DSM Nutritional Products

APPLICATION FOR THE APPROVAL OF ADDITION OF DHA ALGAL OIL FROM *SCHIZOCHYTRIUM* SP. TO INFANT FORMULA PRODUCTS (INCLUDING PRE-TERM INFANT FORMULA, TERM INFANT FORMULA, and FOLLOW ON FORMULA)

Dossier

For Submission to the Food Standards Australia New Zealand, PO Box 7186, Canberra BC ACT 2610 Australia

Australia New Zealand Food Standards Code –

Submitted by DSM Nutritional Products Australia Pty Limited No. 9 Moorebank Avenue, Moorebank, NSW 2170, Australia

7 October 2015

Application to amend the *Australia New Zealand Food Standards Code-*

See attached Executive Summary

PART 1 GENERAL REQUIREMENTS

1.1 APPLICANT DETAILS

(a) Applicant's name/s

[redacted]

(b) Company/organization name

DSM Nutritional Products

(c) Address (street and postal)

6480 Dobbin Rd.
Columbia MD 21045
USA

(d) Telephone and facsimile numbers

[redacted]

(e) Email address

[redacted]

(f) Nature of applicant's business

DSM Nutritional Products is the global market leader in the manufacturing and distribution of nutritional ingredients, in particular vitamins, carotenoids, polyunsaturated fatty acids and nutraceutical ingredients for use in food, pharmaceutical, cosmetic and animal feed applications.

(g) Details of other individuals, companies or organizations associated with the application.

Not applicable.

1.2 PURPOSE OF THE APPLICATION

The purpose of the application is for FSANZ to consider a new DHA-rich Algal Oil as an additional, alternative or replacement oil source of DHA in infant formula products. This may require amendments to Standard 1.3.4 Identity and purity and Standard 2.9.1 Infant formula products.

1.3 JUSTIFICATION FOR THE APPLICATION

(a) the need for the proposed change

The composition of the new strain of *Schizochytrium* sp. microalgae and the oil derived from the new strain of *Schizochytrium* sp. are comparable to other currently permitted sources of DHA. Permitting this new strain of Schizochytrium sp. algae and oil will provide an additional/alternative source of omega-3 fatty acids (e.g. DHA) in foods, specifically infant formula products.

Martek Biosciences Corporation (now DSM Nutritional Products) has commercially manufactured and sold algal oil containing approximately 40% docosahexaenoic acid (DHA), a n-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA), produced via fermentation using *Crypthecodinium cohnii* microalgae since 1994 (DHASCO®). DHA Algal Oil (tradename: DHASCO®) has been added to infant formula sold in Australia and New Zealand since 1998. According to Standard 2.9.1 of the Food Standards Code, n-3 LCPUFA may be present in an infant formula or follow-on formula at a maximum 1% of the total fatty acid content.

Martek Biosciences Corporation (now DSM) has also manufactured and sold algal oil containing approximately 35% DHA produced via fermentation using *Schizochytrium* sp. microalgae (DHA™-S Algal Oil). In 2002, Martek Biosciences Corporation gained approval for this oil, "docosahexaenoic acid (DHA)-rich oil derived from *Schizochytrium* sp." [also known as "Oil

derived from marine micro-algae (Schizochytrium sp.) rich in DHA" (trade name "DHA-S")], as a novel food in Australia and New Zealand (ANZFA, 2002).

In 2012, the Advisory Committee on Novel Foods (ACNF) of FSANZ determined that an improved strain, from another species of *Schizochytrium* microalgae, is not novel in accordance with Standard 1.5.1 of the Australia New Zealand Food Standards Code (ACNF, 2012). This strain produces an oil which contains a minimum 22.5% of the long-chain omega-3 fatty acid, docosahexaenoic acid (DHA; 22:6 n-3) and a minimum 10% of the long-chain omega-3 fatty acid, eicosapentaenoic acid (EPA; 20:5 n-3) as well as saturated and other polyunsaturated fatty acids. This DHA and EPA-rich oil from *Schizochytrium* sp. (hereafter "DHA-O") has a fatty acid profile that more closely represents that of marine/fish sources of n-3 LCPUFA.

DSM has now developed another strain of the *Schizochytrium* species (*i.e.*, the same species of *Schizochytrium* sp. from which the production strain for DHA-O has been derived) to produce a new oil which is rich in DHA and is primarily being targeted as a replacement/alternative to DHASCO® in infant (term and pre-term) and follow-on formula. This new, DHA-rich microalgal oil is given the abbreviation "DHASCO®-B".

Schizochytrium sp. is a thraustochytrid and a member of the Chromista kingdom (Stramenopilia), which includes the golden algae, diatoms, yellow-green algae, haptophyte and cryptophyte algae, and oomycetes. The strain is not genetically-engineered; i.e., it is not a genetically-modified organism (GMO). There are no reports of this organism producing toxins or being pathogenic.

In February 2014, DSM Nutritional Products submitted a novel food questionnaire to the Advisory Committee on Novel Foods (ACNF) to enquire if DHASCO®-B would in the view of the Committee be a novel food or non-novel food ingredient. After consulting with FSANZ, the ACNF informed DSM Nutritional Products that adding new ingredients to infant formula requires consideration outside the scope of ACNF (ACNF, 2014). FSANZ later informed DSM Nutritional Products that an application to amend the Australia New Zealand Food Standards Code is required (FSANZ, 2014).

Status in other countries

In the United States, DHASCO®-B oil was the subject of a New Dietary Ingredient Premarket Notification submitted to the Food and Drug Administration (U.S. FDA) by DSM Nutritional Products in November 2013. The U.S. FDA responded with a letter of no objection in February 2014 (U.S. FDA, 2014). In 2015, DHASCO®-B received approval as a novel food ingredient in the European Union for use in various food categories, including infant formula and follow-on formula (European Commission, 2015). DSM also submitted a novel food pre-market notification to Health Canada for DHASCO®-B in January 2014 which was updated in July 2014. Health Canada approved the use of DHASCO®-B in infant formula, follow-on formula, and foods for special dietary use for children under 3 years old in August 2015 (Health Canada, 2015).

An independent panel of experts has reviewed the safety information contained in this application, as well as characterization of DHASCO®-B oil and concluded that the oil is Generally Recognized as Safe (GRAS) for use as an ingredient in infant formula (term and preterm) and follow on formula (Tarka et al., 2013). DSM Nutritional Products notified the United States Food and Drug Administration (US FDA) of the self-determination of GRAS Status in October 2014. The FDA responded in June 2015 that the agency has no questions regarding DSM's conclusion that this algal oil is GRAS under the intended conditions of use, as an ingredient in preterm and term infant formulas and follow-on formulas at a maximum use level of 1.25% of dietary fat (U.S. FDA, 2015).

(b) the advantages of the proposed change over the status quo, taking into account any disadvantages.

DHA Algal Oil (DHASCO®-B) provides an additional alternative source of DHA for use in infant formula products. It is a consistent, sustainable, and vegetarian source of DHA.

There are no known disadvantages.

A. Regulatory impact information

1. Costs and benefits

(a) the cost and benefits to the consumer e.g. health benefits;

The use of DHA Algal Oil as an alternative source of DHA in infant formula products will not be directly associated with any increase in retail price of these products.

DHA Algal Oil addition to infant formula supports infant DHA tissue levels comparable to human milk and supports term and preterm infant visual development.

(b) the costs and benefits to industry and business in general, noting any specific effects on small businesses e.g. savings in production costs; and

Because the organism used to manufacture DHASCO®-B is more productive when compared to DHASCO®, the oil provides a lower cost alternative source of DHA to infant formula manufacturers using algal oil as an ingredient.

(c) the costs and benefits to government e.g. increased regulatory costs.

Regulatory costs to government beyond this assessment are not expected.

2. Impact on international trade

There will be a positive impact (less trade barrier) to infant formula (term and preterm) products and follow on formula products imported/exported into/from Australia and New Zealand. As mentioned in section 1.3(a) DHASCO®-B is also approved for the intended use in the United States, Canada, and the European Union. The suitability of DHASCO-®B for use in all major global markets, including Australia and New Zealand, would help facilitate international trade by allowing the same products to be marketed globally.

1.4 INFORMATION TO SUPPORT THE APPLICATION

(a) any public health and safety issues related to the proposed change including details of target groups and population groups that may be adversely affected

No public health and safety issues related to the proposed change are expected. Please see Part 2 B for more safety information.

(b) any consumer choice issues related to the proposed change

No consumer choice issues related to the proposed change are expected. Please see Part 2 F for more information regarding consumer impact.

(c) any evidence that the food industry generally or other specific companies have an interest in, or support, the proposed change to the Code (this item is mandatory for applications relating to food additives, processing aids, nutritive substances, novel foods, irradiated foods.)

