*. in other species

7.2.1 Dried Whole Cell Biomass

As noted in Section 6.2, dried whole cell *Schizochytrium* sp. microalgae has been sold for use in animal feed under the tradename DHAgold™ since 1995. The organism used to produce DHA GOLD™, while still a member of the genus *Schizochytrium*, is a different species from that used to produce the algal oil which is the subject of this notification.

Both organisms have been thoroughly examined by Dr. Gunter Bahnweg (Bahnweg, unpublished, 2010; Bahnweg, unpublished, 2009). He concluded that both organisms possess the definitive characteristics of the genus *Schizochytrium*. Morphological examinations, biochemical compositions, and DNA sequence characteristics indicate that these organisms are closely related, and thus, safety studies conducted with either species should be considered relevant.
Published safety studies have shown that algae from *Schizochytrium* sp. is safe in classical toxicology models, including a subchronic rat feeding study (Hammond et al., 2001a),developmental and reproductive toxicity in rats and rabbits (Hammond et al., 2001b) a

single generation rat reproduction study (Hammond et al., 2001c), and gene toxicology studies (Hammond et al., 2002).

Table 30 Summary of Published *Schizochytrium* sp. microalgae Confirmatory Safety Studies

Study Type	System	Doses	Results
***In vitro* Genetic Toxicity**			
• Ames bacterial mutagenicity	*S. typhimurium* (TA98, TA100, TA102, TA1535, TA1537)	500 µg/plate	Not mutagenic[1]
• AS52/XPRT mammalian mutagenicity	Chinese hamster ovary cells	500-5000 (-S9); 200-1000 µg/ml (+S9)	Not mutagenic[1]
• Chromosomal aberration	Human peripheral lymphocytes	125 - 750 µg/ml	Not clastogenic[1]
***In vivo* Genetic Toxicity**			
• Micronucleus assay	Mouse bone marrow	500, 1000 and 2000 mg/kg bw orally	Not clastogenic[1]
Subchronic Toxicity			
• 13-week	Rat (20/sex/group)	400, 1500, 4000 mg/kg bw/day in the diet	No relevant adverse effects were observed at any dose level[2]
Reproductive Toxicity			
• One-generation study	Rat (30/sex/group)	0.6%, 6% and 30% of diet (up to 17,800/ 20,700 mg/kg bw/d for males/females)	No changes in fertility or developmental indices[3]
Developmental Toxicity			
	Rat (25 mated females/group)	0.6%, 6% and 30% of diet on GD 6 - 15.	NOAEL = 22,000 mg/kg bw/d for both maternal and developmental toxicity[4]
	Rabbit (22 mated females/group)	180, 600 and 1800 mg/kg bw/day orally by gavage on GD 6 - 19.	Maternal toxicity NOAEL = 600 mg/kg bw/d; developmental NOAEL = 1800 mg/kg bw/day[4]

1 Hammond et al., 2002
2 Hammond et al., 2001(a)
3 Hammond et al., 2001(c)
4 Hammond et al., 2001(b)

7.2.2 Oil

Studies conducted with algal oil from *Schizochytrium* sp., the same parent organism with which this algal oil (AOCED) is produced, are summarized in the following two tables.

The algal oil was initially developed in 2010 for use in human dietary supplements. Chronic and sub-chronic feeding studies have been conducted in rats using multiple intake levels. The administration of algal oil did not affect health or growth. No adverse effects were noted in the 90-day study, therefore, the no-observed-adverse-effect level (NOAEL) of algal oil in this study was placed at highest level administered viz. at 5% in the diet (equivalent to at least 3250 mg/kg bw/day). Finally, genotoxicity studies demonstrate that the algal oil is not mutagenic when studied in vivo and in vitro.

Table 31 Summary of Algal Oil (*Schizochytrium* sp.) toxicity study (Fedorova-Dahms, et al., 2011a)

Study Type	System	Algal Oil Dose	Results
***In vitro* Genetic Toxicity**			
Ames bacterial mutagenicity	5 strains of *Salmonella typhimurium* and *Escherichia coli*	15.8-5000 µg/plate w/ and w/o metabolic activation	Not mutagenic
Mammalian Chromosome Aberration	Human lymphocytes	1, 2.5, 3, 4, and 5 µL/mL w/ and w/o metabolic activation	Not genotoxic
***In vivo* Genetic Toxicity**			
Micronucleus assay	Mouse peripheral blood	2000 mg/kg bw	Not genotoxic
Subchronic Toxicity			
14 day	Rat (10/sex/group)	1%, 3% and 6% of total diet	Expected tolerance of 6% in a study of longer duration
90 day	Rat (10/sex/group)	0.5%, 1.5%, and 5% of the diet	NOAEL at 5% (equivalent to 3250 mg/kg bw/day)

NOAEL = no-observed-adverse-effect level.

In 2011, a related production strain with lower EPA content (a specification of maximum 10% EPA) from the same *Schizochytrium* species was developed for use in human infant formula. Algal Oil from *Schizochytrium* sp. was evaluated by testing for gene mutations, clastogenicity and aneugenicity, and in a 90-day in utero Sprague-Dawley rat dietary study (Fedorova-Dahms et al, 2011). All studies were performed in accordance with Good Laboratory Practice

Table 32 Summary of Algal Oil (*Schizochytrium* sp.) toxicity study (Fedorova-Dahms, et al., 2011b)

Study Type	System	Algal Oil Dose	Results
***In vitro* Genetic Toxicity**			
Ames bacterial mutagenicity	5 strains of *Salmonella typhimurium* and *Escherichia coli*	Up to 5000 µg/plate w/ and w/o metabolic activation	Not mutagenic
Mammalian Chromosome Aberration	Human lymphocytes	1, 2.5, 3, 4, and 5 µL/mL w/ and w/o metabolic activation	Not genotoxic
***In vivo* Genetic Toxicity**			
Micronucleus assay	Mouse peripheral blood	2000 mg/kg bw	Not genotoxic
Subchronic Toxicity			
90 day (with in utero phase)	Rat (20 F_1/sex/group)	0.5%, 1,5%, and 5% of the diet	NOAEL greater than 5% (equivalent to 4260 mg/kg bw/day)

NOAEL = no-observed-adverse-effect level.

Another set of toxicological studies, geneotoxicity, acute and subchronic with rats, was performed with the oil from *Schizochytrium* strain ONC-T18, (Schmitt et al. 2012).

Table 33 Summary of Algal Oil (*Schizochytrium* sp.) toxicity study (Schmitt et al 2012)

Study Type	System	Algal Oil Dose	Results
***In vitro* Genetic Toxicity**			
Ames bacterial mutagenicity	5 strains of *Salmonella typhimurium* and *Escherichia coli*	Up to 5000 µg/plate w/ and w/o metabolic activation	Not mutagenic
Mammalian Chromosome Aberration	Human lymphocytes	12.5, 25.0, 50.0, 100, 175, 250, 350, 500, 750, and 1000 lg/mL w/o metabolic activation and 100, 250, 500, 750, 1000, 1250, and 1500 lg/mL with activation	Not genotoxic
***Acute* Toxicity**			
Single dose	Female rats	5000 mg/kg bw	Not toxic

Study Type	System	Algal Oil Dose	Results
Subchronic Toxicity			
90 day	Rat (10/sex/group)	1.0%, 2,5%, and 5% of the diet	NOAEL greater than 5% (equivalent to 3305 male and 3679 female,mg/kg bw/day)

7.3 Review of the Safety of DHA and EPA in Cats

Safety of n-3 LCPUFA in Felines

Lenox and Bauer (2013) reviewed "potential" adverse effects of n-3 LCPUFA in felines based on a combination of studies in cats and other species, including humans. The proposed adverse effects include decreased platelet aggregation, increased methyl mercury (MeMg) exposure, weight gain, pancreatitis associated with higher fat intake, potential changes in wound healing related to changes in skin inflammatory responses, and changes in immune function. As outlined above, changes in immune function have been observed but the clinical relevance of these changes is unknown and speculated to be beneficial. There are no known reports of MeMg toxicity, pancreatitis or changes in would healing related to n-3 LCPUFA feeding in cats. A series of studies (Wilkins et al., 2004; Mazaki-Tovi et al., 2011; Mazaki-Tovi et al., 2019) suggest that n-3 LCPUFA may contribute to the maintenance of normal body weight. Two studies, detailed below, have evaluated the impact of n-3 LCPUFA on feline platelet function and coagulation.

Platelet Function and Coagulation

The potential for changes in platelet function and resulting blood coagulation in response to dietary supplementation of n-3 LCPUFA has been studied in cats with mixed results (Lenox and Bauer, 2013). In an earlier study, Bright and co-workers (1994) found no differences in feline platelet function parameters despite a nearly 10-fold increase from baseline in plasma EPA after intake of 1800-2800 mg EPA and DHA daily for 56 days. In a later study, supplementation of feline turkey and rice-based diets for 112 days with 1.03 g/kg diet n-3 LCPUFA from menhaden oil nearly doubled toenail bleeding time compared to diets containing corn oil or white grease (6.1 minutes vs 3.2 minutes; Saker et al., 1998). No differences were seen in activated partial thromboplastin time, one-stage prothrombin time, and fibrinogen concentrations as measured in whole-blood samples from the animals (Saker et al., 1998). The authors did not report blood n-3 LCPUFA levels post-supplementation. Importantly, the increased bleeding time remained with the normal range of up to 8 minutes (Cornell University, 2016). As evidence is mixed regarding the impact of supplemental n-3 LCPUFA on feline coagulation, Lenox and Bauer (2013) suggest caution when considering the combination of a high risk patient (thrombocytopenia) and prolonged n-3 LCPUFA supplementation and recommend monitoring for interactions of n-3 LCPUFA with anti-coagulant drugs.

7.4 Target Animal Safety Studies

7.4.1 Objective of the study

The objective of the study was to evaluate the safety and bioavailability of algal oil containing EPA and DHA as a source of EPA and DHA when administered via diet to domestic short hair cats throughout gestation, lactation, and growth up to 32 weeks old. The study report is in Annex 11 and the summary of the toxicology studies was accepted for publication in December 2019 by the Journal of Animal Physiology and Animal Nutrition, see Vuorinen et al. 2020.
The bioavailability of AOCED is discussed further in Section 7.4.8 as a means of demonstrating efficacy.

7.4.2 Study design

AOCED was incorporated into extruded dry foods for cats at 0, 0.75%, 1.5% and 3.0% levels on a dry matter basis at the expense of chicken fat. A larger high dose was considered during discussions with CVM but a 3X dose resulted in an imbalance in fatty acid levels in the food that could not be overcome and would exceed the current maximum safe dose of EPA plus DHA per the NRC. Therefore, a 2x dose was used as the high dose.

The diets were fed to healthy female cats starting at mating and throughout gestation and lactation. Kittens were fed their maternal corresponding diets for 26 weeks after weaning.
24 female cats with at least one successful breeding were assigned to the study. At the time of randomization, they were between 1 and 7 years of age with body weights ranging from 3.1 to 5.1 kg. One male was housed with three females for a total of 8 different males. All the females became pregnant and the males were returned to the colony. The kittens remained with the queen until six weeks of age. Five males and five females from each dose group were selected at random to continue in the growth phase of the study. The balance of the kittens were returned to the colony.

Table 34 Dosing plan

Study Group	Dosage Level (X of targeted dose)	Target Dosage Level (% of AOCED in the diet on DM basis)	Target DHA+EPA content (% in the diet of DM)	Actual DHA+EPA content† (% in the diet on DM basis)	Number of Animals			
					Parental Generation		Kittens§	
					F	M	F	M
Control	0	0	≥0.012%	0.064%	6	2	5	5
AOCED Low Dose	½X	0.75%	0.39%	0.414%	6	2	5	5
AOCED Mid Dose	X	1.5%	0.79%	0.819%	6	2	5	5
AOCED High Dose	2X	3.0%	1.58%	1.617%	6 (5)*	2	5	5

*Six females used for breeding, all females confirmed pregnant at the end of the mating period remained in the study

The animals in Group 1 received the control diet, which was formulated to meet or exceed (without exceeding any maximums) the AAFCO cat food nutrient profile for growth and reproduction (2016), including the minimum requirement for EPA and DHA (0.05%). Instead of being achieved through the addition of algal oil, the EPA and DHA level in the control diet was achieved through the addition of fish oil. Fish oil was not used in the diets fed to Groups 2-4.

The finished diet was produced by coating the base kibble (table 35) with 6.0 % fat with a composition shown in table 33.

Table 35 Treatment composition

Test diets	Chicken Fat, %	Fish Oil, %	AOCED, %
Group 1 (Control)	5.93	0.07	0
Group 2 (1/2 X)	5.27	0	0.72
Group 3 (1 X)	4.55	0	1.45
Group 4 (2 X)	3.11	0	2.89

Table 36 Study design for the F_1 generation

	No. of Animals					
Diet Code	Male	Female	Dose Level	Target Dose (%AOCED in Diet DM)	Target DHA + EPA (% in Diet DM)	Actual DHA + EPA (% diet, As Is)
FD828	5	5	0	0	≥0.012%	0.06%
FD128	5	5	0.5 x	0.75%	0.39%	0.39%
FD016	5	5	1 x	1.50%	0.79%	0.77%
FD854	5	5	2 X	3.00%	1.58%	1.53%

7.4.4 Diet Offered During the Study

The dry extruded test diets were manufactured at [redacted] (b) (4) from May 15-23, 2017. The diets were produced once for the entire study duration. The basal diet, which was formulated to meet the AAFCO Cat Food Nutrient Profile for Growth and Reproduction (2016), was coated with chicken fat, fish oil, or algal oil to produce the test diets. The lot number of the algal oil used to make the diets for Groups 2-4 was [redacted] The diets were analyzed to confirm that they met the AAFCO Cat Food Nutrient Profile for Growth and Reproduction (2016) as well as the targeted DHA+EPA concentrations. The diets were made isocaloric with the control diet.
Ingredient composition of the base kibble is presented in Table 35 while the test diets nutritional composition is shown in Table 37.