DSM is working with several international infant formula manufacturers to replace DHASCO® with DHASCO®-B in their formulations. This is highly desirable due to cost savings.

1.5 ASSESSMENT PROCEDURE

We are in favour of the adoption of the General Procedure to assess the application as we are applying to include an additional/alternative source of DHA infant formula products that is currently not specified in the Code.

1.6 CONFIDENTIAL COMMERCIAL INFORMATION (CCI)

Not applicable.

1.7 EXCLUSIVE CAPTURABLE COMMERCIAL BENEFIT (ECCB)

Not applicable

1.8 INTERNATIONAL AND OTHER NATIONAL STANDARDS

A. International Standards

Table. 1 Standards And Regulations For Use Of LCPUFA In Infant Formula*		
Regulatory Body	**Long-chain omega-3/DHA Levels**	**Long-chain omega-6/ARA Levels**
Codex (2007)	DHA Upper limit 0.5% total fat content EPA should not exceed DHA Minimum level not specified	ARA Upper limit not specified ARA required to meet or exceed added DHA Minimum level not specified

EU Commission (2006)	Upper limit 1% of total fat content for n-3 LCP[1] DHA shall not exceed ARA EPA shall not exceed DHA 0.2% minimum for DHA/if LCP nutrition claim is made	Upper limit of 1% of total fat content for ARA Minimum level not specified ARA ≥ DHA
Food Standards Australia New Zealand (2014)	Upper limit 1% of total fat content for LC omega-3[2] Total long chain omega 6:long-chain omega-3 ratio that is not less than 1	Upper limit of 1% of total fat content for ARA Total long chain omega 6:long-chain omega-3 ratio that is not less than 1
Indonesian National Agency for Food and Drug Control (2010)	0.2% minimum DHA Upper limit 0.5% DHA addition must be accompanied by addition of ARA according to the ratio of 1-2:1 EPA must not exceed DHA	ARA Upper limit not specified
Estados Unidos Mexicanos.- Secretaría de Salud (2012)	Minimum level not specified. Upper limit 0.5%. EPA must not exceed DHA	Minimum level and Upper limit not specified. ARA ≥ DHA

[1]LCP=long-chain (20 and 22 carbon atoms) polyunsaturated fatty acids

[2]LC omega-3 = long chain omega 3 series fatty acid (C>=20)

- Australia New Zealand - Standard 2.9.1 Infant Formula Products mandates that the ratio of total long chain omega-6 fatty acids (C>=20) to total long chain omega-3 series fatty acids (C>=20) is not less than 1 in an infant formula or follow-on formula which contains these fatty acids and where long chain polyunsaturated fatty acids are present in an infant formula or follow-on formula, an eicosapentaenoic acid (20:5 n-3) content of no more than the docosahexaenoic acid (22:6 n-3) content.
    - Maximum 1% of the total fatty acid content for omega-3 series fatty acids, and
    - Maximum 2% of the total fatty acid content for omega-6 fatty acids (1% of the total fatty acid content for arachidonic acid).

- Codex STAN 72 – 1981 (Rev 2007) Standard for Infant Formula and Formulas For Special Medical Purposes Intended for Infants allows the addition of DHA (0.5% Guidance Upper Level) to infant formula.
    - If docosahexaenoic acid (22:6 n-3) is added to infant formula, arachidonic acid (20:4 n-6) contents should reach at least the same concentration as DHA. The content of eicosapentaenoic acid (20:5 n-3), which can occur in sources of LC-PUFA, should not exceed the content of docosahexaenoic acid. National authorities may deviate from the above conditions, as appropriate for the nutritional needs.

- European Union - Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC allows the addition of long-chain (20 and 22 carbon atoms) polyunsaturated fatty acids (LCP). Their content shall not exceed:
    - 1% of the total fat content for n-3 LCP, and
    - 2% of the total fat content for n-6 LCP (1% of the total fat content for arachidonic acid (20:4 n-6))
        - The eicosapentaenoic acid (20:5 n-3) content shall not exceed that of docosahexaenoic (22:6 n-3) acid content.
        - The docosahexaenoic acid (22:6 n-3) content shall not exceed that of n-6 LCP.

- European Union - Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC requires a minimum level of DHA as a condition of use for added LCP or an equivalent nutrition claim related to the addition of DHA
    - Nutrition claim related to added LCP or an equivalent nutrition claim related to the addition of docosahexaenoic acid
        - The docosahexaenoic acid content is not less than 0.2% of the total fatty acid content.

B. Other National Standards and Regulations

DHA Algal Oil (tradename: DHASCO®-B; DHA-B) is proposed to be added to infant formula products [infant formula (term and preterm) and follow-on formula] at a level that provides n-3 LCPUFA up to 1% of formula fat. This level is therefore consistent with the maximum level of n-3 LCPUFA of 1.0% of formula fat specified in Standard 2.9.1 Infant Formula Products for Australia and New Zealand.

1.9 STATUTORY DECLARATION

Refer to end of document for statutory declaration.

1.10 CHECKLIST

Refer to Appendix 1 for checklist.

PART 2 STANDARDS RELATED TO SUBSTANCES ADDED TO FOOD

NUTRITIVE SUBSTANCES

A. Technical information on the nutritive substance

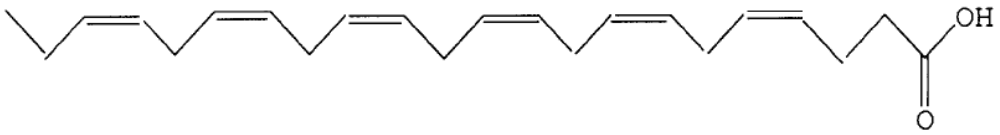
All data provided in this section is representative of the product intended for commercialization.

1. Information to enable identification of the nutritive substance

Chemical name: Docosahexaenoic acid Algal Oil (from *Schizochytrium* sp.)

Structural Formula: The chemical structure of DHA is shown in Figure 1.

Figure 1. Chemical Structure of DHA



Common Name: DHA Algal Oil

Synonyms: Algal Oil

Manufacturers' code: XP55100000

Marketing trade name: DHA-B or DHASCO®-B

CAS No.: The CAS number for fatty acids containing 14-22 carbons (C14-C22), and 16-22 carbons (C16-C22) esterified to glycerol is 68424-59-9 (described in the CAS registry as "glycerides", C14-C22 and C16-C22-unsatd.)

EINECS No.: Not applicable

Empirical formula: $C_{22}H_{32}O_2$

Molecular mass: The molar mass of docosahexaenoic acid (DHA) is 328.488 g/mol.

2. Information on the chemical and physical properties of the nutritive substance

Appearance and Color:

DHASCO®-B, a free flowing, light yellow-orange oil, is comprised of oil extracted from the microalgae *Schizochytrium* sp. DHASCO®-B is predominantly triglyceride in composition, along with some monoglyceride, diglyceride and nonsaponifiable material, as is typical for food-grade vegetable oils. The oil contains approximately 40% DHA by weight.

Odor: Marine

Melting point/range: < 10°C

Water solubility: Insoluble in water

Solubility in other solvents: Soluble in non-polar organic solvents (e.g. hexane) and partially soluble in polar organic solvents (e.g. ethanol)

Partition coefficient: N/A

Thermal decomposition: Same as that for all fats and oils (carbon dioxide, water, CO, and H_2)
Particle size, size distribution, morphology any size-dependent properties are not relevant.

Application Methodology of DHA Algal Oil in formula

DHASCO®-B can be used in both liquid and powder infant formula products.

Stability of DHA Algal Oil in formula and overages

DHASCO®-B was added to liquid ready-to-feed infant formula and studied for 12 months at ambient temperature.

Table 2 Stability of DHASCO®-B in Ready to Feed Infant Formula

Nutrient	Unit	Label Claim (LC)	0 Month		3 Month		6 Month		12 Month	
Docosahexaneoic Acid (DHA)	Mg/100 kcal	19	Result	%LC	Result	%LC	Result	%LC	Result	%LC
			21.6	113	20.4	107	21.8	114	19.5	102

Stability data for DHASCO®-B in infant formula powders is not available. As the ingredient supplier, we do not manufacture the finished infant formula product. The infant formula

producer is responsible for assuring that when the oil is combined with approved ingredients that the finished infant formula product is stable throughout shelf life.

3. Information on the impurity profile

Nitrogen and Protein Content in Oil – The protein content of DHA Algal Oil was non-detectable (<0.1%).

Testing was carried out at Covance (Madison, WI) and the Certificates of Analysis are presented in Appendix 2.

Elemental Analysis – Heavy metals were not detected at the limit of detection/limit of quantification (LOD/LOQ) in DHA Algal Oil.

Testing was conducted by Eurofins Central Analytical Laboratories (Kingstree, SC) and Columbia Analytical Services (WA). Certificates of Analysis are presented in Appendix 2.