Table 37 Composition of Base Kibble (on As Is basis)

Ingredient Name	%
Chicken by-product meal	23.3
Corn gluten meal	21.1
Dried eggs	15.1
Brewers rice	10.8
Corn	9.9
Chicken fat	4.55
Wheat	4.3
Flavor	3.0
Sorghum	2.0
Beet pulp	1.9
Potassium chloride	0.75
Salt	0.7
Dicalcium phosphate	0.5
Verdilox™ GT Dry[1]	0.1
Vitamin premix[2]	0.3
Taurine	0.21
Mineral premix[3]	0.14
Choline choride	0.08
Total	100

[1]Verdilox™ GT Dry is commercial antioxidant and flavoring product from Kemin Industries, Inc (b) (4)).

[2]Contains Vitamin A supplement, Vitamin D_3, Vitamin E, Vitamin B_{12}, Riboflavin, Pantothenic acid, Niacin, Folic Acid, Vitamin B6, Thiamine, Biotin.

[3]Contains ferrous sulfate, potassium iodide, zinc oxide, manganous oxide, copper sulfate, sodium selenite, cobalt carbonate, mineral oil.

Table 38 Nutrient composition of the diets compared to the AAFCO recommendation for cats

	AAFCO Cat Food Nutrient Profile Based on Dry Matter Growth and Reproduction Minimum	FDS002 (DM basis)	FDS003 (DM basis)	FDS004 (DM basis)	FDS005 (DM basis)
Moisture*	N/A	5.9%	5.7%	6.0%	5.4%
Crude Protein	30.0%	42.55%	43.72%	43.59%	42.97%
Arginine	1.24%	2.22%	2.23%	2.28%	2.24%
Histidine	0.33%	0.90%	0.92%	0.94%	0.91%
Isoleucine	0.56%	1.80%	1.85%	1.85%	1.82%
Leucine	1.28%	4.39%	4.55%	4.48%	4.43%
Lysine	1.20%	2.20%	2.16%	2.24%	2.17%
Methionine	0.62% - 1.5%	1.01%	1.06%	1.04%	1.04%
Methionine-cystine	1.10%				
Cystine		0.63%	0.65%	0.65%	0.64%
Phenylalanine	0.52%	2.06%	2.12%	2.13%	2.08%
Phenylalanine-tyrosine	1.92%				
Tyrosine		1.42%	1.43%	1.45%	1.47%
Threonine	0.73%	1.67%	1.72%	1.71%	1.67%
Tryptophan	0.25% - 1.7%	0.45%	0.46%	0.46%	0.44%
Valine	0.64%	2.20%	2.26%	2.26%	2.21%
Crude fat	9.0%	18.01%	19.53%	17.88%	18.50%
Linoleic acid	0.6%	3.24%	3.20%	3.18%	2.90%
Alpha-linolenic acid	0.02%	0.13%	0.13%	0.14%	0.12%
Arachidonic acid	0.02%	0.16%	0.18%	0.20%	0.22%
Eicosapentaenoic + Docosahexaenoic Acid	0.012%	0.06%	0.42%	0.82%	1.62%
Calcium	1.0%	1.17%	1.15%	1.16%	1.17%
Phosphorus	0.8%	1.17%	1.16%	1.18%	1.17%
Potassium	0.6%	0.87%	0.90%	0.89%	0.89%
Sodium	0.2%	0.682%	0.682%	0.714%	0.703%
Chloride	0.3%	1.19%	1.22%	1.21%	1.18%
Magnesium	0.08%	0.11%	0.11%	0.11%	0.11%
Iron	80 ppm	279 ppm	301 ppm	298 ppm	295 ppm
Copper	15 ppm (extruded)	22 ppm	23 ppm	23 ppm	23 ppm
Manganese	7.6 ppm	92 ppm	93 ppm	93 ppm	92 ppm
Zinc	75 ppm	336 ppm	334 ppm	369 ppm	360 ppm
Iodine	1.8 ppm – 9.0 ppm	3.33 ppm	3.27 ppm	3.43 ppm	3.54 ppm
Selenium	0.3 ppm	1.15 ppm	1.18 ppm	1.15 ppm	1.18 ppm

	AAFCO Cat Food Nutrient Profile Based on Dry Matter Growth and Reproduction Minimum	FDS002 (DM basis)	FDS003 (DM basis)	FDS004 (DM basis)	FDS005 (DM basis)
Vitamin A	6,668 IU/kg - 333,300 IU/kg	33,050 IU/kg	34,677 IU/kg	30,638 IU/kg	29,387 IU/kg
Vitamin D	280 IU/kg - 30,080 IU/kg	2,891 IU/kg	2,853 IU/kg	2,521 IU/kg	4,461 IU/kg
Vitamin E	40 IU/kg	379 IU/kg	413 IU/kg	399 IU/kg	370 IU/kg
Vitamin K	0.1 ppm	0.319 ppm	0.329 ppm	0.362 ppm	0.275 ppm
Thiamine	5.6 ppm	35.6 ppm	23.6 ppm	27.0 ppm	21.4 ppm
Riboflavin	4.0 ppm	22.3 ppm	21.6 ppm	23.4 ppm	23.3 ppm
Pantothenic acid	5.75 ppm	37.6 ppm	37.6 ppm	39.4 ppm	38.2 ppm
Niacin	60 ppm	259 ppm	308 ppm	274 ppm	274 ppm
Pyridoxine	4.0 ppm	22.74 ppm	23.86 ppm	23.72 ppm	22.41 ppm
Folic acid	0.8 ppm	3.70 ppm	3.81 ppm	3.89 ppm	3.64 ppm
Biotin	0.07 ppm	0.647 ppm	0.679 ppm	0.660 ppm	0.672 ppm
Vitamin B_{12}	0.020 ppm	0.113 ppm	0.121 ppm	0.121 ppm	0.117 ppm
Choline	2400 ppm	3305 ppm	3680 ppm	3351 ppm	3689 ppm
Taurine	0.10% (extruded)	0.31%	0.33%	0.33%	0.32%

* Moisture content was used to calculate values on a dry matter basis.

Upon receipt at the breeding and main research facilities, diets were stored at room temperature in temperature-controlled rooms. Fatty acid analysis of dietary samples collected throughout the study confirmed acceptable levels of EPA and DHA which ensured proper algal oil dosing of animals.

7.4.5 Animal Care

This study was conducted at [redacted] (b) (4) under (b) (4) Standard Operating Procedures, in compliance with the Animal Welfare Act (9 CFR, Subchapter A), and the Guide for the Care and Use of Laboratory Animals. The study protocol was approved by the [redacted] (b) (4).

7.4.6 Housing

Prior to mating, the females were individually housed in caging (ca. 2,5' x 2,5' x 2,5' in size). During mating, the animals were group housed (cage size ca. 7.6' x 3.0' x 2.0' in size) per dietary group. During pregnancy females were housed in individual cages with HDPE surfaces included perches, stainless steel water/food bowls, litter pans and enrichment toys. After weaning, two kittens, one male and one female to reduce variability in food consumption, were housed in stainless steel cages (ca. 2.6' x 3.0' x 2.3') until the age of approximately 13 weeks to avoid stress of single housing during

early development. Thereafter the kittens were housed individually in stainless steel cages (ca. 2.6' x 3.0' x 2.3') until study termination at 32 weeks of age. All cages were clearly labeled with cage cards, indicating a study number, animal tattoo number, blinded animal assignment number, sex, and replicate. All animals were transferred to the colony upon completion of the study.

7.4.7 Clinical Observations

Clinical observations were conducted on queens once daily from the baseline until weaning of the kittens. The kittens were sexed at 7 days of age, and thereafter clinical observations were collected once daily until the end of the study. The observations included, but were not limited to, changes in skin and fur, eyes and mucus membranes, ears, nose, overall body condition and behavioral pattern. Beside the clinical observations, all animals were observed throughout the study twice daily for morbidity, mortality, injuries, and availability of food and water. Prior to 7 days of age, kittens were examined to determine litter size, number of stillborn/live kittens and for any gross visceral or skeletal abnormalities. Any animal in poor health was further monitored under the supervision of a veterinarian.

A detailed clinical examination was performed on each animal on the day of estrous and on the day of treatment initiation, and once within the week prior to transfer to (b) (4), and monthly thereafter. A detailed clinical examination was also performed on the day prior to or on the day of release from the study. During the detailed examination, animals were first observed in the cage for behavior, general appearance, and coordination. Then they were removed from the cage for close evaluation of general condition of the whole body including skin, fur, eyes, mucus membranes, ears, nose, overall body condition and behavior. Body temperature, heart rate, and respiratory rate were also evaluated.

Kittens were examined by the veterinarian at approximately 7-10 days of age and again at 6, 16, 24 and 32 weeks of age(± 3 days). These assessments included the following: general condition and behavior; ocular; oral cavity; integument; musculoskeletal; gastrointestinal; body temperature; cardiovascular rate; respiratory rate (including assessment by auscultation); abdominal palpation and external genitalia.

7.4.7.1. Mortality

Mortality checks were recorded concomitantly with the cage-side clinical signs observations during all phases of the study. Two kittens, one in the mid-dose group and one in the high-dose group, were found dead during the first 4 days post-partum. These deaths were not considered related to dietary treatments as such accidents occur relatively often shortly after birth, especially in larger litters. Three kittens were euthanized during the study: one kitten in the low-dose group on day 8 post-partum and one kitten in the high-dose group on day 9 due to overlicking by their queens, and the third kitten with an umbilical hernia on day 39 post-parturition.

7.4.7.2 Body Weights

Body weights were recorded for all queens on the day of estrous and on the day of treatment

initiation, and weekly thereafter.
Kitten body weights were recorded at 7 days of age and weekly thereafter until study termination.

7.4.7.3 Food Consumption

Individual food intake was measured on all animals daily starting at initiation of feeding the reference/control diet (3-14 days prior to treatment start). In addition to the daily food consumption, achieved intakes were calculated on a weekly basis. Any spilled food was also weighed daily for each cage, unless clearly contaminated with liquid or solid material (e.g. water, urine, feces, etc.). The amount of contaminated food (excluded from the spillage measured value) was visually estimated and documented.

The average of daily individual achieved intake of the test item in mg/kg/bw per week was calculated for both generations based on daily food consumption taking into account any spilled food.

The average exposure to AOCED in mg/Kg BW for the queens from week 3 to week 8 was 0 for the control group, 132.4 for the low dose, 250.8 for the mid-dose and 504.7 for the high dose.
For the kittens, the average exposure to AOCED in mg/Kg BW from week 1 to 26 was zero for the controls, for the treatment groups exposure decreased over time from 818.2 – 264.1 for the low dose group, 1730.0 – 584.2 for the mid-dose group and 3905.0 – 1122.4 for the high dose group.

7.4.7.4 Parturition and litter observations

The time of onset and completion of delivery was recorded for each animal when possible. The day when parturition was completed was termed as PPD 0. Any signs of difficulty in parturition were recorded. Number of live, dead and malformed kittens was recorded at birth. Gross abnormalities were recorded, if present. Number of male and female kittens in each litter was determined by visually judging the anogenital distance and/or presence of a vulva or penis. Recordings of live and dead kittens continued daily throughout the study.

7.4.7.5 Clinical Pathology

Clinical pathology evaluations (hematology and clinical chemistry) were performed on all queens once during pretreatment and at weaning. Kittens were assessed at weeks 8, 16, 24 and 32.

7.4.7.6 Statistical Analysis

The statistical analyses for this study were designed with reference to the Guidance for Industry #226: “Target Animal Safety Data Presentation and Statistical Analysis” of the Center of Veterinary Medicine (CVM), a division of the US Food and Drug Administration (FDA). As recommended by the guidance, no statistical adjustments for multiplicity were applied to avoid false negative findings and the significance level for comparisons against the control group was α=0.1 for safety endpoints. The interpretation of the results was based both on statistical significance and clinical relevance, as recommended by the guidance.

The statistical analyses were independently performed for the following three periods: gestation, pre and post weaning. The treatment group was used as a fixed effect in all models.

Analysis of variance (ANOVA), analysis of covariance (ANCOVA) as well as repeated measures ANOVA/ANCOVA and linear mixed models were employed, depending on the available number of timepoints within a period and whether a baseline measurement was available.

For parameters with more than one timepoint within a period, repeated measures equivalents of the above methods were employed to take into account potential effects over time. The fixed effects in the models were the treatment group, the time and their interaction, with the animal as subject and, when applicable, the pre-treatment value as covariate. For the efficacy parameters (EPA and DHA plasma levels), mixed effects models with random intercept by animal were used.
Three-way repeated measures ANOVA were used for longitudinal analyses on kittens, where males and females had to be differentiated, and when more than one follow-up timepoint within a period was available, e.g. for body weight of kittens post-weaning. The fixed effects in the model were the treatment group, sex, time and their two-way and three-way interactions with the animal as subject. For the pre-weaning period, the statistical unit was the kitten nested within litter.
Inter-group differences of the time to event curve estimates were tested using the log-rank test.

7.4.8 Results and Discussion

7.4.8.1 General observations

No statistically significant differences in physical examination parameters were observed in the queens in any treatment group when compared to the control. In kittens, there was a treatment effect on body temperatures and heart rate values based on the treatment-by-time statistical analysis, indicating that the AOCED diets affected different groups differently over time. Body temperatures in the high-dose group were significantly lower compared to the control at weeks 16 and 32 ($P \leq 0.1$) but unaffected at weeks 6 and 24. In the mid-dose group, body temperatures were significantly lower than those of the control at the study completion, week 32, only ($P < 0.1$). Further, heart rate values in the mid and high-dose groups were significantly lower compared to those in the control if data were analyzed over the whole time period rather than at certain timepoints ($P \leq 0.1$). All these parameters were within a normal physiological range for kittens of this age and were not considered adverse by the veterinarian.

No AOCED-related effects (overall treatment effects over all timepoints) were observed on the food consumption of the queens or their kittens compared to the control. A significant treatment by time interaction for the food consumption of the queens revealed a significantly higher food consumption in the high-dose group compared to the control at the later stages of lactation ($p = 0.003$ for the study day 126). This is thought to be attributable to the larger litter sizes in this group that consequently increased energy demands of the nursing queens. More kittens in a litter could have also contributed to the higher food consumption values at this period since kittens typically start consuming solid food shortly before weaning.

No statistically significant differences were observed in the body weights of the queens when compared to the control in any dose group during pregnancy and lactation . Although the queens in the high-dose group tended to be heavier during gestation and lighter during lactation due again to the larger litter sizes in this group, these differences did not reach statistical significance. In

kittens, there were statistically significant differences in body weights before weaning when compared to the control: body weights were higher in the low-dose group on days 7 and 14 post-partum, lower in the mid dose group on days 21, 28, 35, and 42 post-partum, and lower in the high dose group at all time points from day 14 to 42 post-partum. During the growth phase (from weaning to the week 32), body weights of the kittens of either sex in any treatment group were not affected by the treatment. Toward the study end, males in the mid-dose AOCED group and females in the high-dose group appeared to be lighter than the controls, however, the differences did not reach statistical significance (P>0.1).

In the queens, hematological and coagulation parameters were not affected by the AOCED treatment. Mean monocyte counts were statistically significantly increased in the mid and high-dose groups compared to the control at weaning (P<0.1); however, the mean values were within the laboratory reference range and, therefore, this was not considered adverse.

The AOCED treatment did not affect hematological and coagulation parameters of the kittens. The parameters remained within laboratory reference ranges at every time point. None of the queens showed excessive or prolonged bleeding during birth and none of the kittens showed excessive or prolonged bleeding from the umbilical cord.

7.4.8.2 Effect of AOCED on blood levels of EPA and DHA

The concentration of DHA and EPA in the blood plasma of the test article treatments groups increased over time and with dose level versus the control as shown in the tables and charts below, indicating that AOCED is an effective source of those omega-3 fatty acids when incorporated into food for cats, which is the intended use of the material. The more detailed information regarding the analysis in Appendix 18 of the [redacted] (b) (4) report, Annex 11.

Table 39 Blood Plasma EPA concentration, Queens

EPA	Description	Mean Value µg/mL
Group 1	Control	18.75
Group 2	Low dose	51.33
Group 3	Mid dose	76.17
Group 4	High dose	142.48

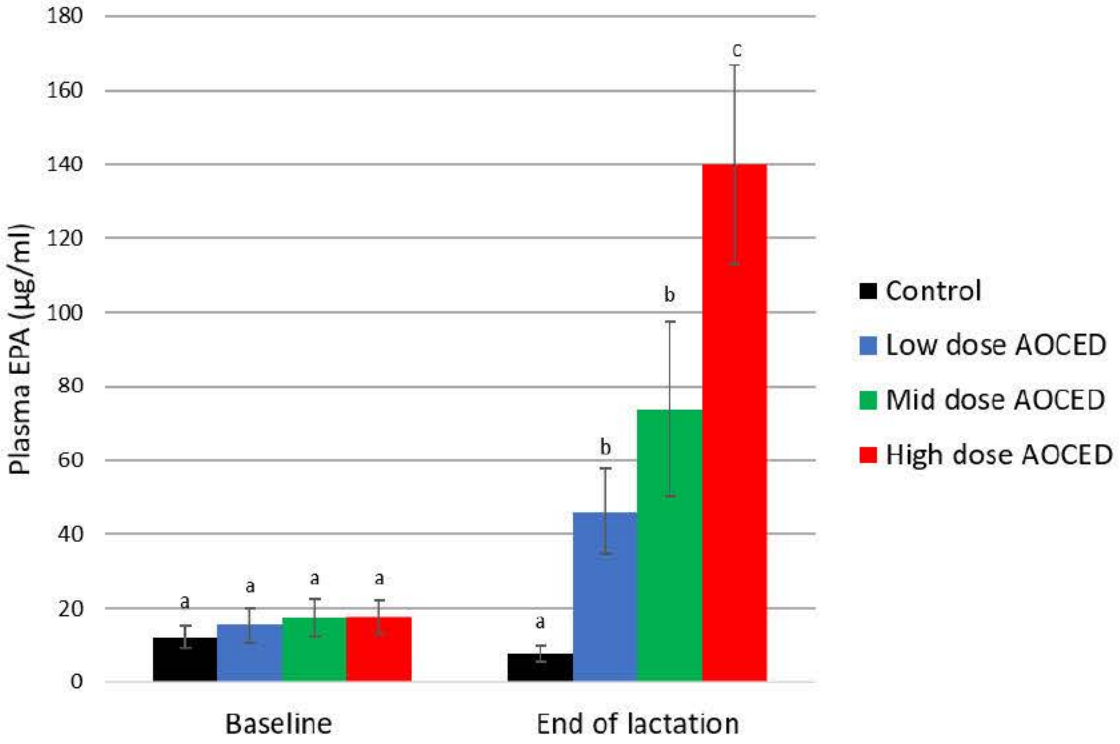


Figure 14 Blood Plasma EPA Concentration over time, Queens

Table 40 Blood Plasma DHA concentration, Queens

DHA	Description	Mean Value µg/mL
Group 1	Control	24.12
Group 2	Low dose	82.55
Group 3	Mid dose	99.45
Group 4	High dose	165.66

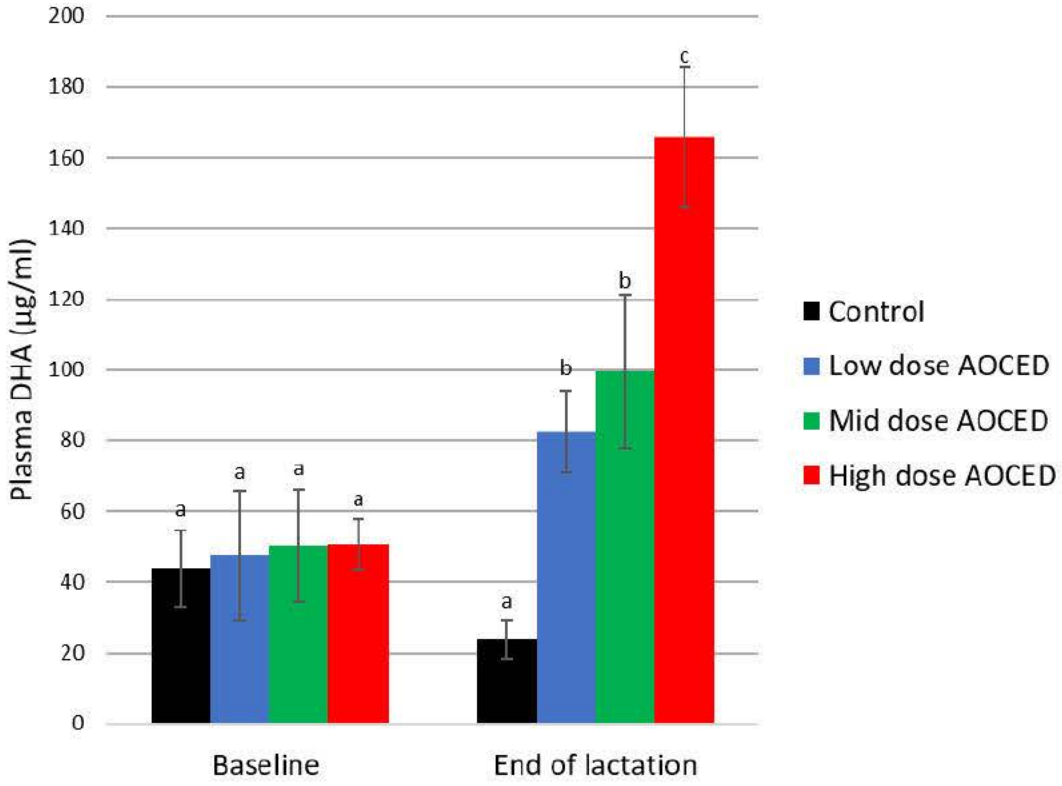


Figure 15 Blood Plasma DHA Concentration over time, Queens

Kitten Data

Table 41 Blood Plasma EPA concentration, kittens

EPA	Description	Week 8 µg/mL	Week 24 µg/mL	Week 32 µg/mL
Group 1	Control	4.33	6.80	6.43
Group 2	Low dose	25.94	45.54	48.19
Group 3	Mid dose	58.27	77.70	79.39
Group 4	High dose	96.11	148.99	139.41

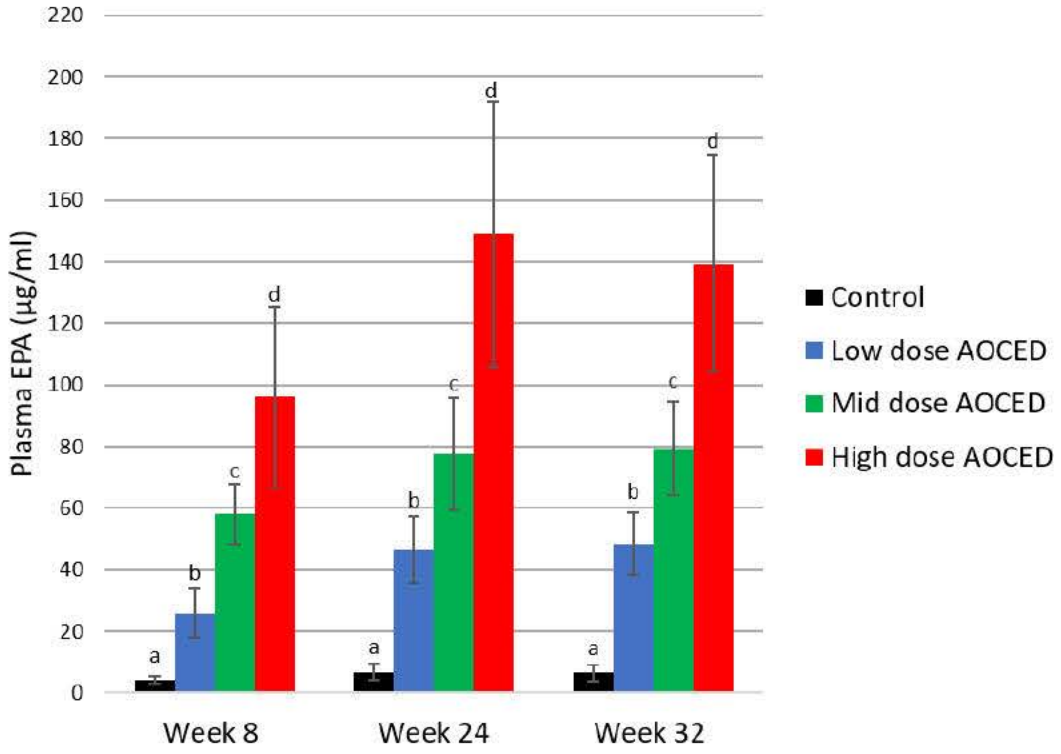


Figure 16 Blood Plasma EPA concentration over time, kittens

Table 42 Blood Plasma DHA concentration, kittens

DHA	Description	Week 8 µg/mL	Week 24 µg/mL	Week 32 µg/mL
Group 1	Control	37.71	26.64	25.12
Group 2	Low dose	131.37	92.85	78.29
Group 3	Mid dose	134.77	120.32	106.21
Group 4	High dose	170.89	134.33	116.88

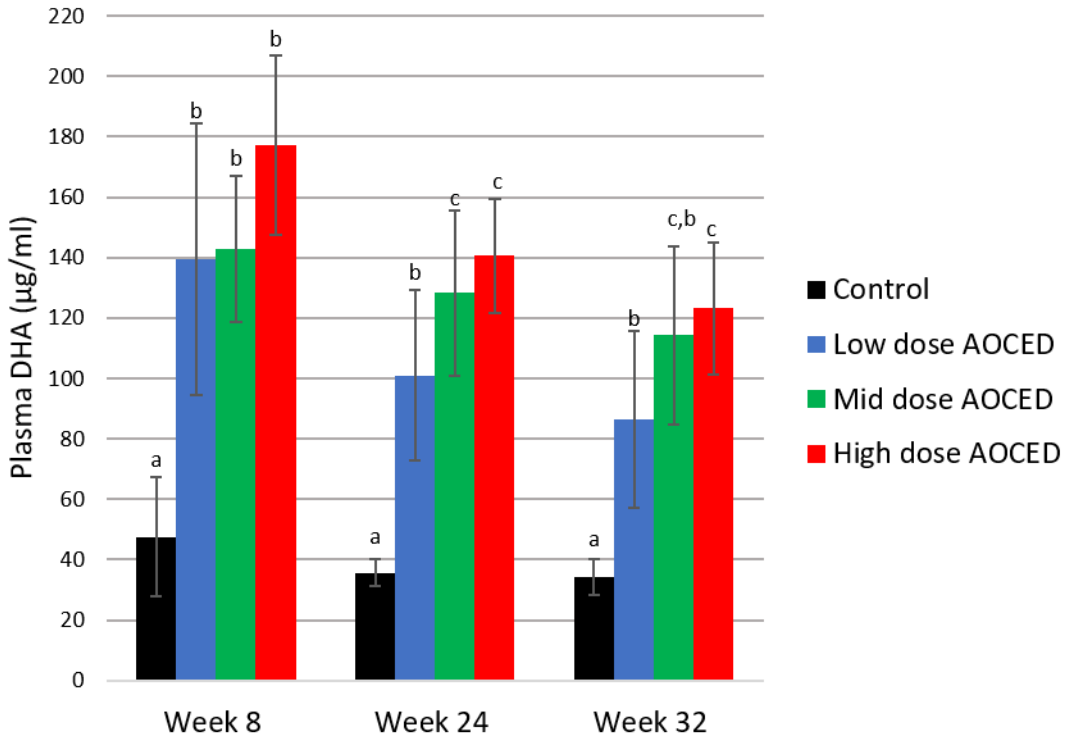


Figure 17 **Blood Plasma DHA concentration over time, kittens**

7.4.8.3 Statement of compliance with GLP

The GLG study was not conducted in accordance with 21 CFR Part 58, good laboratory practice (GLP) for nonclinical laboratory studies as CVM had been informed during a study protocol review in late 2016. However, the study site was visited and monitored by sponsor personnel familiar with GLP. The study facility works according to standard operating procedures and the study differed from GLP only in that the study did not have a quality assurance unit. Veramaris confirms that the results of the study have not been compromised by the lack of a QA unit.