Other Analyses – The total amount of dioxins in DHA Algal Oil was below 2 pg/g, the EU maximum residual limit. The total dioxins and PCBs in the DHA Algal Oil were below 10 ng/kg fat. All individual polycyclic aromatic hydrocarbon (PAH) components were below the limit of detection except for lot 08-6592 which contained 11.4 ppb of naphthalene, 5.5 ppb of phenanthrene, and 1 ppb of pyrene. There were no detectable levels of mycotoxins in DHA Algal Oil, and the levels of total aflatoxins and total fumonisins were below 4.0 µg/kg and 200 µg/kg, respectively. No detectable levels of acrylamide (<50 µg/kg) were present, nor were pesticides detected in DHA Algal Oil. Except for the single PAH detection noted above, results from analyses conducted by Eurofins CAL (LA) and Eurofins (Germany) demonstrated the absence of dioxins, PCBs, PAHs, acrylamide, mycotoxins, and pesticides. The Certificates of Analysis are presented in Appendix 2.

4. Manufacturing process

DHA Algal Oil is produced via fermentation from a new production strain of *Schizochytrium* sp. microalgae. A flow chart of the manufacturing process is shown in Figure 2. Following fermentation, the broth is collected in a centrifuge for further processing and pasteurization.

Enzymatic degradation of the algal cell walls by a food grade protease is used to facilitate the release of the oil from the algae, followed by sodium chloride addition and centrifugation. The oil from the centrifuge is collected as the top (light) phase and the spent broth as the lower (heavy) phase. The recovered oil is collected in the oil recovery tank and pumped into a vacuum dryer, where it is dried under maximum vacuum. The separated algal oil then undergoes the standard food oil industry downstream processing operations of filtration, refining, physical bleaching and deodorization.

DHA Algal Oil is made under appropriate food current good manufacturing practices (cGMPs). DSM maintains material control specifications for all raw materials used in the manufacture of DHA Algal Oil. The quality of all raw materials used is verified from the manufacturers' Certificates of Analysis. All raw materials used in the manufacturing process are food grade (see Table 3).

Table 3. Raw Materials used in the Manufacturing of DHA Algal Oil (DHASCO®-B)

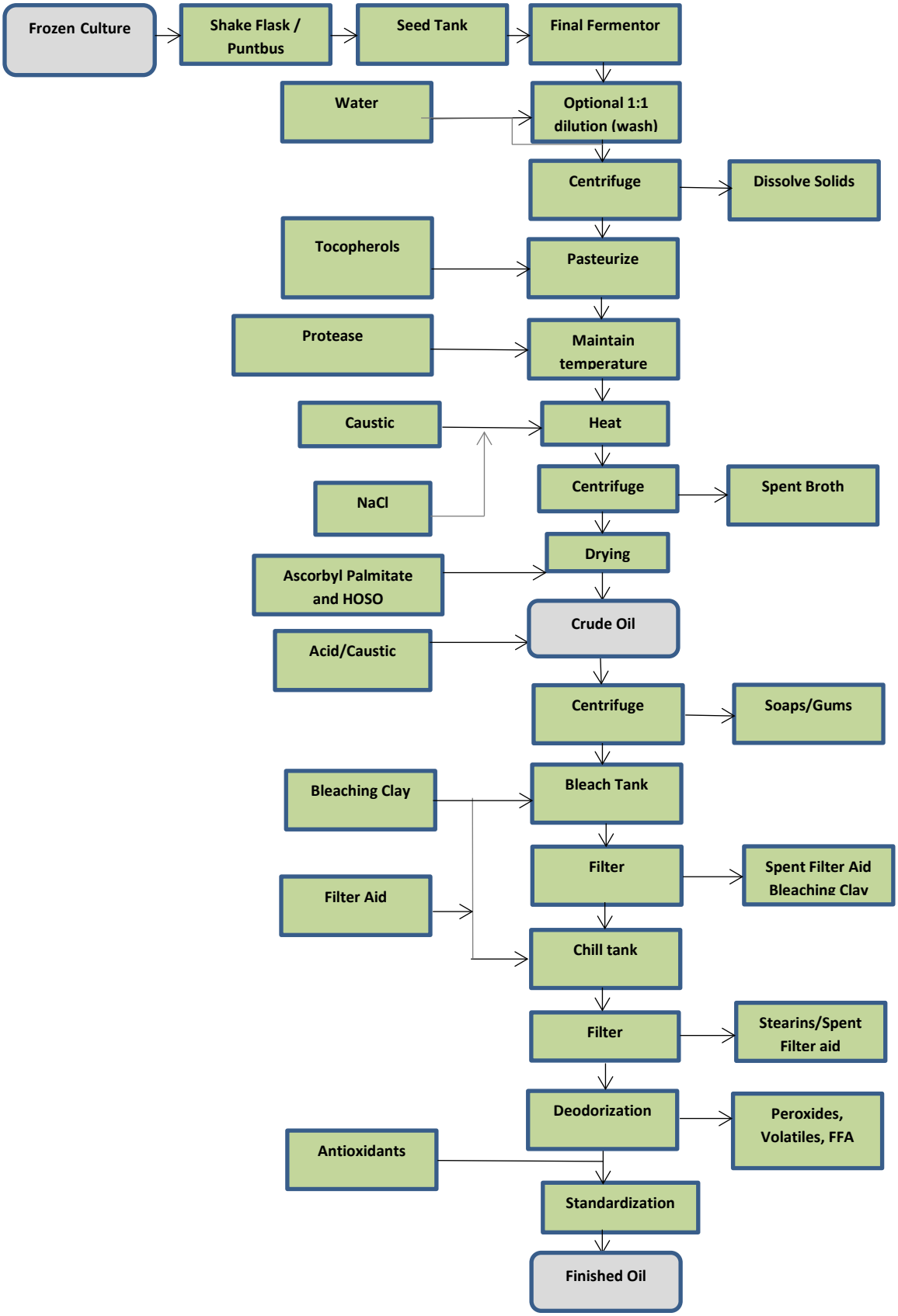
Ingredient	Status	Purpose	Approved Use	Ingredient	Status	Purpose	Approved Use
Ammonium hydroxide	Metallurgical grade	Nitrogen Source	Standard 1.3.3 Table to Clause 3	Ammonium Sulfate	FCC	Nitrogen Source	Standard 1.3.3 Table to Clause 18
Mono-Potassium Phosphate	FCC	Macronutrient Source	Standard 1.3.1 Schedule 2	Manganese Chloride	ACS	Micronutrient source	Standard 1.3.3 Table to Clause 18
Calcium Chloride	FCC	Macronutrient source	Standard 1.3.1 Schedule 2	Nickel Sulfate	ACS	Micronutrient source	Nickel is a permitted catalyst in Standard 1.3.3 Table to Clause 5
Calcium Pantothenate	FCC	Micronutrient source	Standard 1.3.3 Table to Clause 18	Potassium Chloride	USP	Macronutrient source	Standard 1.3.1 Schedule 2
Dextrose and glucose	FCC	Carbon source	Food Ingredient	Sodium Chloride	FCC	Macronutrient source	Food Ingredient
Sodium Sulfate	USP	Macronutrient	Standard 1.3.1	Sodium	ACS	Micronutrient	Standard 1.3.3

Table 3. Raw Materials used in the Manufacturing of DHA Algal Oil (DHASCO®-B)							
Ingredient	**Status**	**Purpose**	**Approved Use**	**Ingredient**	**Status**	**Purpose**	**Approved Use**
		source	Schedule 2	Molybdate		source	Table to Clause 18
Citric Acid	USP/FCC	Chelating agent for metal micronutrients	Standard 1.3.1 Schedule 2	Yeast Extract	Food grade	Complex nutrient source	Food Ingredient
Cupric Sulfate	ACS	Micronutrient source	Standard 1.3.1 Schedule 2 and Standard 1.3.3 Table to Clause 18	Thiamine HCl	USP/Ph. EUR/FCC	Micronutrient source	Standard 1.3.3 Table to Clause 18
D-Biotin	USP	Micronutrient source	Standard 1.3.3 Table to Clause 18	Zinc Sulfate	ACS	Micronutrient source	Standard 1.3.3 Table to Clause 18
Ascorbyl Palmitate	USP/NF, FCC, Ph. Eur.	Antioxidant	Standard 1.3.1	High Oleic Sunflower Oil (HOSO)	N/A	Standardization	Food Ingredient
Ferrous Sulfate	USP	Micronutrient source	Standard 1.3.3 Table to Clause 18	Sodium hydroxide	FCC	pH adjustment	Standard 1.3.3 Table to Clause 3
Magnesium Sulfate	USP	Macronutrient source	Standard 1.3.1 Schedule 2	Tocopherols	FCC, USP, JECFA	Antioxidant	Standard 1.3.1 Schedule 1
Bentonite	None*	Bleaching clay	Standard 1.3.1 Schedule 2	Silicon dioxide	FCC	Filter aid	Standard 1.3.3 Table to Clause 3 and Standard 1.3.1 Schedule
Protease (Serine proteinase from	JECFA, FCC	Cell wall breakage					

Table 3. Raw Materials used in the Manufacturing of DHA Algal Oil (DHASCO®-B)							
Ingredient	**Status**	**Purpose**	**Approved Use**	**Ingredient**	**Status**	**Purpose**	**Approved Use**
Bacillus subtilis)		during oil recovery	Standard 1.3.3 Table to Clause 17				2

***** The supplier of the bentonite cannot confirm compliance with the FCC specification. The product complies with FDA regulation 21 CFR §184.1155 for bentonite and the supplier is ISO certified.