7.5 Information that may appear to be inconsistent with the GRAS determination

Important potential adverse effects of omega-3 fatty acid supplementation from fish oil include altered platelet function, gastrointestinal adverse effects, detrimental effects on wound healing, lipid peroxidation, potential for nutrient excess and toxin exposure, weight gain, altered immune function, effects on glycemic control and insulin sensitivity, and nutrient-drug interactions. (Lenox and Bauer, 2013) The potential adverse effects noted by the authors in the studies reviewed were typically seen where diets for cats or dogs contained concentrations of EPA and DHA greater than 2.0 gm/Kg of food. Saker et al. (1998) reported a two fold increase in bleeding time from toe nail cuts in cats fed a diet containing 1030 mg/Kg of Omega-3 fatty acids when compared to control at 70 mg/Kg. At this time there is no safe upper limit established by the NRC for EPA plus DHA for cat food. (Bauer, 2011)

AOCED is at a minimum 50% EPA plus DHA, see section 2.5 in this dossier and the lot used to produce the cat food used in the GLG study was (b) (4), with a combined DHA + EPA concentration of 52.5%, see Annex 1.

The exposure to the combined EPA and DHA was 504.7 to 925.6 mg/Kg BW for the queens and 1122.4 to 3905.0 for kittens, see table 24 in section 3.1.1 of this dossier. These exposures at the high dose, which did not result in any clinical physiological or blood chemistry adverse effects, and were slightly higher than the NRC maximum recommended dose, indicates that the mid-dose, the highest expected commercial dose will not induce any adverse effects in cats.

Veramaris USA, LLC is not aware of any additional information that would be inconsistent with a finding that the proposed use of AOCED in food for cats that meets appropriate specifications, and is used according to cGMP, is GRAS. Recent reviews of the scientific literature revealed no potential concerns for adverse health effects.

7.6 Summary

The organism used to produce the substance is not known to be toxic to animals and analysis of parent and sister strains has shown that modifications have not cause the organism to produce known algal toxins. The 90-day rat study and GLG study with felines also did not reveal any harmful effects from consumption of the purified oil produced by the *Shizochytrium sp.* organism within the anticipated use levels; no more than 1.5% of the diet in both extruded (dry) food and canned (wet) food. Efficacy studies indicate that the product, an algal oil referred to as AOCED in this dossier delivers the anticipated effect, elevation of the DHA and EPA concentrations in the blood plasma of cats.

8 Annexes

Annex 1: Certificates of Analysis for AOCED

Annex 2: Prymnesin analysis

Annex 3: Domoic acid analysis

Annex 4: Raw Material Specifcations

Annex 5: AOCED Stability Report

Annex 6: AOCED Stability Summary 25 and 40 C

Annex 7: Stat-Analysis_1153_Cat_Kibble_Post-extrusion

Annex 8: Stat-Analysis_1310_Cat_Kibble_pre-extrusion

Annex 9: Heavy Metals Analysis

Annex 10: Pesticide Analysis

Annex 11: Cat Gestation/Lactation/Growth (GLG) Final Report

Annex 12: Literature search terms

Annex 13: Domoic Acid Report

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10 Expert Panel Statement

GRAS Panel Consensus Statement on the Generally Recognized as Safe (GRAS) Determination of the Use of Algal Oil produced by *Schizochytrium* sp.

for use as a source of DHA and EPA in Food for Cats

March 17, 2020

The undersigned, an independent panel of experts, qualified by their scientific training and national and international experience to evaluate the safety of food and food ingredients (the "GRAS Panel"), was specially convened by DSM Nutritional Products ("DSM") on behalf of Veramaris LLC to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a nutritive additive in the food of cats, algal oil produced by *Schizochytrium* sp. as an oil soluble liquid, is safe and "generally recognized as safe" ("GRAS") based on scientific procedures as stated under FDA's GRAS Notification Program for Animal Feed (21 CFR §570.30 (U.S. FDA, 2016). For purposes of this evaluation, "safe" or "safety" as it relates to GRAS within the terms of the Federal Food, Drug, and Cosmetic Act means that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2012a).

DSM Nutritional Products ("DSM") performed a comprehensive search of the scientific literature, through January, 2020, relating to the safety of algal products. DSM summarized the results of the literature search and prepared a safety dossier, **"The Safety and Generally Recognized as Safe (GRAS) Status of Algal Oil produced by Schizochytrium sp. As a Source of DHA and EPA in Food for Cats"** for consideration by the GRAS Panel.

The GRAS Panel consisted of the following individuals: Michael Pariza, Ph.D. (Emeritus Professor, University of Wisconsin-Madison, Food Research institute), Michael Carakostas, DVM, Ph.D. (Consultant) and Stanley M. Tarka Jr., Ph.D., F.A.T.S. (The Pennsylvania State University College of Medicine, Tarka Group, Inc. and Panel Chair). The GRAS Panel critically evaluated the safety documentation (the dossier), and other available data and information that the members of the GRAS Panel believed to be pertinent to the safety of the algal oil preparation and its intended use as a nutritive additive in food for cats.

On March 17, 2020 the GRAS Panel convened via teleconference, and independently, jointly, and unanimously concluded that algal oil, internally referenced as AOCED (algal oil containing EPA and DHA) for use as a nutritive additive in food for cats, produced consistent with current good manufacturing practice ("cGMP") and meeting the stated specifications, is safe for its intended use as a nutritive additive in food for cats. The GRAS Panel further concluded unanimously that the intended use of the algal oil from *Schizochytrium sp.*, and internally referenced as AOCED, is GRAS based on scientific procedures. It is also the unanimous consensus opinion of this GRAS Panel that other qualified experts would concur with these conclusions.

Summarized below are the data, information, and interpretive analysis supporting the GRAS Panel's conclusions.

Summary and Basis for GRAS Determination

Algal oil (AOCED) produced by *Schizochytrium sp.* is intended for use as a nutritive additive in food for cats. The product contains over 50% by weight of the omega-3 fatty acids EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid) in a ratio of at least 1:2.5. The balance of the product is other saturated and unsaturated fatty acids with the majority (~25%) being palmitic acid.

EPA and DHA are found predominately in marine products, flax seed oil and borage seed oil, the concentration and ratio produced by the production strain of *Schizochytrium sp.* closely resembles that of fish oil. EPA and DHA have been added to cat food via fish meal for decades and via the dried *Shizochytrium sp.* Algal for over 10 years.

AOCED (Algal oil) is manufactured following cGMP in a multistep process starting with the fermentation by the *Schizochytrium sp.* organism followed by enzymatic hydrolysis, de-emulsification and separation by centrifugation. Any remaining water is removed by drying with nitrogen under vacuum. The fermentation was previously described in Animal Food Additive Petition Federal Register /Vol. 83, No. 88, pg. 19934 (21 CFR §573.615). Raw materials used in the fermentation medium are acceptable for use in animal food. The purified final product is a clear, orange-to-golden brown, mobile fluid that can be incorporated into both dry and canned cat food products.

Process Flow for Manufacture of Algal Oil from Marine Microalgae

Four pre-production lots were evaluated and found to meet the specification necessary for use in food for cats. Lot (b) (4) was used in the manufacture of the food used in the target animal study.

Specification		Method	Lot Number			
			VY00014962	VY00015240	VY00015465	VY00015572
DHA + EPA content (mg/g)	Min. 500	AOCS Ce 1b-89	(b) (4)			
DHA content (mg/g)	Min. 250	AOCS Ce 1b-89				
EPA content (mg/g)	Min. 100	AOCS Ce 1b-89				
Free fatty acids (%)	Max. 5	AOCS Ca 5a-40				
Moisture (%)	Max. 0.75	AOAC 930.15				
Crude Fat (%)	Min. 92	AOAC 954.02				

Schizochytrium sp. are thraustochytrids that belong to the Chromista kingdom (Bennett et al., 2017). The genus *Schizochytrium* is characterized by multiple binary cell divisions leading to clusters of sporangia. *Schizochytrium* spores are biflagellate at discharge and the number of spores discharged can range from two-to-one hundred. Thraustochytrids are microscopic, unicellular algae. The earliest research of thraustochytrids placed them in the fungi because of their heterotrophic nature and superficial resemblance to chytrids (Sparrow, 1936). Current analysis using molecular biology techniques have demonstrated that thraustochytrids are not fungi, but are related to the heterokont algae (Cavalier-Smith, 2018) and as a result are now considered microalgae. Current phylogenic analysis indicates that the members of the Chromista are all photosynthetic, but some groups such as the Thraustochytridae have lost their chloroplast and the capability of photosynthesis and as a result, are exclusively heterotrophic (Tsui et al. 2009).

Veramaris has developed an improved strain from the patented wild-type parent strain using a classical mutagenesis screening program. This program utilized well-accepted techniques commonly used in industrial strain improvement programs. No recombinant DNA technology was employed. The parent, ATCC PTA-10208, underwent three successive mutagenesis steps using a standard UV exposure procedure. The production strain was selected as a single colony out of the surviving population after screening for oil production ability and DHA to EPA ratio. The selected strain has consistent DHA and EPA content, and high biomass density under large scale fermentation conditions. Further manipulation of the fermentation media composition, pH and temperature led to an optimal oil production level without adversely affecting the DHA to EPA ratio.

Although some members of the Chromista are known to produce toxins, domoic acid and prymnesin, there are no literature reports of this organism producing toxins or being pathogenic nor does the sponsor have any data indicating the initially isolated strains nor the daughter after over 30 years of strain improvement have produced toxins under the conditions of large-scale fermentation. Analysis of related and parent strains in the DSM collection used for the manufacture of algal oil for human consumption did not reveal the presence of domoic acid (Annex 3). Toxicology studies with the production strain confirmed the absence of toxins. The production strain was also evaluated for domoic acid production and none was found using the current analytical method (Annex 13) in the dossier.

The AOCED was shown to be stable in commercial packaging for up to 12 months at 25° C and 6 months at 40° C.

The intended conditions of use are as follows:

The algal oil will be applied to dry, extruded kibble or in canned food at a level of no more than 1.5 % wt/wt of dry matter. AOCED will be used as a replacement for fish oil in cat food. The algal oil will be added to the food of cats at no more than what is necessary following good animal feeding practices, NRC recommendations. When added to a model canned cat food, the concentrations of EPA and DHA remained stable for 18 months at 25° C and at 40° C. When applied topically to extruded kibble, the EPA and DHA levels

fell slightly over two years and indicated a shelf life of at least 18 months. When AOCED was added to the mash before extrusion, shelf life ws similar to the post-extrusion application.

The safety evaluation of the algal oil included the assessment of detailed literature including pivotal published safety studies conducted with the oil from parent and sister strains. These included studies addressing *In Vitro* Genotoxicity, (Ames test and Chromosomal Aberration) *In Vivo* Genotoxicity (mammalian chromosome aberration) and short-term acute and repeated-dose toxicity, 90-day rat and Reprotoxicity (rat) and Developmental toxicity with the rat and rabbit. The results of these investigations were previously provided in human GRAS Notices for the use of algal oil in infant formula.

Algal oil from the production strain was evaluated in a combined target animal and efficacy study, a Gestation-Lactation-Growth study with cats, utilizing three doses of algal oil, ½ x, 1x and 2x of the anticipated highest amount to be added to commercial cat food. Clinical observations were made twice daily on the queens and one a day with the litters; no adverse effects were noted. Detailed clinical evaluations of the kittens starting at one-week post-weaning revealed no adverse effects. The body weight of the animals was measured in weekly intervals as well as the feed intake. All kittens in the four groups (control plus treatments) gained weight continually; no growth depression could be recognized.

The purpose of adding the Algal oil to cat food is to act as a replacement for fish oil/meal in the diet. The monitoring of blood lipid profile was performed and revealed an increase in DHA and EPA content compared to control in the queens at all dose levels. Treatment kittens also had elevated levels of EPA and DHA following the same pattern as the queens indicating that AOCED is effective as a source of EPA and DHA in the diet of cats. Other clinical blood chemistry parameters were not affected in an adverse manner.

Blood Plasma DHA concentration, Queens

DHA	Description	Mean Value µg/mL
Group 1	Control	24.12
Group 2	Low dose	82.55
Group 3	Mid dose	99.45
Group 4	High dose	165.66

Blood Plasma EPA concentration, Queens

EPA	Description	Mean Value µg/mL
Group 1	Control	18.75
Group 2	Low dose	51.33
Group 3	Mid dose	76.17
Group 4	High dose	142.48

Blood Plasma DHA concentration, kittens

DHA	Description	Week 8 µg/mL	Week 24 µg/mL	Week 32 µg/mL
Group 1	Control	37.71	26.64	25.12
Group 2	Low dose	131.37	92.85	78.29
Group 3	Mid dose	134.77	120.32	106.21
Group 4	High dose	170.89	134.33	116.88

Blood Plasma EPA concentration, kittens

EPA	Description	Week 8 µg/mL	Week 24 µg/mL	Week 32 µg/mL
Group 1	Control	4.33	6.80	6.43
Group 2	Low dose	25.94	45.54	48.19
Group 3	Mid dose	58.27	77.70	79.39
Group 4	High dose	96.11	148.99	139.41

Based upon this collection of data, AOCED (Algal oil) produced by a strain of *Schyzochytrium sp.* and its marketed forms are safe and efficacious as a GRAS food ingredient for use as a nutritive additive in the food for cats. The proposed uses of Veramaris' AOCED as a nutritive additive in food for cats is safe and "generally recognized as safe" (GRAS) based on scientific procedures as stated under FDA's GRAS Notification Pilot Program in Animal Feed (21 CFR §570.36(c)(1) (U.S. FDA, 2016) as the pivotal data and information are generally available, satisfying the "common knowledge" element of a GRAS determination.