Figure 2. Process Flow Chart for the Manufacture of DHA Algal Oil (DHASCO®-B)



5. Specification for identity and purity

DHA Algal Oil is a mixture of triglycerides derived from fermentation by the alga *Schizochytrium* sp. It contains approximately 40% of the n-3 LCPUFA, DHA (22:6 n-3) as well as saturated and unsaturated fatty acids. Analyses of five separate lots (at least three of which were non-consecutive) indicated that the manufacturing process results in a consistent product (see Table 4).

DHASCO®-B also meets the "Specification for oil derived from marine micro-algae (*Schizochytrium* sp.) rich in docosahexaenoic acid (DHA)" included in Standard 1.3.4 (Identity and Purity) of the Australia New Zealand Food Standards Code (ANZFSC). Detailed specifications for DHA Algal Oil (DHASCO®-B) are also presented in Table 4.

Table 4. Specifications for DHA Algal Oil (DHASCO®-B)

Tests	**Specification**	**ANZFSC Standard 1.3.4[2]**	**LOD[1]**	**DHA Algal Oil**				
				08-6530	**08-6586**	**08-6585**	**08-6592**	**08-6643**
DHA (%)	Min. 35.0	Min. 32	N/A	44.35	42.65	42.96	41.23	45.71
EPA (%)	Max. 10.0		0.1	5.90	6.10	6.43	6.10	6.61
Free fatty Acids	Max. 0.4		0.005	0.07	0.05	0.05	0.07	0.13
Peroxide Value	Max. 5.0		0.1	0.4	<0.1	<0.1	<0.1	<0.1
Moisture and Volatiles (%)	Max. 0.02		0.01	<0.1	<0.1	<0.1	<0.1	<0.1
Unsaponifiables	Max. 3.5		0.05	0.97	0.96	0.88	0.78	0.88
Trans-fatty Acids (%)	Max. 1.0	Max. 2.0	1	<1	<1	<1	<1	<1
Arsenic (mg/kg)	Max. 0.1	Max. 0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Cadmium (mg/kg)	Max. 0.1	Max. 1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Copper (mg/kg)	Max. 0.1		0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Iron (mg/kg)	Max. 0.2		0.02	0.04	0.02	0.05	0.03	0.04
Mercury (mg/kg)	Max. 0.04	Max. 0.1	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Lead (mg/kg)	Max. 0.1	Max. 0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1

[1] Limit of Detection

[2] Hexane is also included in Standard 1.3.4 but it is not applicable to this product. Hexane is not used in the manufacturing process.

6. Analytical method for detection

Determination of DHA in formula and other matrixes

AOCS Ce 1b-89 is a method of choice for identifying and quantifying DHA in oils, but the method is not applicable to food matrices.

For fortified foods, the method of choice is AOAC 996.06, "Fat (Total, Saturated and Unsaturated) in Foods". Item d in the calculation section outlines how to determine DHA potency of a sample.

7. Information on the proposed food label

Because DHASCO®-B is a replacement for/alternative to DHASCO® as a source of DHA in infant formula products [including infant (term and preterm) and follow on formula], no change in the proposed food label is expected.

B. Information related to the safety of the nutritive substance

1. Information on the toxicokinetics and metabolism of the nutritive substance and, if necessary, its degradation products and major metabolites

DHASCO®-B is composed primarily of triglycerides and triglycerides represent the principal source of dietary lipid in the human diet. A general review of the absorption, distribution, metabolism and excretion (ADME) of triglycerides can be found in several textbooks (Christophe et al., 2000; Grundy et al., 1996; Innis, 1996; Nelson, 1991). The main points are reviewed below.

Absorption

Ingested triglycerides are generally emulsified in the stomach and upper intestine (duodenum) where they are mixed with bile salts and enzymatically hydrolyzed by pancreatic lipase. The pancreatic lipase has a high degree of specificity for the sn-1 and sn-3 positions of the triglyceride, and the digested triglycerides are generally absorbed by the intestinal mucosa either as free fatty acids or as the sn-2 monoglyceride.

Distribution

The intestinal mucosal cells absorb the free fatty acids and sn-2 monoglycerides, and "retailor" these components into new triglycerides and phospholipids. Triglycerides accumulate in large 1 mm droplets containing about 88% triglyceride, 8% phospholipid, 3% cholesterol ester, and 1-2% protein (apolipoprotein B-48) which are ejected from the gut mucosal cells by exocytosis as chylomicrons (CMs). These CMs are too large to cross the basement membranes of capillaries, so they enter the large-pored lacteal (lymph) vessels and

leave the intestine with lymph via the thoracic duct before being emptied into the venous circulation. The CMs are then tagged with Apolipoprotein E (ApoE) and Apolipoprotein C (ApoC) following an interaction with circulating high density lipoprotein (HDL). This makes the CMs vulnerable to attack by vascular lipoprotein lipase, which hydrolyses the triglyceride, releasing individual fatty acids to the tissues for metabolism. The liver, via the ApoE receptor, resorbs CM remnants. Excess fat and ApoB-100 (+ApoE and ApoC) results in very low density lipoprotein (VLDL) synthesis in liver. VLDL is also attached by lipoprotein lipase, providing free fatty acids to the tissues.

Metabolism

Fatty acids are building blocks for new membranes as well as energy rich fuel sources for cellular metabolism. In adipose cells, for example, the free fatty acids produced by lipoprotein lipase are retailored to triglycerides and stored as fat (triglyceride) for later use. Most of the fatty acids in tissues throughout the body, however, are oxidized in mitochondria by ß-oxidation to produce 2-carbon substrates for the Kreb's cycle. The Kreb's metabolic cycle produces CO_2 and reducing power in the form of NADPH. NADPH is then oxidized in the mitochondria to produce ATP, releasing two electrons (added to O_2 to make H_2O). The ATP produced in this way provides energy for all cellular functions.

Essential fatty acids (the n-3 and n-6 families) may be metabolized for energy as described above or retained by tissues, since they cannot be synthesized de novo. In tissue, they may be incorporated into membrane phospholipids. The n-3 and n-6 fatty acids, particularly the 20-carbon fatty acids arachidonic (AA; 20:4 n-6) and eicosapentaenoic acids (EPA; 20:5 n-3), serve as precursors to eicosanoids, bioactive molecules important in modulating immunologic, inflammatory, vascular and thrombotic responses and homeostasis.

Excretion

A majority of lipid consumed during a meal is metabolized in tissue and excreted as carbon dioxide and water. In cases of malabsorption due to certain pathologies (e.g., pancreatic insufficiency, short bowel, etc.), the triglycerides may not penetrate the gastrointestinal tract and are excreted in the stools.

2. Information from studies in animals and humans that is relevant to the toxicity of the nutritive substance and, if necessary, its degradation products and major metabolites

To corroborate the existing safety data on DHA Algal Oil (DHASCO®-B**)** from *Schizochytrium* sp., DSM Nutritional Products sponsored studies to assess its safety and to complement the existing published literature on the safety of algal oil produced by fermentation. An overview of all preclinical toxicology studies conducted with DHASCO®-B are presented in Table 5, followed by more detailed descriptions of each study.

While some of these DSM-sponsored studies have not been published, they were conducted under GLP and have been reviewed by GRAS (Generally recognized as safe) expert panels comprised of individuals with expertise in food safety and toxicology. Taken together, these studies provide additional support for the safety of DHA Algal Oil from *Schizochytrium* sp. DHA Algal Oil from *Schizochytrium* sp. was evaluated by testing for gene mutations, clastogenicity (structural chromosome activity) and aneugenicity (numerical chromosome activity), and also in a bioequivalence (DHASCO®-B compared to DHASCO®) study in piglets, and in a 90-day rat dietary study with an *in utero* phase. All studies were performed in accordance with Good Laboratory Practice.

An independent panel of experts reviewed this safety information, as well as characterization of DHASCO®-B oil and concluded that the oil is Generally Recognized as Safe (GRAS) for use as an ingredient in infant formula and follow on formula (Tarka et al., 2013).

While DSM will only market the aqueous extracted oil, additional studies conducted on DHASCO®-B prepared *via* extraction by isopropyl alcohol (85 to 100%) are available. DSM originally developed DHASCO®-B manufactured *via* isopropyl alcohol (IPA) extraction. In order to maximize sustainability in view of the environment and in response to a commercial need for non-solvent extracted infant formula ingredients, DSM developed a recovery process that does not utilize solvents.

DHASCO®-B produced by IPA extraction meets the same specifications of DHASCO®-B produced by aqueous extraction and is compositionally similar. Due to the similarities between the two substances, studies conducted on the IPA preparation provide additional supporting evidence of the safety of DHASCO®-B (aqueous extraction) and are also discussed in the following sections.

An independent panel of experts reviewed the studies conducted on the oil from IPA extraction, as well as characterization of DHASCO®-B oil, available through January 2012 and concluded that the oil is Generally Recognized As Safe (GRAS) for the intended uses as an ingredient in infant formula and in food for the general population (Borzelleca, 2011).

Table 5 Summary of Preclinical Studies with DHASCO®-B

Test System	Type	Results	Concentration	Reference
DHASCO®-B (Aqueous Extraction)				
In vitro Studies				
Escherichia coli WP2uvrA *Salmonella typhimurium* TA98, TA100, TA1535, TA1537	Ames reverse mutation (+/-S9), plate incorporation method	Non-mutagenic	31.6, 100, 316, 1,000, 2,500, or 5,000 µg/plate	BSL Bioservice Study No. 105886 - BSL, 2011a
Human lymphocytes	CA (+/-S9)	Equivocal[a]	250, 500, 1,000, 2,500, or 5,000 µg/mL (4-hr; +/-S9) 250, 500, 1,000, 2,500, or 5,000 µg/mL (24-hr; -S9) 3,000, 4,000, or 5,000 µg/mL (4-hr; +/-S9) 400, 500, 750, or 1,000 µg/mL (4-hr; +/-S9)	BSL Bioservice Study No. 105887 - BSL, 2011b
In vivo Studies				
NMRI mice	MN	Non-genotoxic	2,000 mg/kg bw (oral)	BSL Bioservice Study No. 105888 - BSL, 2011c
Subchronic Toxicity Studies				
90 day including *in utero* phase	Rat (20/sex/group)	NOAEL at 5% (equivalent to 3278.9 mg/kg bw/day (males) and 3788.4 mg/kg bw/day (females))	1%, 3%, and 5% of the diet	Bauter, 2013

Table 5 Summary of Preclinical Studies with DHASCO®-B				
Test System	**Type**	**Results**	**Concentration**	**Reference**
Bioequivalence	Piglet (6/sex/group randomized by weight)	Well tolerated and DHASCO®-B and DHASCO® were bioequivalent	Diets 1 and 2 contained DHASCO®-B while Diets 3 and 4 contained DHASCO®. Diets 1 and 3 targeted 0.32% DHA and 0.64% ARA in the formula; Diets 2 and 4 targeted 0.96% DHA and 1.92% ARA in the formula. Control group was fed commercial piglet formula which contained no DHA and 0.21% of ARA.	Fedorova-Dahms *et al.*, 2014
DHASCO®-B (IPA Extraction)				
In vitro Studies				
E. coli WP2uvrA *S. typhimurium* TA98, TA100, TA1535, TA1537	Ames reverse mutation (+/-S9) plate incorporation method	Non-mutagenic	50, 158, 500, 1,580, 4,000, or 5,000 µg/plate	Fedorova-Dahms *et al.*, 2011; BSL Bioservice Study No. 101021 - BSL, 2010a
Human lymphocytes	CA (+/-S9)	Non-genotoxic	1,0, 2.5, or 5.0 µL/mL (4-hr; +/-S9) 1,0, 2.5, or 5.0 µL/mL (24-hr; -S9) 3, 4, or 5 µL/mL (4-hr; +S9)	Fedorova-Dahms *et al.*, 2011; BSL Bioservice Study No. 101022 - BSL, 2010b
In vivo Studies				
NMRI mice	MN	Non-genotoxic	2,000 mg/kg bw (oral)	Fedorova-Dahms *et al.*, 2011; Bioservice Study No. 101023 - BSL, 2010c
Subchronic Toxicity Studies				
90 day including *in utero* phase	20 animals/sex/group	NOAEL at 5% (equivalent to 4122 mg/kg bw/day (males) and 4399 mg/kg bw/day (females))	0.5%, 1.5%, and 5% of the diet	Fedorova-Dahms *et al.*, 2011; Bauter, 2011

bw = body weight; CA = chromosomal aberration; MN = micronucleus; Mut = mutation; S9 = metabolic activation

[a] Increased frequency of chromosomal aberrations was observed at concentrations of 500 μg/mL and greater in the absence of S9; however, no dose-response relationship was observed.

Safety of the Source Organism

Based on existing published and unpublished scientific data, the source microalgae, *Schizochytrium* sp., is a thraustochytrid, a member of the kingdom Chromista (stramenopiles) which includes the heterokont algae. *Schizochytrium* sp. occurs widely in the aquatic environment and is an indirect component of the human food chain through consumption of fish and other marine animals which feed on the microalgae. There have never been any reports of toxic compounds being produced by members of the thraustochytrids. The safety is further supported by published confirmatory safety studies of the dried microalgal source (Table 6).

Martek Biosciences Corporation (now DSM Nutritional Products) has also manufactured and sold algal oil containing approximately 35% DHA produced via fermentation using *Schizochytrium* sp. microalgae (Martek DHA™-S Algal Oil) since 2004, which is GRAS for use in a variety of foods for the general population (GRN 000137) (U.S. FDA, 2001d).

Table 6: Summary of *Schizochytrium* sp. toxicity studies

Study Type	System	Maximum Biomass Dose	Results
***In vitro* Genetic Toxicity** Ames bacterial mutagenicity	5 strains of *Salmonella typhimurium*	500 μg/plate	Not mutagenic[1]
AS52/XPRT mutation assay	Chinese hamster ovary cells	5000 μg/mL without S9 1000 μg/mL with S9	Not mutagenic[1]
Chromosomal aberration	Human peripheral lymphocytes	750 μg/mL	Not clastogenic[1]
***In vivo* Genetic Toxicity** Micronucleus assay	Mouse bone marrow	2000 mg/kg bw	No micronucleus formation[1]
Subchronic Toxicity 13-week	Rat (20/sex/group)	4 g/kg bw/day in the diet.	Not toxic. Sporadic findings attributed to high polyunsaturated fatty acid content of diet[2]

Reproductive Toxicity One-generation study	Rat (30/sex/group)	30% of diet through mating, gestation, and lactation. (0.4 to 17.8 g/kg bw/day for males and 0.48 to 20.7 g/kg bw/day for females)	No dose-dependent changes in fertility or developmental indices[3]
Developmental Toxicity	Rat (25 mated females/group)	30% of diet on gestation days 6 to 15.	Not teratogenic; NOAEL = 22 g/kg bw/day[4]
	Rabbit (22 mated females/group)	800 mg/kg bw/day orally by gavage on gestation days 6 to 19.	Not teratogenic; maternal toxicity NOEL= 600 mg/kg bw/day; developmental toxicity NOEL = 1800 mg/kg bw/day[4]

1 Hammond et al., 2002
2 Hammond et al., 2001a
3 Hammond et al., 2001b
4 Hammond et al., 2001c
NOAEL = no-observed-adverse-effect level.

Preclinical Safety Studies (Aqueous Extraction)

1. 90 day toxicity study with *in utero* phase (Aqueous Extraction)

The potential toxicity of DHASCO®-B was investigated in a 90-day dietary toxicity study, preceded by an *in utero* phase, in Sprague-Dawley CRL:CD®IGS rats (Bauter, 2013 [unpublished study]). This study was conducted in accordance with the Organization for Economic Cooperation and Development (OECD) Test Guideline No. 408 (OECD, 1998) and U.S. Redbook Guideline IV.C.4.a (U.S. FDA, 2003).

During the *in utero* phase, DHASCO®-B was administered at dietary levels of 1.0% (low-dose), 3.0% (mid-dose), or 5.0% (high-dose) % to F_0 rats (13 males and 26 females/group). Two control groups also were included in the study, one that received a standard low fat basal diet and one a basal diet supplemented with 5.0% tuna oil. For the *in utero* phase, parental males and females received the experimental diet while housed separately for a 28-day pre-mating period, followed by feeding through a 14-day co-habitation period. Upon determination of pregnancy, females were removed to a separate cage and continued to be fed through the gestation period of pregnancy and Day 22 of lactation. Males were sacrificed following the weaning of their respective litters. In the subsequent 90-day F_1 phase, the test diets were fed to randomly selected offspring from each litter (generally, 1 male and 1 female/litter) according to their respective original *in utero* treatment groups. Twenty F_1 animals/sex/group were selected to proceed to the 90-day dietary phase. Parameters evaluated in the F_0 generation included viability, signs of gross toxicity, behavioral changes,

body weights, food consumption, fertility, reproductive, and developmental indices. Parameters assessed in the F_1 generation included viability, signs of gross toxicity, behavioral changes, ophthalmology, body weights, food consumption, functional observation battery (FOB), motor activity, haematology, clinical chemistry, urinalysis, organ weights, and gross pathology. Histopathological examination was performed on selected organs and tissues from both control and high-dose groups.