The use of AOCED as a nutritive additive in food for cats is also supported by years of use of the dried algae itself in food for cats, small mammals and chickens. It is therefore reasonable to conclude that the proposed use of AOCED is safe within the meaning of the FD&C Act, i.e., meets the standard of reasonable certainty of no harm.

CONCLUSION

We, the undersigned independent qualified members of the GRAS Panel, have individually and collectively critically evaluated the data and information summarized above, as well as other data and information that we deemed pertinent to the safety of the intended conditions of use of Algal oil containing EPA and DHA, referred to in this document and accompanying GRAS dossier as AOCED, in diets of cats. We unanimously conclude that the proposed use of this algal oil referred to as AOCED, produced via fermentation by a strain of *Schizochytrium sp.* in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate established specifications as presented in the supporting dossier *["The Safety and Generally Recognized as Safe (GRAS) Status of Algal Oil produced by Schizochytrium sp. As a Source of DHA and EPA in Food for Cats"]* is safe for consumption at the maximum use levels specified in diets of cats. The AOCED will be present in the diet of cats at no more that 1.5% of the diet in both extruded (dry) food and canned (wet) food.

We, the members of the GRAS Panel, further unanimously conclude that the intended use of this algal oil referred to as AOCED, produced via fermentation by a strain of *Schizochytrium sp.* in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate established specifications as presented in the supporting dossier is Generally Recognized as Safe (GRAS) based on scientific procedures, under the intended conditions of use in the diet of cats at no more that 1.5% of the diet in both extruded (dry) food and canned (wet) food.

It is our opinion that other qualified experts would concur with this conclusion.

Michael Pariza, Ph.D.
(Panel Member)

Date

Michael Carakostas., DVM, Ph.D.
(Panel Member)

Date

Stanley M. Tarka, Jr., Ph.D., F.A.T.S.
The Tarka Group, Inc.(Chair),
The Pennsylvania State University College of Medicine

17 March 2020

Date

Annexes

(b) (4)

DSM NUTRITIONAL PRODUCTS/MARTEK
ATTN: COLEMAN BRAXTON
1416 N. WILLIAMSBURG COUNTY HWY
KINGSTREE, SC 29556

Reporting Date 11/08/2018

REPORT OF ANALYSIS (b) (4)

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Client Sample Code: VY00014962

(b) (4)

REPORT OF ANALYSIS

(b) (4)

Test Results	Result
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Client Sample Code: VY00014962

REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00014962

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REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00014962

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REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00014962

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REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00014962

(b) (4)

REPORT OF ANALYSIS

(b) (4)

Test Results **Result**

Respectfully Submitted,
(b) (4) Central Analytical Laboratories

(b) (6)

(b) (4), (b) (6) Analytical Service Manager

Results shown in this report relate solely to the item submitted for analysis.
Uncertainty can be obtained upon request.

ACCREDITED

TESTING CERT
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DSM NUTRITIONAL PRODUCTS/MARTEK
ATTN: COLEMAN BRAXTON
1416 N. WILLIAMSBURG COUNTY HWY
KINGSTREE, SC 29556

Reporting Date 11/08/2018

REPORT OF ANALYSIS

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Sample Code (b) (4)

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Client Sample Code: VY00015240

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REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00015240

REPORT OF ANALYSIS

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Test Results	Result

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Client Sample Code: VY00015240

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REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00015240

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REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00015240

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REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00015240

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REPORT OF ANALYSIS

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Test Results	Result

Respectfully Submitted,

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Results shown in this report relate solely to the item submitted for analysis.
Uncertainty can be obtained upon request.

ACCREDITED

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Reporting Date 11/08/2018

REPORT OF ANALYSIS (b) (4)

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Client Sample Code: VY00015465

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REPORT OF ANALYSIS

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Client Sample Code: VY00015465

REPORT OF ANALYSIS

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Test Results	Result
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Client Sample Code: VY00015465

REPORT OF ANALYSIS

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Test Results	Result

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Client Sample Code: VY00015465

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REPORT OF ANALYSIS

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Test Results	Result
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(b) (4)

Client Sample Code: VY00015465

(b) (4)

REPORT OF ANALYSIS

AR-16-QA-048471-03

This analytical report supersedes AR-16-QA-048471-02.

Test Results	Result
* OctaCDF	(b) (4)
* WHO(2005)-PCDD/F TEQ (lower-bound)	
* WHO(2005)-PCDD/F TEQ (upper-bound)	

A7347 - Dioxin-like PCBs (12 WHO-PCBs)
Completion Date: 09/15/2016 **Method:** EC Reg 589/2014 (food) and EC Reg 709/2014 (feed)

* PCB 77	(b) (4)
* PCB 81	
* PCB 105	
* PCB 114	
* PCB 118	
* PCB 123	
* PCB 126	
* PCB 156	
* PCB 157	
* PCB 167	
* PCB 169	
* PCB 189	
* WHO(2005)-PCB TEQ (lower-bound)	
* WHO(2005)-PCB TEQ (upper-bound)	

GF004 - WHO-PCDD/F+PCB TEQ
Completion Date: 09/15/2016 **Method:** EC Reg 589/2014 (food) and EC Reg 709/2014 (feed)

* WHO(2005)-PCDD/F+PCB TEQ (lower-bound)	(b) (4)
* WHO(2005)-PCDD/F+PCB TEQ (upper-bound)	

**This is not covered by our current A2LA accreditation.*

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

[redacted]

[redacted]

Client Sample Code: VY00015465

[redacted]

REPORT OF ANALYSIS

AR-16-QA-048471-03

This analytical report supersedes AR-16-QA-048471-02.

Test Results	Result

Respectfully Submitted,

[redacted]

[redacted]

[redacted] Analytical Service Manager

Results shown in this report relate solely to the item submitted for analysis.
Uncertainty can be obtained upon request.

ACCREDITED

[redacted]

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

(b) (4)

Reporting Date 11/08/2018

(b) (4)

AR-16-QA-049459-04

REPORT OF ANALYSIS

This analytical report supersedes AR-16-QA-049459-03.

Sample Code 468-2016-0909B132

Sample Description VY00015572
Client Sample Code VY00015572
Sample Reference 5015816001

Reception Date 09/09/2016
Reception Temperature -41 (Celsius)
Sample Condition Acceptable
Purchase Order 4701594417

Test Results

Result

QA377 - Moisture (Air Oven 135C 2 hrs)
Completion Date: 09/12/2016 **Method:** AOAC 930.15
* Moisture

QA271 - Crude Fat (Acid Hydrolysis)
Completion Date: 09/14/2016 **Method:** AOAC 954.02
* Crude Fat

QA270 - Total Fat (as FAME)
Completion Date: 09/16/2016 **Method:** AOAC 996.06
* FAME total (%fat)

QA133 - Arsenic (ICP-MS)
Completion Date: 09/16/2016 **Method:** AOAC 2013.06
Arsenic (As)

QA205 - Cadmium (ICP-MS)
Completion Date: 09/16/2016 **Method:** AOAC 2013.06
Cadmium (Cd)

(b) (4)

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation

(b) (4)

(b) (4)

Client Sample Code: VY00015572

(b) (4)

REPORT OF ANALYSIS

AR-16-QA-049459-04

This analytical report supersedes AR-16-QA-049459-03.

Test Results	Result
QA230 - Copper (ICP-AES) **Completion Date:** 09/14/2016 **Method:** AOCS Ca 17-01 * Copper (Cu)	(b) (4)
QA278 - Iron (ICP-AES) **Completion Date:** 09/14/2016 **Method:** AOCS Ca 17-01 * Iron (Fe)	
QA419 - Lead (ICP-AES) **Completion Date:** 09/14/2016 **Method:** AOCS Ca 17-01 * Lead (Pb)	
QD610 - Mercury (ICP-MS) **Completion Date:** 09/16/2016 **Method:** AOAC 2013.06 Mercury (Hg)	
QA01L - p-Anisidine Value **Completion Date:** 09/12/2016 **Method:** AOCS Cd 18-90 p-Anisidine Value	
QA282 - Free Fatty Acid, as Oleic **Completion Date:** 09/12/2016 **Method:** AOCS Ca 5a-40 Free fatty acids as oleic acid	
QA328 - Insoluble Impurities **Completion Date:** 09/12/2016 **Method:** AOCS Ca 3a-46 Insoluble impurities	
QA00F - Peroxide Value **Completion Date:** 09/12/2016 **Method:** AOCS Cd 8-53 Peroxide value	
QA188 - Eicosapentaenoic Acid (EPA) **Completion Date:** 09/16/2016 **Method:** AOCS Ce 1b-89 C 20:5n3 Eicosapentaenoic acid	

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

(b) (4)

(b) (4)

Client Sample Code: VY00015572

(b) (4)

REPORT OF ANALYSIS

AR-16-QA-049459-04

This analytical report supersedes AR-16-QA-049459-03.

Test Results	Result
QA196 - Docosahexaenoic Acid (DHA)	
Completion Date: 09/16/2016 **Method:** AOCS Ce 1b-89	
DHA Docosahexaenoic acid C22:6	(b) (4)
QA01N - Fatty Acid Profile (relative area %)	
Completion Date: 09/16/2016 **Method:** AOCS Ce 1b-89	
C 6:0 (Caproic acid)	
C 8:0 (Caprylic acid)	
C 10:0 (Capric acid)	
C 12:0 (Lauric acid)	
C 13:0 (Tridecanoic acid)	
C 14:0 (Myristic acid)	
C 14:1 (Myristoleic acid)	
C 15:0 (Pentadecanoic acid)	
C 15:1 (Pentadecenoic acid)	
C 16:0 (Palmitic acid)	
C 16:1 (Palmitoleic acid)	
C 16:2 (Hexadecadienoic acid)	
C 16:3 (Hexadecatrienoic acid)	
C 16:4 (Hexadecatetraenoic acid)	
C 17:0 (Margaric acid)	
C 17:1 (Margaroleic acid)	
C 18:0 (Stearic acid)	
C 18:1n9 (Oleic acid)	
C 18:1n7 (Vaccenic acid)	
C 18:2n6 (Linoleic acid)	
C 20:0 (Arachidic acid)	
C 18:3n6 (gamma-Linolenic acid)	
C 20:1 (Eicosenoic acid)	
C 20:1n9 (Eicosenoic acid)	
C 18:3n3 (alpha-Linolenic acid)	
C 21:0 (Heneicosanoic acid)	
C 18:4n3 (Stearidonic acid)	
C 20:2n6 (Eicosadienoic acid)	

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

[redacted]

[redacted]

Client Sample Code: VY00015572

[redacted]

REPORT OF ANALYSIS

AR-16-QA-049459-04

This analytical report supersedes AR-16-QA-049459-03.

Test Results	Result
C 22:0 (Behenic acid)	[redacted]
C 22:1n9 (Erucic acid)	
C 22:1n11 (Cetoleic acid)	
C 20:3n6 (homo-gamma-Linolenic acid)	
C 20:3n3 (Eicosatrienoic acid)	
C 20:4n5 (Eicosatetraenoic)	
C 20:4n6 (Arachidonic acid)	
C 23:0 (Tricosanoic acid)	
C 22:2n6 (Docosadienoic acid)	
C 20:4n3 (Eicosatetraenoic acid)	
C 22:4n3 (Eicosatetraenoic acid)	
C 24:0 (Lignoceric acid)	
C 20:5n3 (Eicosapentaenoic acid)	
C 24:1 (Nervonic acid)	
C 21:5n3 (Heneicosapentaenoic acid)	
C 22:4n6 (Docosatetraenoic acid)	
C 22:5n6 (Docosapentaenoic acid)	
C 22:5n3 (Docosapentaenoic acid)	
C 22:6n3 (Docosahexaenoic acid)	
Unknown Components	

QA052 - Polychlorinated Biphenyls (Oils & Fats)
Completion Date: 09/14/2016 **Method:** ASU L00.00-34

* PCB 1	
* PCB 8	
* PCB 18	
* PCB 28	
* PCB 29	
* PCB 44	
* PCB 50	
* PCB 52	
* PCB 66	
* PCB 77	
* PCB 87	
* PCB 101	

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

[redacted]

[redacted]

[redacted]

Client Sample Code: VY00015572

[redacted]

REPORT OF ANALYSIS

AR-16-QA-049459-04

This analytical report supersedes AR-16-QA-049459-03.

Test Results	Result
* PCB 104	[redacted]
* PCB 105	
* PCB 118	
* PCB 126	
* PCB 128	
* PCB 138	
* PCB 153	
* PCB 170	
* PCB 180	
* PCB 187	
* PCB 188	
* PCB 195	
* PCB 201	
* PCB 206	
* PCB 209	
* Total PCB	

A7158 - Dioxins and Furans: PCDD/F (17 Congeners)

Completion Date: 09/21/2016 **Method:** EC Reg 589/2014 (food) and EC Reg 709/2014 (feed)

* 2,3,7,8-TetraCDD	[redacted]
* 1,2,3,7,8-PentaCDD	
* 1,2,3,4,7,8-HexaCDD	
* 1,2,3,6,7,8-HexaCDD	
* 1,2,3,7,8,9-HexaCDD	
* 1,2,3,4,6,7,8-HeptaCDD	
* OctaCDD	
* 2,3,7,8-TetraCDF	
* 1,2,3,7,8-PentaCDF	
* 2,3,4,7,8-PentaCDF	
* 1,2,3,4,7,8-HexaCDF	
* 1,2,3,6,7,8-HexaCDF	
* 1,2,3,7,8,9-HexaCDF	
* 2,3,4,6,7,8-HexaCDF	
* 1,2,3,4,6,7,8-HeptaCDF	
* 1,2,3,4,7,8,9-HeptaCDF	

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

[redacted]

[redacted]

Client Sample Code: VY00015572

[redacted]

REPORT OF ANALYSIS

AR-16-QA-049459-04

This analytical report supersedes AR-16-QA-049459-03.