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

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

Fertility and reproductive performance parameters of males and females were comparable between DHASCO®-B groups and the controls. No significant effects on mean gestation length, gestation index, number of implantation sites, number of corpora lutea, pre-implantation loss, post-implantation loss, stillbirths, live births, or viability indices were observed compared to controls.

No significant differences were noted compared to controls in litter loss, litter size, litter or pup weight, sex ratio, time and body weight to attainment of developmental indices and sexual maturity, or pup survival. Taken together, there were no pre-mating, mating, reproductive, or early developmental effects attributed to DHASCO®-B in the *in utero* phase of the study, and all indices remained within historical control values for age- and strain-matched rats.

In the 90-day dietary phase of the study, intakes of DHASCO®-B in the 1.0, 3.0 and 5.0 % dietary regimens were equivalent to doses of 645, 1,973, or 3,279 mg/kg body weight/day in males, and 754, 2,331, and 3,788 mg/kg body weight/day in females, respectively. Intakes of

the tuna oil controls were equivalent to 3,237 and 3,761 mg/kg body weight/day in males and females, respectively.

No mortalities were observed and no clinical signs of toxicity were noted during the 90-day dietary phase. No test article-related ophthalmoscopic findings or test article-related differences in the functional observational battery or motor activity were observed compared to controls. No test article-related adverse changes in haematology, clinical chemistry, coagulation, or urinalysis parameters were observed, and all differences in these parameters from the basal diet control such as cholesterol concentrations reductions in all dose levels in females and the high dose males were determined to be within historical control data or without histological correlates and thus were deemed to be incidental.

Moderate granulomatous infiltration of retroperitoneal fat was observed in 2 high-dose (5 % DHASCO® -B) males. Similar granulomatous infiltration of minimal to slight intensity was noted in the adipose tissue of the mammary gland fat pad in 4 high-dose males and 2 high-dose females. These were considered to be possibly related to the test article. However, the authors considered these effects to be non-adverse and of little biological relevance.

Slight/moderate cytoplasmic vacuolation of cortical cells in the zona fasciculata of the adrenal glands was noted with increased incidence in the tuna oil control and the high dose DHASCO®-B males. Vacuolation microscopically was characterized by the presence of large, single to multiple vacuoles within the cytoplasm of cortical cells consistent with lipid. The increased incidence of this finding in the high dose males was likely due to increased dietary fat content in males fed dietary tuna oil and DHASCO®-B compared to Basal Diet control. These histologic changes were not accompanied by any changes in adrenal organ weight or secondary changes in the affected adrenal glands and were therefore, considered non-adverse. The remaining macroscopic and microscopic findings were not considered to be test substance-related and were considered to be incidental.

Compared to the basal diet control, some changes in liver, heart, testes, kidney, and spleen weight were reported; however, these were without histological correlates, without a dose-response relationship, and thus were deemed to be toxicologically insignificant by the authors. Based on the results of the study, the authors derived a NOAEL of 3,279 and 3,788 mg/kg body weight/day for male and female rats respectively, the highest doses tested.

Toxicokinetic results indicated that DHA from DHASCO®-B was bioavailable as demonstrated by the elevated DHA concentrations in plasma, brain and liver compared to the Basal Diet control levels. Tissue DHA levels were higher than those of the Basal Diet control both at weaning and at the end of the 90-day study and in both genders. The increases were generally dose-dependent.

Similar results were reported by Schmitt et al. (2012) in 14 and 90-day subchronic dietary studies with an *in utero* exposure phase, where a similarly produced (extracted with IPA) DHA-rich algal oil administered at concentrations of 0, 10,000, 25,000, and 50,000 ppm in the diet for 2 and 13 weeks did not produce any significant toxicological manifestations. The algal oil test article was well tolerated at these dietary levels as evidenced by the absence of major treatment-related changes in the general condition and appearance of the rats, neurobehavioral endpoints, growth, feed and water intake, ophthalmoscopic examinations, routine hematology and clinical chemistry parameters, urinalysis, or necropsy findings. The no observed adverse effect level (NOAEL) was determined to be 50,000 ppm, the highest dose tested, and equivalent to at least 3,305 and 3,679 mg/kg bw/day, for male and female rats, respectively.

2. Bioequivalence Study in Piglets (Aqueous Extraction)

The study sought to evaluate the bioequivalence and safety of DHASCO®-B (Aqueous extracted) relative to DHASCO® when administered in a blend with ARASCO (ARA Single Cell Oil) in the neonatal pig (Fedorova-Dahms et al., 2014). The neonatal pig model was selected as the most appropriate for testing substances new to infant formula (IOM, 2004; Flamm, 2013). The study was conducted in compliance with Good Laboratory Practice (GLP) Regulations and according to the following guidelines: FDA Center for Drug Evaluation and Research Guidance on Nonclinical Safety Evaluation of Pediatric Drug Products (2006); European Medicines Agency Guideline on the Need for Non-Clinical Testing in Juvenile Animals of Pharmaceuticals for Pediatric Indications (2008).

Piglets were fed one of four formulas with added DHA and ARA, and a control commercial formula ProNurse milk replacer with no added LC-PUFA. Formulas provided DHA at two dose levels: approximately 0.32% and 0.96% of total fatty acids blended with twice this amount of ARA. Diets 1 and 2 contained 0.32% DHA + 0.64% ARA targeting the maximum expected human

levels (1x); Diets 3 and 4 contained 0.96% DHA + 1.92% ARA and were designed to provide three times the maximum expected human levels (3x). Diets 1 and 2 contained DHASCO®-B, while Diets 3 and 4 - DHASCO®. The diets were administered from day 2 to 22 after birth, upon which tissues were harvested and analyzed for DHA and ARA accretion. In addition, piglet growth and development (clinical observations, body weights, food consumption), and clinical pathology parameters (hematology, clinical chemistry, coagulation and urinalysis) were evaluated. Macro- and microscopic pathology evaluations were also performed at the end of the study to assess safety of DHASCO®-B. The bioequivalence of DHASCO®-B to DHASCO® was determined by DHA accretion in cerebral cortex, liver, and heart as well as circulating levels of DHA and ARA in red blood cells (RBC) and plasma.

Based on food consumption and body weight data, there were no differences in calculated compound consumption between the corresponding doses of DHASCO®-B and DHASCO®: consumption of Diet 1 was very close to that of Diet 3, and of Diet 2 – to Diet 4. There were no gender differences in the DHA oil consumption, therefore data were combined for both genders: Diet 1 animals consumed 98.2 mg/kg/day of DHASCO®-B; Diet 2 – 294.7 mg/kg/day of DHASCO®-B; Diet 3 – 101.0 mg/kg/day of DHASCO®; Diet 4 – 291.4 mg/kg/day of DHASCO®.
Dietary administration of DHASCO®-B (Diets 1 and 2) and DHASCO® (Diets 3 and 4) was well tolerated by the preweaning piglets during the three week dosing period right after birth. All animals survived to scheduled necropsy and no test article-related changes were noted in clinical observations. All piglets were in good health and grew and developed as expected for their age.

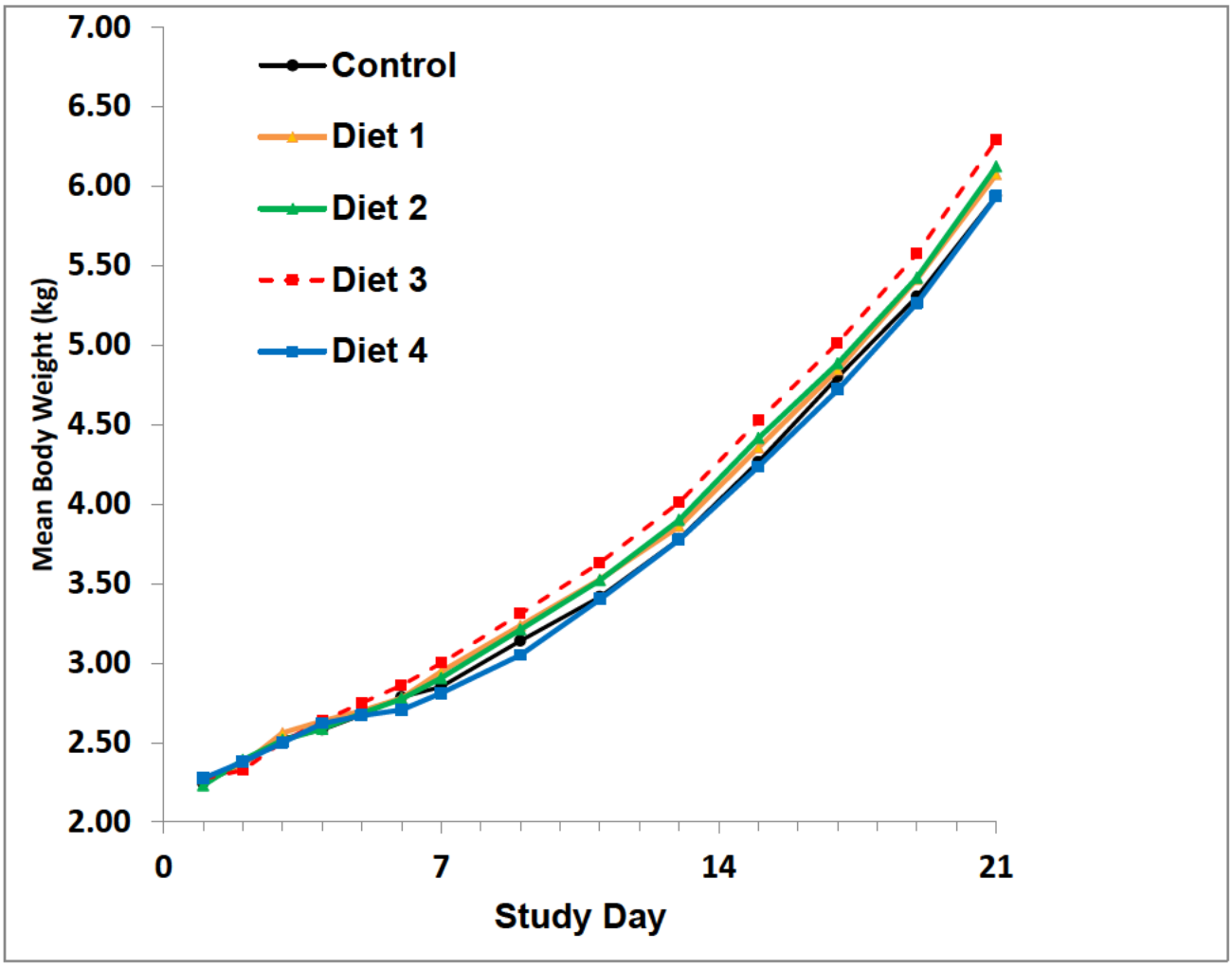
Body weight, body weight gains as well as food consumption and food efficiency were unaffected by dietary treatment in both males and females (Figure 3).

There were no adverse changes in hematology, clinical chemistry, coagulation, or urinalysis parameters in male and female piglets that were attributable to the administration of any DHA algal oils tested (DHASCO®-B or DHASCO®).

No adverse macroscopic changes related to DHASCO®-B or DHASCO® administration were observed at the end of the study.

There were no DHASCO®-B or DHASCO®-related microscopic findings. The mild red focus/foci of the lung observed in one Diet 2 (3x DHASCO®-B) female and in one Diet 4 (3x DHASCO®) female correlated with mild focal hemorrhage microscopically in both females and was considered incidental and of the type occasionally observed as spontaneous/background lesions of swine of this age and breed.

Figure 3: Mean body weight values (kg). Control Diet had no added DHA and ARA; Diet 1 contained DHASCO®-B (1x); Diet 2 - DHASCO®-B (3x); Diet 3 - DHASCO® (1x); Diet 4 - DHASCO® (3x)

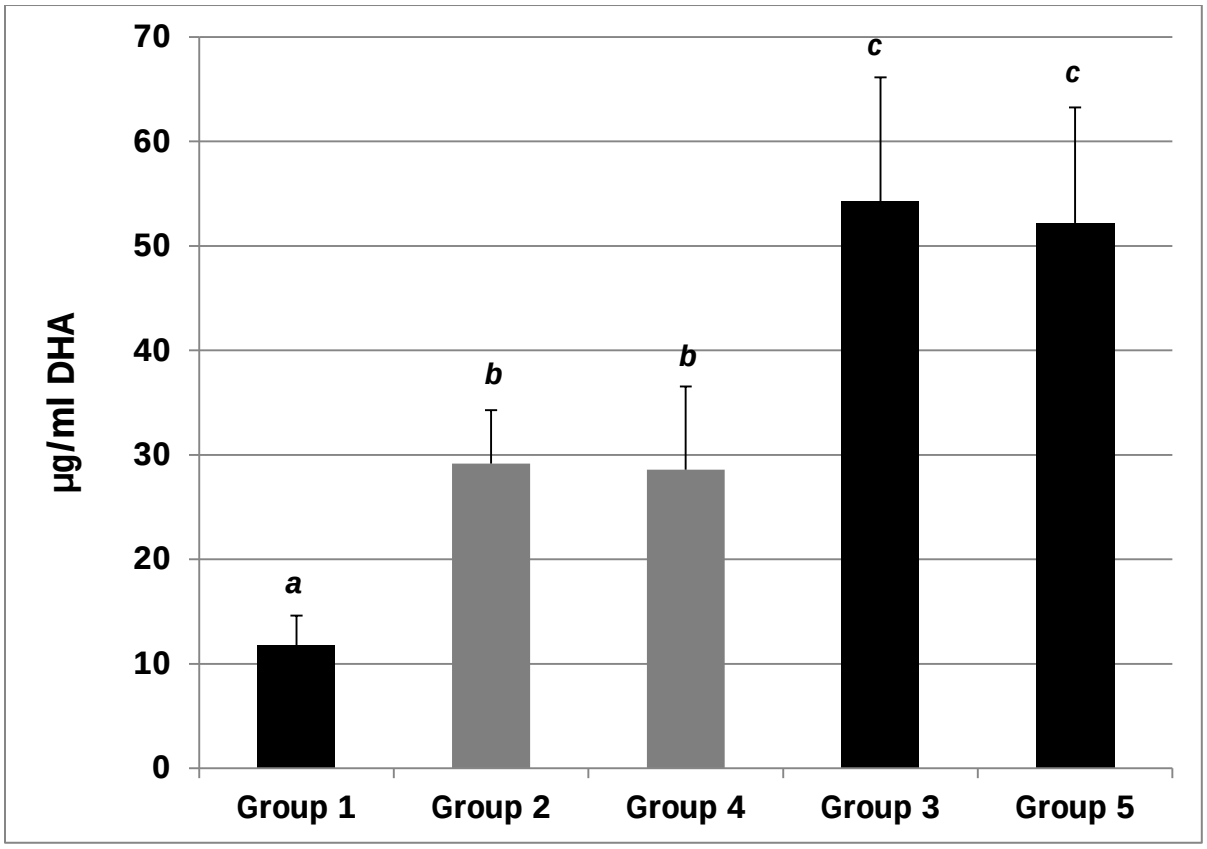


There were no DHASCO®-B and DHASCO®-related organ weight changes. Absolute body weights did not differ between diets. Increases in relative liver to body weights were observed in Diet 2 males (3x DHASCO®-B) by 10%, Diet 3 males (1x DHASCO®) by 12%, and Diet 4 males (3x DHASCO®) by 10%; however, there were no histological correlates to account for these

changes. As the changes were small in magnitude, with no dose relationship and no histological correlates, they were considered toxicologically insignificant. There were no other organ weight changes observed in either sex or treatment group.