Test Results	Result
* OctaCDF	[redacted]
* WHO(2005)-PCDD/F TEQ (lower-bound)	
* WHO(2005)-PCDD/F TEQ (upper-bound)	

A7347 - Dioxin-like PCBs (12 WHO-PCBs)
Completion Date: 09/21/2016 **Method:** EC Reg 589/2014 (food) and EC Reg 709/2014 (feed)

* PCB 77	[redacted]
* PCB 81	
* PCB 105	
* PCB 114	
* PCB 118	
* PCB 123	
* PCB 126	
* PCB 156	
* PCB 157	
* PCB 167	
* PCB 169	
* PCB 189	
* WHO(2005)-PCB TEQ (lower-bound)	
* WHO(2005)-PCB TEQ (upper-bound)	

GF004 - WHO-PCDD/F+PCB TEQ
Completion Date: 09/21/2016 **Method:** EC Reg 589/2014 (food) and EC Reg 709/2014 (feed)

* WHO(2005)-PCDD/F+PCB TEQ (lower-bound)	[redacted]
* WHO(2005)-PCDD/F+PCB TEQ (upper-bound)	

**This is not covered by our current A2LA accreditation.*

Comments Sum of EPA+DHA = 525 mg/g

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

[redacted]

[redacted]

Client Sample Code: VY00015572

[redacted]

REPORT OF ANALYSIS

AR-16-QA-049459-04

This analytical report supersedes AR-16-QA-049459-03.

Test Results	Result

Respectfully Submitted,

[redacted]

[redacted] Analytical Service Manager

Results shown in this report relate solely to the item submitted for analysis.
Uncertainty can be obtained upon request.

[redacted]

Any opinions/interpretations expressed on the Report of Analysis are outside the scope of this lab's A2LA accreditation.

(b) (4)

From: (b) (4)

To:

Subject: Toxicity Study on Orca using *Artemia nauplii* to Detect Presence/Absence of Prymnesin Toxin

Date: December 21, 2009

Summary

Orca strain is a new species of Schizochytrium. While no evidence from the literature indicates prymnesin toxin potential Schizochytrium sp, artemia bioassay was performed to confirm absence of prymnesin toxin by Orca. Multiple experiments using different fermentation broths were performed and it was concluded that Orca strain exhibited no evidence of prymnesin toxin production.

Introduction

Prymnesin toxins are acidic polar phosphor-proteolipids limited to two species of *Prymnesium* (*P. parvum* and *P. patelliferum*). These toxins exhibit a broad spectrum of activity including lethal effects on gill breathing animals, cytotoxic effects on erythrocytes, mammalian cells, protozoa and bacteria. The major economic impact of prymnesin toxins to humans to date has been related to fish kills in aqua-culture ponds. All gill breathing animals tested to date have proved sensitive to prymnesin toxin. As a result, a sensitive toxicity test for prymnesin toxin has been developed using nauplii of the brine shrimp *Artemia* (Larsen et. al. 1993).

According to taxonomic system of Cavalier-Smith (1981, 1983, 1993), in Chromista kingdom, Thraustochytriales (including *Schizochytrium* sp.) are found in separate division (Heterokonka) than the known prymnesin toxic producers (found in the Prymnesiophyta division). Furthermore, in the four orders of Prymnesiophyta, all known toxin producers are found in only one order, Prymnesiales. Orca strain was identified as a new species of *Schizochytrium* and no evidence from the literature indicates prymnesin toxin potential in *Schizochytrium* sp. However, the artemia bioassay was performed to confirm absence of prymnesin toxin.

Methods

The overall experiment and more precisely the cells/ml were based on two previously published papers:
1) Levels Toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta)
A. HOUDAN*, A. BONNARD, J. FRESNEL, S. FOUCHARD, C. BILLARD AND I. PROBERT
JOURNAL OF PLANKTON RESEARCH 26: 875–883 (2004)
2) Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions; Edna Granéli , Niclas Johansson; Harmful Algae 2: 135–145 (2003)

Orca Preparation

(b) (4)

(b) (4)

(b) (4)

Toxicity Test

- A volume of 10 mL 25 ppt Instant Ocean was added to a 60x15 mm sterile petri dish.
- 10 artemia nauplii were added to each petri dish.
- For each condition, a target number of cells were added while the control contained only the artemia in the 25 ppt Instant Ocean and did not include any Orca cells. Each condition was run in five replicates and the plates were randomized to avoid bias.
- The plates were incubated at 25°C and observed after approximately 24 hours and 48 hours. The number of dead artemia were counted and tabulated.
- The 25°C was chosen as the experimental temperature based on 1) This was the growth condition stated in the Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions; Edna Granéli , Niclas Johansson; Harmful Algae 2: 135–145 (2003) 2) It was believed best to keep the nauplii growth conditions the same as used to hatch the cysts. A temperature of 17°C was used in "Levels Toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta)", Houdan et. al., sited earlier.

Results and Discussion

Three different Orca fermentation broths were used for toxicity studies and some broths were retested to confirm the results. Each study contained a control with no Orca broth addition and each control exhibited numberous deaths. The most basic explanation is starvation. As cysts are hatched they rely on a yolk sac to sustain them for the first 48 hours. After 48 hours the newly hatched artemia begin to consume outside nutrients for substance. If food has not been provided within the 48 hour allotment of the experiment it is not unreasonable for the artemia to begin dying. As each artemia hatches and matures at a slightly different pace it would also follow that they will die at differing times. The mortality of each experimental condition was compared to the experimental control. If an experimental condition had mortality comparable to or lower than the control, the condition would be considered non-toxic. The experimental results are discussed below.

1. Artemia Feeding Trial using 10L Fermentation Broth NBS1- Nx1020et09(1-4)

A broth sample from the 10L fermentation run NBS1 Nx1020et09(1-4) was obtained from the (b) (4) . The cell concentrations for each condition and the experimental results are summarized below.

(b) (4)

10/28/09 Artemia Feeding Trial

Fermentation broth from lab fermentation lot #Nx 1020et09 (1-4) was tested on 10/28/09 using the procedures described in the Methods section. The cell concentrations and results are summarized in the table below.

24 hr	1	2	3	4	5	Total dead	avg	
Control	0	0	2	0	1	(b) (4)	0.6	control
A					(b) (4)		1.4	0.025ml in 10ml= $9*10^4$ cells/ml
D							2.8	0.05ml in 10ml= $1.8*10^5$ cells/ml
E							4.8	0.1 ml in 10ml= $3.6*10^5$ cells/ml
C							2.8	0.25 ml in 10 ml= $9*10^5$ cells/ml
B							0.8	0.5 ml in 10 ml= $1.8*10^6$ cells/ml

48 hr	1	2	3	4	5	Total dead	avg	
Control	4	3	3	1	2	13	2.6	control
A					(b) (4)	(b) (4)	3	0.025ml in 10ml= $9*10^4$ cells/ml
D							4.4	0.05ml in 10ml= $1\ 8*10^5$ cells/ml
E							5.4	0.1 ml in 10ml= $3.6*10^5$ cells/ml
C							3.4	0.25 ml in 10 ml= $9*10^5$ cells/ml
B							2.2	0.5 ml in 10 ml= $1.8*10^6$ cells/ml

Based on the experimental results, there was no clear correlation between Orca cell concentrations and average artemia death. Therefore, the experiment yielded inconclusive results. Additional experiments were performed to determine the Orca toxicity to artemia.

2. Artemia Feeding Trials Using Production Run F884 (Pasteurized Broth)

A frozen pasteurized broth sample from production run #F884 was obtained from (b) (4). The broth was thawed in hot water and then used for the experiments on 11/4/09. The broth was refrozen and retested on 11/11/09. The cell concentrations for each condition and the experimental results are summarized below.

11/04/09 Artemia Feeding Trial

-ORCA cell count- $3.97x\ 10^8$ cells/ml

		dH2O added
Control-	No ORCA adds.	
E	0.45 ml in 10 ml= $1.8*\ 10^7$ cells/ml	
B	0.225 ml in 10 ml= $9*10^6$ cells/ml	225ul
D	0.09 ml in 10ml= $3.6*10^6$ cells/ml	360ul
A	0.045ml in 10ml= $1.8*10^6$ cells/ml	405ul
C	0.0225ml in 10ml= $9*10^5$ cells/ml	449ul

[redacted] (b) (4)

5-Nov	1	2	3	4	5	Total dead	Avg dead per plate	Volume of ORCA add
Control	1	2	0	0	2	5	1	0
C	[redacted] (b) (6)						0.4	0.0225 ml
A							1	0.045 ml
D							0	0.09 ml
B							0.2	0.225 ml
E							0.6	0.45 ml

6-Nov	1	2	3	4	5	Total dead	Avg dead per plate	Volume of ORCA add
Control	1	4	0	0	4	9	1.8	0
C	[redacted] (b) (6)						1	0.0225 ml
A							1	0.045 ml
D							1	0.09 ml
B							0.8	0.225 ml
E							0.8	0.45 ml

This feeding trial shows no adverse effects on artemia at a wide range of ORCA concentrations.

11/11/09 Artemia Feeding Trial

The above experiment was repeated using the same broth on 11/11/09, Fermentation lot #F-884. The cell concentrations and results are summarized in the tables below.

-ORCA cell count- 2.81x 10^8 cells/ml

Control- No ORCA adds.

E 0.03ml in 10ml= $9*10^5$ cells/ml

A 0.064ml in 10ml= $1.8*10^6$ cells/ml

B 0.128 ml in 10ml= $3.6*10^6$ cells/ml

C 0.32 ml in 10 ml= $9*10^6$cells/ml

D 0.64 ml in 10 ml= $1.8* 10^7$cells/ml

12-Nov	1	2	3	4	5	Total dead	Avg dead per plate	Volume of ORCA add
Control	1	0	0	0	0	1	0.2	na
E	[redacted] (b) (4)						0.8	0.03 ml
A							0.8	0.064ml
B							0	0.128 ml
C							0.5	0.32 ml
D							0.2	0.64 ml

13-Nov	1	2	3	4	5	Total dead	Avg dead per plate	Volume of ORCA add
Control	1	0	1	2	1	5	1	na
E	[redacted] (b) (4)						0.8	0.03 ml
A							1	0.064ml
B							0.6	0.128 ml
C							1	0.32 ml
D							0.5	0.64 ml

[redacted] (b) (4)

The results obtained were similar to those found in the 11/4/09 experiment.
Based on the two trials, broth from production run F884 exhibited no adverse effects on artemia at a wide range of ORCA concentrations.

3. Artemia Feeding Trials Using 10L Fermentation Broth NBS1 Nx1105et09(1-4)

A broth sample from the 10L fermentation run NBS1 1 Nx1105et09(1-4) was obtained from the [redacted] (b) (4) The broth was used for the artemia feeding trials on 11/18/09, 11/23/09 and 12/09/09, and the broth was frozen in between test dates. The cell concentrations for each condition and the experimental results are summarized below.

Artemia Feeding Trial- ORCA Strain- 11/18/09

- Fermentation lot #Nx1105et09(1-2) [NBS1] was tested on 11/18/09.

-ORCA cell count- 2.2x 10^7 cells/ml

Control-	No ORCA adds.
E	0.04ml in 10ml= $9*10^4$ cells/ml
A	0.08ml in 10ml= $1.8*10^5$ cells/ml
B	0.16 ml in 10ml= $3.6*10^5$cells/ml
C	0.41 ml in 10 ml= $9*10^5$ cells/ml

No dH2O was added to normalize the total volume added to each plate.

19-Nov	1	2	3	4	5	Total dead	Avg dead per plate	Volume of ORCA add
Control	2	1	1	1	2	7	1.4	
E	[redacted]					(b) (4)	1	0.04 ml
A							0.6	0.08 ml
B							1.4	0.16 ml
C							1.2	0.41 ml
D							1.4	0.81 ml
20-Nov	1	2	3	4	5	Total dead	Avg dead per plate	Volume of ORCA add
Control	4	4	1	2	3	14	2.8	
E	[redacted]					(b) (4)	2.2	0.04 ml
A							0.8	0.08 ml
B							2.4	0.16 ml
C							2.2	0.41 ml
D							1.8	0.81 ml

*** At 4:30 pm on 11/19/09 I came in and found the incubator power turned off and the temperature was down to 21.5 C.***

This feeding trial shows no adverse effects on artemia at a wide range of ORCA concentrations.

(b) (4)

[redacted]

Artemia Feeding Trial- ORCA Strain- 11/23/09

- Fermentation lot #Nx1105et09(1-4) [NBS1] was tested on 11/23/09.

-ORCA cell count- 2.03x 10^7 cells/ml

Control- No ORCA adds.

B 0.91ml in 10 ml= 1.8* 10^6 cells/ml

E 0.44ml in 10 ml= 9*10^5 cells/ml

C 0.18 ml in 10ml= 3.6*10^5cells/ml

D 0.09ml in 10ml= 1.8*10^5 cells/ml

A 0.04ml in 10ml= 9*10^4 cells/ml

No dH2O was added to normalize the total volume added to each plate.

24-Nov	1	2	3	4	5	Total dead	Avg dead per plate	Volume of ORCA add
Control	0	0	1	0	0	1	0.2	
A	[redacted]					(b) (4)	0.4	40 ul
B							0.2	910 ul
C							0	180 ul
D							0.2	90 ul
E							0	440 ul

25-Nov	1	2	3	4	5	Total dead	Avg dead per plate	Volume of ORCA add
Control	0	1	2	0	0	3	0.6	
A	[redacted]					(b) (4)	0.2	40 ul
B							0.8	910 ul
C							0.6	180 ul
D							1.2	90 ul
E							1.2	440 ul

*** At 4:30 pm on 11/19/09 I came in and found the incubator power turned off and the temperature was down to 21.5 C.***

Based on the experimental results, there was no clear correlation between Orca cell concentrations and average artemia death. Also to be noted is a lower than usual number of the control artemia died makes for a questionable experiment. Therefore, the experiment yielded inconclusive results. A third repeat of this broth is planned for the week of 12/07/09.

(b) (4)

Artemia Feeding Trial- ORCA Strain- 12/09/09

- Fermentation lot #Nx1105et09(1-4) [NBS1] was tested on 12/09/09.

-ORCA cell count- 4.51x 10^7 cells/ml										
	Control-	No ORCA adds.								
	C	0.02ml in 10ml= 9*10^4 cells/ml (20uL)				**No dH2O was added to normalize the total				
	E	0.04ml in 10ml= 1.8*10^5 cells/ml (40uL)				volume added to each plate.**				
	A	0.08 ml in 10ml= 3.6*10^5cells/ml (80 uL)								
	D	0.20 ml in 10 ml= 9*10^5 cells/ml (200 uL)								
	B	0.4ml in 10 ml= 1.8* 10^6 cells/ml (400uL)								

10-Dec		1	2	3	4	5	Total dead		Avg dead per plate		Volume of ORCA add
	Control	0	2	0	2	1	5		1		
	C						(b) (4)		0.2		20uL
	E								1.2		40 uL
	A								1.6		80 uL
	D								0.6		200 uL
	B								1.6		400 uL
11-Dec		1	2	3	4	5	ad		Avg dead per plate		Volume of ORCA add
	Control	1	4	2	3	3	13		2.6		
	C						(b) (4)		0.4		20uL
	E								1.6		40 uL
	A								1.8		80 uL
	D								1.2		200 uL
	B								1.8		400 uL

This feeding trial shows no adverse effects on artemia at a wide range of ORCA concentrations.

Based on the three trials, broth from run Nx1105et09(1-4) exhibited no adverse effects on artemia at a wide range of ORCA concentrations. The inconclusive results observed in the 11/23/09 experiment were not dose responsive, hence points to experimental variability.

Statistical Analysis

Statistcal analysis was performed using the artemia testing results above (see report " Results of Toxicity Study", Tracy Brown to Paul Behrens, Susan Cheney, Tracey Stahl, Erin Sylvester and Nathan Yang, dated Jaunuary 15, 2010). ANOVA and Dunnett's Comparison was used to determine if the mean number of dead Artemia in the Control plates was statistically different than the mean number of dead Artemia in the plates containing Orca. Analysis was conducted for all trials at 24 hours and 48 hours with a 5% risk. The conclusions are summarized below.

- Results from the trial performed October 28th indicate conditions C, D and E are statistically different than the Control at 24 hours. At 48 hours, condition E is statistically different than the Control.
- Results from the trials performed November 4th, November 11th and November 23rd indicate all conditions are statistically equal to the corresponding Control at 24 hours and 48 hours.
- Results from the trial performed November 18th indicate all conditions are statistically equal to the Control at 24 hours. At 48 hours, condition A is statistically different than the Control. Although statistically significant, condition A has a lower average compared to the Control (0.8 vs. 2.8), indicating Orca did not have an adverse affect on the Artemia.
- Results from the trial performed December 9th indicate all conditions are statistically equal to the Control at 24 hours. At 48 hours, condition C is statistically different than the Control. Although statistically significant, condition C has a lower average compared to the Control (0.4 vs. 2.6), indicating Orca did not have an adverse affect on the Artemia

Conclusion

Based on the results and discussion above, it was concluded that Orca strain exhibited no evidence of prymnesin toxin production.

[redacted]

Domoic Acid Data Report

Project: [redacted]

Date: 1/15/2010
Report Prepared by: [redacted]
Samples Submitted by: [redacted], [redacted]

Sample Identification:

NB61105et09
NBx1220dt09

Toxins: Domoic Acid (DA)

Toxin extraction: Vials received 1/7/2010 were stored in the fridge at 20°C until they were emptied into weigh boats and mixed in preparation of extraction. Dried material was extracted in 1.0 gram subsets. The sample NBx1220dt09 was less dense than the material from NB61105et09, so extraction solution volumes were doubled for NBx1220dt09. NB61105et09 was extracted with10 mL of acidified methanol-water solution (75%-25%) while NBx1220dt09 was extracted with 20 mL. Duplicate spiked samples were also prepared prior to extraction with the addition of a Domoic Acid certified standard provided by the National Research Council of Canada. A spiked sample for NB61105et09 was prepared with the addition of 0.01 µg Domoic Acid (DA) to the dried material and NBx1220dt09 was spiked with 0.1 µg DA. After the addition of acidified MeOH, samples were vortexed then sonicated via water bath for 25 minutes. The samples were then centrifuged @ 3,000 RPM for 10 minutes and the supernatant retained. Extraction on the pellet was repeated two additional times with 75% acidified methanol. The pooled supernatant was filtered through GF/C and blown to dryness under a stream of nitrogen at 60°C. Samples and spiked samples were then reconstituted at a concentration of 1g/mL in 10% acetonitrile.

An initial extraction of NBx1220dt09 resulted in a positive response (~40 ng/g DA) with the Domoic Acid Enzyme Linked Immunosorbent Assay (ELISA). This could not be confirmed with Liquid Chromatography-Mass Spectrometry, so the sample was re-extracted as detailed above, and further cleanup using Solid Phase Extraction was employed. Strata-X 200 mg Polymeric Reversed Phase SPE (Phenomenex) was conditioned and equilibrated with methanol and DI water, loaded, rinsed and eluted with 100% MeOH. The resultant eluant was blown to dryness, reconstituted in 10% acetonitrile and diluted appropriately for analysis. Samples were analyzed via ELISA (Biosense) in duplicate after sufficient dilutions of extract were made using the kit protocol.

[redacted]

[redacted] (b) (4)

Analytical Methodology: A Domoic Acid ELISA (Biosense) was utilized for the quantitative detection of DA. The current assay is sensitive down to a detection/quantification limit of 0.5 ng/g for DA as determined from spike recoveries, dilution factors, and kit sensitivity. The standard recoveries were 88% and 97%.

Summary of Results

Sample	DA levels (ng/g)
NB61105et09	ND
NBX1220dt09	ND

ND = Not detected above the detection limit
Detection/Quantification Limit = 0.5 ng/g
Sample Spikes = 10 & 100 ng/g
Standards = 0.5 & 1.0 ng/mL

Data Interpretation: Although an initial extraction of NBx1220dt09 resulted in a positive response with ELISA, an additional extraction employing Solid Phase Extraction resulted in acceptable spike recovery (76%) and a non-detect when re-analyzed. The initial results appear to be due to matrix interference, as indicated by the inability to detect domoic acid via LC/MS. Domoic acid was not detected in either sample above our detection limit of 0.5 ng/g.

Submitted by: [redacted] (b) (6)

[redacted] (b) (4), Ph.D.

Date: 1/15/10

[redacted] (b) (4)

(b) (4)

(b) (4)

To: Jan 12, 2010

Cc:

From:

Subject: Domoic Acid Analysis of Orca Biomass by Monsanto Method

Summary

Previously, we determined the amount of domoic acid in Orca oils with an independent method using LC-MS (see report from (b) (4) "Domoic Acid in Bell and Orca" on 12/4/2009). Here, the domoic acid was quantified in one Orca biomass (NB61105et09) using Monsanto Corporate Research's method (code: AASC-SOP-96-0010) which was derived from AOAC official Method 991.26 using HPLC/diode array detector. The Orca biomass sample was found to have less than 0.5 ppm domoic acid.

Experimental

Reagent preparation and standard calibration

Domoic Acid Stock Solution: 5 mg of domoic acid (90%) was dissolved in 100 mL volumetric flask with 0.1 N HCl. The stock concentration is 0.045 mg/mL = 45 ppm.

Domoic Acid Standard Calibration: Using stock solution to make 5, 4, 2, 1, 0.5, 0.05 ppm domoic acid for standard calibration. Each sample was injected into the HPLC system to get the area reading.

Sample preparation

A) Hexane Wash:
About sixty grams of Orca biomass were treated with 10 mL/g hexane wash in a 1 L beaker. The sample was ultrasonicated for 10 minutes followed by filtration. The hexane filtrate was discarded and the biomass powder was dried under vacuum overnight for the neat biomass extraction.

B) Biomass Spike recoveries:
Five grams of biomass was spiked with 0.5 ppm of domoic acid by adding 250 µL of 10 ppm domoic acid stock solution. The sample is rolled to mix the domoic acid uniformly and dried overnight under vacuum for the neat biomass extraction step.

C) Neat Biomass Extraction:
Two portions of 2.5 grams of biomass (regular and spiked) were weighed into two 25 mL glass tubes and heated to 80-90˚C in the heating block for 5 minutes with 10 mL of 0.1N HCl. Transfer the extract into a 50 mL volumetric flask with 0.1 N HCl and cooled in an ice bath. Each of the samples was then transferred into 50 mL Falcon tube for 2500 rpm centrifugation for 10 minutes. The supernatants were filtered through 0.45 µm syringe filters into autosampler vials for HPLC analysis. All samples were prepared in duplicate and labeled as Orca, Orca-spiked, Orca Dup, and Orca Dup-spiked.

HPLC instrumental parameters

System: Waters Acquity UPLC®
Column: Waters Atlantis dC18 (part # 186001346), 4.6×250 mm, 5µm, ambient temperature

Flow rate: 1 mL/min
Injection volume: 20 µL
Detection: Waters Photodiode Array Detector, 235-245 nm, UV.
Mobile phase: A. 100% water with 0.2% phosphoric acid
B. 100% acetonitrile with 0.2% phosphoric acid
Gradient

Time (min)	% A	% B
0	100	0
5	100	0
20	88	12
30	88	12
31	10	90
35	10	90
35.1	100	0
45	100	0

Results and Discussion

The linear regressions for the calibration curve have exceeded 0.999. The instrument detection limit was 0.05ppm; since the sample was diluted 10-fold during the extraction, the method detection limit for domoic acid was 0.5ppm.

Under this condition, domoic acid eluted out from the column at around 27 min. From the HPLC chromatogram, there was no peak observed for un-spiked Orca sample (Figure 1). We concluded that domoic acid was not detected in Orca NB61105et09 at the method detection limit of 0.5 ppm.

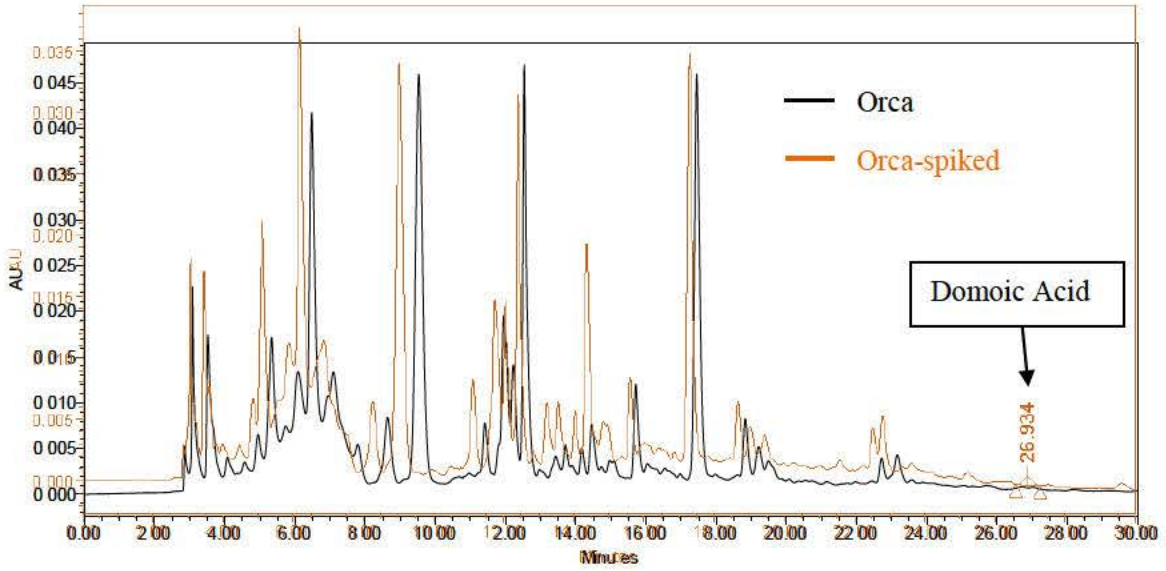

Figure 1. The Orca sample showed no domoic acid above method detection limit (0.5 ppm). The peak for Orca was too small to be integrated and quantitated.

DSM BRIGHT SCIENCE. BRIGHTER LIVING.	MATERIAL CONTROL SPECIFICATION	Page 1 of 2
	Document Name: MCS.5070266BAG.03	Product Code: (b) (4)

Raw Material: Sodium Chloride Granular - MCS

Section 1. RECEIVING / RECEIVING INSPECTION

Approved Manufacturer(s):

- Avantor Performance Materials (Doe and Ingalls), Part Number N/A: Description: Sodium Chloride
- Morton Salt, Part Number N/A: Description: Sodium Chloride

Date Received:	**Quantity Received:**	**Manufacturer Batch Number:**

Packing List Received matches PO and material identification o YES o NO	**COA Received?** o YES -- Attach to MCS o NO
Kosher Group 1 (Kosher Marking Not Required) o YES o NO o N/A	**Shipping Criteria:** o Acceptable o Damaged
Temperature abuse evident? o YES o NO	**Truck seals intact & match Truck Bill (Fwd seals to Lab with COA)?** o YES o NO o N/A
Shortage of more than 1% (Bulk only)? o YES o NO o N/A	**Evidence of moisture, insects, rodents, spillage, foreign odors, or other concern?** o YES o NO **If YES, explain:**_____
Materials require allergen labeling according to plant Allergen Control Policy. Labels applied? o YES o NO o N/A	**Note:** if one of the above is nonconforming, initiate nonconformance and forward to QA.
Inspected By:	**Date:**

Section 2. SAMPLE CONTROL

Sample Use	Sample Quantity	Packaging	Storage	Shipped To
NA	NA	NA	NA	NA

Section 3. MANUFACTURE DATE & EXPIRATION

Manufacture Date:	**Expiration Date:**

Section 4. PRODUCT PACKAGING, LABELING & STORAGE

Batch Number: N / A	**Container or Packaging Instructions:** 50 lb. bag	
Labeling Instructions: 1. Label as "Quality Inspection, as needed"	**Storage Condition:**	Keep in a tightly closed container, stored in a cool, dry, ventilated area. (per Manufacturer's instructions)
	Product Shelf Life:	2 years from date of manufacture

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Section 5. QUALITY CONTROL

Test	Responsible Party	Method No.	Result	Specification	Initial / Date
Assay	Manufacturer	N/A	Attach COA	9.0 – 100.5%	
Identification • Sodium • Chloride		USP		Passes Test	
Acidity/Alkalinity				Passes Test	
Loss on drying		N/A		Max. 0.5%	
Bromides				Max. 0.010%	
Phosphates				Max. 0.0025%	
Potassium				Max. 0,05%	
Iodides		USP		Passes Test	
Aluminum		N/A		Max. 0.2 ppm	
Mg/Alkaline Earth Metals				Max. 100 ppm	
Arsenic		USP		Max. 1 ppm	
Iron		N/A		Max. 2 ppm	
Barium		USP		Passes Test	
Ferrocyanides				Passes Test	
Sulfate		N/A		Max. 0.020%	
Nitrites (absorbance 0.01 max.)		USP		Passes Test	
Heavy Metals		N/A		Max. 5 ppm	
Grade		COA or Letter on File		Record	

o **Pass** o **Fail** **QA/QC Signature and Date:**

Remarks:

Section 6. PRODUCT RELEASE CONTROL

o **Unrestricted** o **Restricted**

o **Blocked**

QA/QC Signature and Date:

Remarks/Conditions:

Section 7. QUALITY STATUS

o **Update Status in SAP**

QA/QC Signature and Date:

Section 8. DOCUMENT APPROVALS & CHANGE CONTROL

Revision: Approval Date	Change Control History	Author	Materials	Operations	QC	QA
01: 08/31/12	New document	(b) (4)	NA	NA	NA	(b) (4)
02: 12/12/12	Revised expiration to match DSM defined expiration		NA	NA	NA	
03: 01/06/15	Biennial Review; no changes necessary		NA	NA	NA	

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Mon Oct 10 13:57:13 CDT 2016

DSM BRIGHT SCIENCE. BRIGHTER LIVING.	MATERIAL CONTROL SPECIFICATION	Page 1 of 2
	Document Name: MCS.5070332BAG.03	Product Code: (b) (4)

Raw Material: Potassium Phosphate Monobasic - MCS

Section 1. RECEIVING / RECEIVING INSPECTION

Approved Manufacturer(s):

- o Innophos (Brenntag Southeast Inc.), Part Number 5111: Description : MonoPotassium Phosphate
- o ICL Performance Products LP, Part Number N/A: Description: MonoPotassium Phosphate
- o Prayon, Part Number 5111: Description: MonoPotassium Phosphate

Date Received:	Quantity Received:	Manufacturer Batch Number:

Packing List Received matches PO and material identification o YES o NO	**COA Received?** o YES -- Attach to MCS o NO
Kosher Group 1 (Kosher Marking Not Required) o YES o NO o N/A	**Shipping Criteria:** o Acceptable o Damaged
Temperature abuse evident? o YES o NO	**Truck seals intact & match Truck Bill (Fwd seals to Lab with COA)?** o YES o NO o N/A
Shortage of more than 1% (Bulk only)? o YES o NO o N/A	**Evidence of moisture, insects, rodents, spillage, foreign odors, or other concern?** o YES o NO **If YES, explain:**_____
Materials require allergen labeling according to plant Allergen Control Policy. Labels applied? o YES o NO o N/A	**Note:** if one of the above is nonconforming, initiate nonconformance and forward to QA.
Inspected By:	**Date:**

Section 2. SAMPLE CONTROL

Sample Use	Sample Quantity	Packaging	Storage	Shipped To
NA	NA	NA	NA	NA

Section 3. MANUFACTURE DATE & EXPIRATION

Manufacture Date:	Expiration Date:

Section 4. PRODUCT PACKAGING, LABELING & STORAGE

Batch Number: N / A	**Container or Packaging Instructions:** 50 lb. bag	
Labeling Instructions: 1. Label as "Quality Inspection, as needed"	**Storage Condition:**	Cool and dry (per Manufacturer's instructions)
	Product Shelf Life:	18 months from production date

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Mon Oct 10 14:03:06 CDT 2016

Section 5. QUALITY CONTROL

Test	Responsible Party	Method No.	Result	Specification	Initial / Date
Assay	Manufacturer	NA	Attach COA	Min. 98.0%	
Arsenic				Max. 3 mg/kg	
Fluoride				Max. 10 mg/kg	
Insoluble Substances				Max. 0.2%	
Lead				Max. 2 mg/kg	
Loss on drying				Max. 1%	
Grade		COA or Letter on File		Record	
o Pass o Fail	QA/QC Signature and Date:				

Remarks:

Section 6. PRODUCT RELEASE CONTROL

o Unrestricted o Restricted o Blocked	QA/QC Signature and Date:

Remarks/Conditions:

Section 7. QUALITY STATUS

o Update Status in SAP	QA/QC Signature and Date:

Section 8. DOCUMENT APPROVALS & CHANGE CONTROL

Revision: Approval Date	Change Control History	Author	Materials	Operations	QC	QA
01: 08/31/12	New document	(b) (4)	NA	NA	NA	C. Panker
02: 11/29/12	Revised expiration period to match DSM defined expiration from 24 to 18 months.		NA	NA	NA	C. Panker
03: 01/05/15	Biennial Review; no changes necessary		NA	NA	NA	K. Janes

DSM BRIGHT SCIENCE. BRIGHTER LIVING.	MATERIAL CONTROL SPECIFICATION	Page 1 of 2
	Document Name: MCS.XP00021001.03	Product Code: (b) (4)

Raw Material: Ammonium Sulfate - MCS

Section 1. RECEIVING / RECEIVING INSPECTION

Approved Manufacturer(s):

o GAC: Part Number 515518: Description : Ammonium Sulfate

Date Received:	Quantity Received:	Manufacturer Batch Number:

Packing List Received matches PO and material identification o YES o NO	**COA Received?** o YES -- Attach to MCS o NO
Kosher Group 1 (Kosher Marking Not Required) o YES o NO o N/A	**Shipping Criteria:** o Acceptable o Damaged
Temperature abuse evident? o YES o NO	**Truck seals intact & match Truck Bill (Fwd seals to Lab with COA)?** o YES o NO o N/A
Shortage of more than 1% (Bulk only)? o YES o NO o N/A	**Evidence of moisture, insects, rodents, spillage, foreign odors, or other concern?** o YES o NO **If YES, explain:**_____
Materials require allergen labeling according to plant Allergen Control Policy. Labels applied? o YES o NO o N/A	**Note:** if one of the above is nonconforming, initiate nonconformance and forward to QA.
Inspected By:	**Date:**

Section 2. SAMPLE CONTROL

Sample Use	Sample Quantity	Packaging	Storage	Shipped To
NA	NA	NA	NA	NA

Section 3. MANUFACTURE DATE & EXPIRATION

Manufacture Date:	Expiration Date:

Section 4. PRODUCT PACKAGING, LABELING & STORAGE

Batch Number: N / A	**Container or Packaging Instructions:** 22.7 kg bag	
Labeling Instructions: 1. Label as "Quality Inspection, as needed"	**Storage Condition:**	Cool, dry area
	Product Shelf Life:	2 years from production date

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Section 5. QUALITY CONTROL

Test	Responsible Party	Method No.	Result	Specification	Initial / Date
Moisture	Manufacturer	NA	Attach COA	≤ 0.15%	
Assay-Ammonium Sulfate				≥ 99.0%	
Water Insoluble				≤ 0.3%	
Residue on Ignition				≤ 0.25%	
Iron as Fe				≤ 15 ppm	
Lead				≤ 3 ppm	
Selenium				≤ 5 ppm	
Arsenic				≤ 0.5 ppm	
Grade		COA or Letter on File		Record	
o Pass o Fail	QA/QC Signature and Date:				

Remarks:

Section 6. PRODUCT RELEASE CONTROL

o Unrestricted o Restricted o Blocked	QA/QC Signature and Date:

Remarks/Conditions:

Section 7. QUALITY STATUS

o Update Status in SAP	QA/QC Signature and Date:

Section 8. DOCUMENT APPROVALS & CHANGE CONTROL

Revision: Approval Date	Change Control History	Author	Materials	Operations	QC	QA
01: 08/31/12	New document	(b) (4)	NA	NA	NA	C. Panker
02: 11/29/12	Revised expiration period to match DSM defined expiration (from 3 to 2 years).		NA	NA	NA	C. Panker
03: 01/06/15	Biennial Review; no changes necessary		NA	NA	NA	K. Janes

Version: 4.0

Effective Date: 14-Jul-2015

DNP

DNP OPS Americas Full Lifecycle Specification

(b) (4) Phosphoric Acid (b) (4) - MCS

Owner: Janes, Ken

Authors: (b) (6)

Reviewed By

Aurora ID	User Name	Date	Version
191665	Janes, Ken	09-Jun-2015 14:07:14 UTC	3.4
413100	McCutcheon, Amy	08-Jul-2015 11:34:59 UTC	3.4

Electronically Approved By

Aurora ID	User Name	Date	Signature Justification	Department
191665	Janes, Ken	09-Jul-2015 19:41:12 UTC	Approve	Kingstree Quality Assurance
413100	McCutcheon, Amy	13-Jul-2015 12:36:17 UTC	Approve	Kingstree Production

Location: US Kingstree, US Winchester

Material Type: ☒ Raw Material ☐ Finished Goods ☐ Resale ☐ Intermediate ☐ Packaging Component

Section 1. RECEIVING / RECEIVING INSPECTION	
Approved Manufacturer(s): ☐ Innophos, Part Number N/A: Description: Phosphoric Acid 75% Bu k ☐ ICL Performance Products LP, Part Number N/A: Description: Phosphoric Acid 75% Bulk ☐ Rhodia, Part Number N/A: Description: Phosphoric Acid 75% Bulk ☐ Prayon, Part Number N/A: Description: Phosphoric Acid 75% Bulk	
Date Received: **Quantity Received:**	**Manufacturer Batch Number:**
Packing List Received matches PO and material identification ☐ YES ☐ NO	**COA Received?** ☐ YES -- Attach to MCS ☐ NO
Kosher Group 1 (Kosher Marking Not Required) ☐ YES ☐ NO ☐ N/A	**Shipping Criteria:** ☐ Acceptable ☐ Damaged
Temperature abuse evident? ☐ YES ☐ NO	**Truck seals intact & match Truck Bill (Fwd seals to Lab with COA)?** ☐ YES ☐ NO ☐ N/A
Shortage of more than 1% (Bulk only)? ☐ YES ☐ NO ☐ N/A	**Evidence of moisture, insects, rodents, spillage, foreign odors, or other concern?** ☐ YES ☐ NO **If YES, explain:**_____
Materials require allergen labeling according to plant Allergen Control Policy. Labels applied? ☐ YES ☐ NO ☐ N/A	**Note:** if one of the above is nonconforming, initiate nonconformance and forward to QA.
Inspected By:	**Date:**

Section 2. SAMPLE CONTROL				
Sample Use	**Sample Quantity**	**Packaging**	**Storage**	**Shipped To**
NA	NA	NA	NA	NA

Section 3. MANUFACTURE DATE & EXPIRATION	
Manufacture Date:	**Expiration Date:**

Section 4. PRODUCT PACKAGING, LABELING & STORAGE

Batch Number: N / A	**Container or Packaging Instructions:** Bu k	
Labeling Instructions: 1. Label as "Quality Inspection, as needed"	**Storage Condition:**	Store in an area that is cool, dry, and well-ventilated (per Manufacturer's instructions)
	Product Shelf Life:	24 months from date of production

Section 5. QUALITY CONTROL

Test	Responsible Party	Method No.	Result	Specification	Initial / Date
Concentration	Manufacturer	N/A	Attach COA	≥75%	
Grade		COA or Letter on File		Record	
☐ Pass ☐ Fail	QA/QC Signature and Date:				

Remarks:

Section 6. PRODUCT RELEASE CONTROL

☐ Unrestricted ☐ Restricted ☐ Blocked	**QA/QC Signature and Date:**

Remarks/Conditions:

Section 7. QUALITY STATUS

☐ Update Status in SAP	**QA/QC Signature and Date:**

Section 8. DOCUMENT APPROVALS & CHANGE CONTROL

Revision: Approval Date	Change Control History	Author	Materials	Operations	QC	QA
01: 08/31/12	New document	(b) (4)	NA	NA	NA	C. Panker
02: 11/29/12	Revised expiration to match DSM defined expiration		NA	NA	NA	C. Panker
03; 01/06/15	Biennial Review; no changes necessary		NA	NA	NA	K. Janes
04: 06/08/15	Scheduled review - no changes		NA	Amy McCutcheon	NA	K. Janes