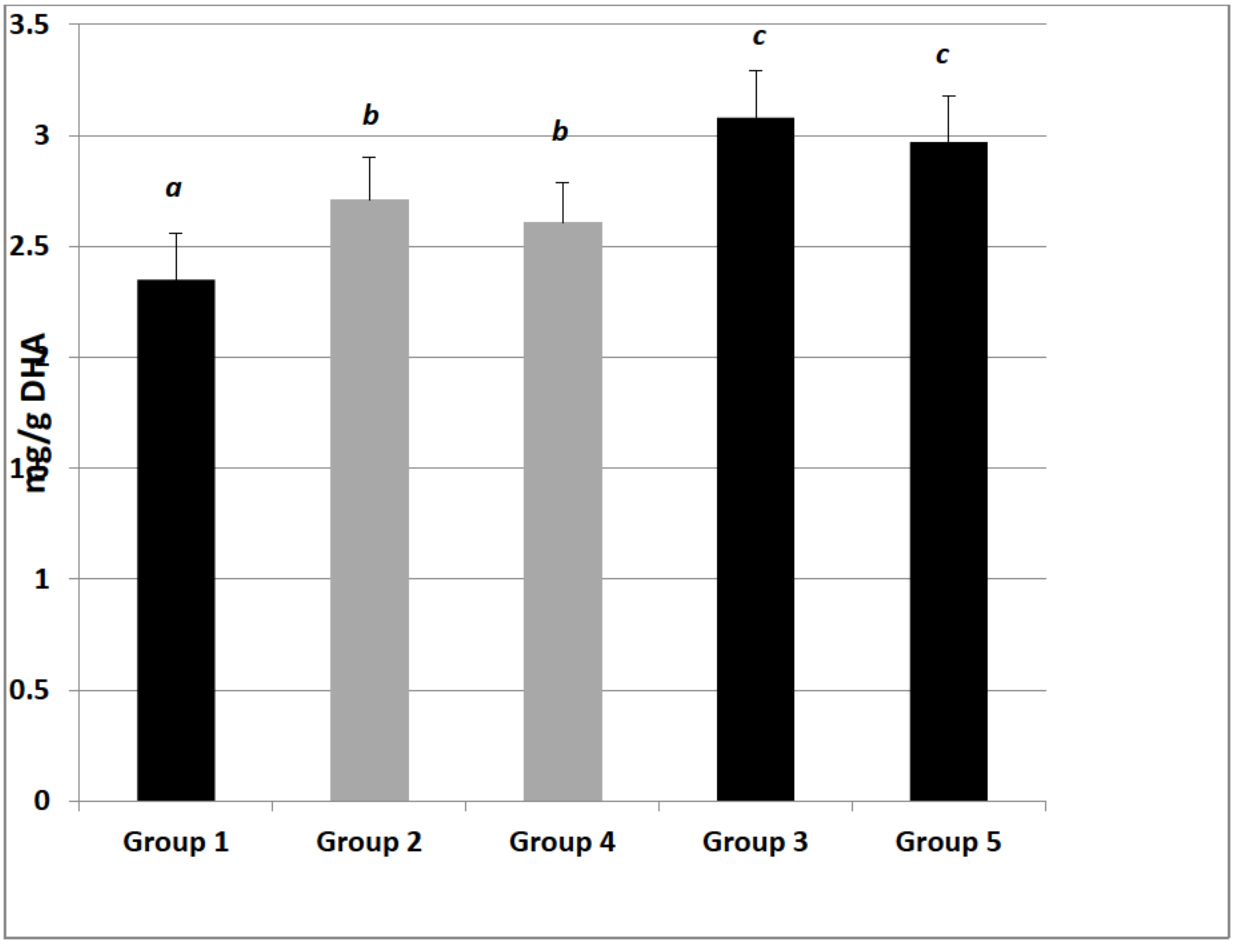
Further, DHASCO®-B was bioequivalent to DHASCO® as indicated by the piglet growth and tolerance data, as well as DHA accretion in tissues. In plasma and RBC, dose-dependent increases in DHA levels were observed in the DHASCO®-B and DHASCO® treated animals. There were no differences between the corresponding DHASCO®-B and DHASCO® groups: the Diet 1 DHA level (1x DHASCO®-B) did not differ from that of Diet 3 (1x DHASCO®); as also, the Diet 2 DHA level (3x DHASCO®-B) did not differ from Diet 4 (3x DHASCO®). Similar to plasma and RBC, DHA increases in the liver, heart, and brain cortex were dose dependent regardless of DHA source and DHASCO®-B was bioequivalent to DHASCO®.

Figure 4: Plasma total lipid content of DHA after 3 weeks of DHASCO® and DHASCO®-B supplementation in piglet formula. Values are given as mean +SD. Data combined for both genders (n=12/group). Group 1 is control (no added DHA and ARA); Group 2 is 1x DHASCO®-B; Group 3 is 3x DHASCO®-B; Group 4 is 1x DHASCO®; Group 5 is 3x DHASCO®.



[a,b,c] signify between group differences as determined by ANOVA (p<0.05)

Figure 5: Brain cortex total lipid content of DHA after 3 weeks of DHASCO® and DHASCO®-B supplementation. Values are given as mean +SD. Data combined for both genders (n=12/group). Group 1 is control (no added DHA and ARA); Group 2 is 1x DHASCO®-B; Group 3 is 3x DHASCO®-B; Group 4 is 1x DHASCO®; Group 5 is 3x DHASCO®.



[a,b,c] signify between group differences as determined by ANOVA (p<0.05)

3. Genotoxicity studies (Aqueous Extraction)

Reverse Mutation (Ames) Assay

A bacterial reverse mutation test (Ames test) was conducted to assess the potential mutagenicity of DHASCO®-B in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2*uvr*A (BSL Bioservice Study No. 105886 - BSL, 2011a). This study was conducted in compliance with the OECD Test Guideline No. 471 (OECD, 1997a), Commission

Regulation (EC) No. 440/2008 (Commission of the European Communities, 2008), and the Environmental Protection Agency (EPA) Test Guideline OPPTS 870.5100 (U.S. EPA, 1998a). No biologically relevant increases in revertant colony numbers of any of the 5 tester strains were observed following treatment with DHASCO®-B at any concentration level, neither in the presence nor absence of metabolic activation. Positive control agents substantially induced the number of revertant colonies compared to the negative control, confirming the sensitivity of the assay. Based on these findings, the investigators concluded that DHASCO®-B did not induce gene mutations by base-pair changes or frameshifts in the genomes of the tester strains used and therefore was non-mutagenic.

***In-vitro* Mammalian Chromosome Aberration Test**

An *in vitro* mammalian chromosome aberration test in human lymphocytes was conducted to further evaluate the genotoxic potential of DHASCO®-B in the presence and absence of metabolic activation (BSL Bioservice Study NO. 105887 – BSL, 2011b). This study was conducted in accordance with OECD Test Guideline No. 473 (OECD, 1997b), Commission Regulation (EC) No. 440/2008 (Commission of the European Communities, 2008), EPA Test Guideline OPPTS 870.5375 (U.S. EPA, 1998b), and the International Conference on Harmonisation (ICH) Guideline S2(R1) (ICH, 2011).

The chromosomes were prepared 24 h after start of treatment with DHASCO®-B at concentrations up to 5,000 µg/mL. The treatment interval was 4 h with and without metabolic activation (experiment I) and 4 h with and 24 h without metabolic activation (experiment II). Two parallel cultures were set up. 100 metaphases per culture were scored for structural chromosomal aberrations. In both experiments, an extension of evaluation was performed due to inconsistent results within some concentrations.

The test item was tested as suspension. Precipitates were seen at concentrations of 750 µg/mL and higher. No toxic effects of DHASCO®-B were noted at all concentrations evaluated in both experiments with an exception of 5000 µg/mL in the experiment II without metabolic activation (24 h treatment) when a decrease of the mitotic index was observed.

DHASCO®-B did not induce chromosomal aberrations in human lymphocytes in both experiments conducted in the absence of metabolic activation. In both experiments with metabolic activation, an increase in the frequency of chromosomal aberrations was noted at

concentrations of 500 µg/mL and greater; however, no dose-response relationship was observed. Some increases were within the historical control data of the negative controls. In both experiments, positive controls induced distinct and biologically relevant increases in the incidence of cells with structural chromosomal aberrations. No biologically relevant increase in the frequency of polyploidy cells was observed in any experiment.

Thus, DHASCO®-B did not induce structural chromosomal aberrations in human lymphocytes in the absence of metabolic activation, but induced an increase in the frequency of chromosomal aberrations in the presence of metabolic activation; however, given that the clastogenic effect was relatively moderate, observed mostly at concentrations beyond the solubility limit and a dose-response relationship was not observed, the study authors concluded that the results of the *in vitro* chromosomal aberration test were equivocal.

***In-vivo* Mouse Micronucleus Test**

A micronucleus test was performed in the immature erythrocytes in the bone marrow of the mouse to investigate the genotoxic potential of DHASCO®-B *in vivo* (BSL Bioservice Study Report No. 105888 – BSL, 2011c). This study was conducted in accordance with OECD Guideline No. 474 (OECD, 1997c), Commission Regulation (EC) NO. 440/2008 (Commission of the European Communities, 2008), and EPA Test Guideline OPPTS 870.5395 (U.S. EPA, 1998c). In a preliminary dose-range finding study, NMRI mice (1/sex) were administered the test article at a single dose of 2,000 mg/kg body weight *via* intraperitoneal (i.p.) injection with no signs of toxicity observed. Therefore, this dose was selected as the maximum tolerable dose in the main micronucleus test. In the main micronucleus test, NMRI mice (5/sex) were administered DHASCO®-B at a single dose of 2,000 mg/kg body weight *via* i.p. injection. The negative and positive control groups were administered cottonseed oil and 40 mg/kg body weight of CPA, respectively. No toxicity was observed in animals administered the test article. DHASCO®-B did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse as no increases in micronuclei was found after the treatment. The incidence of micronuclei in the negative control group was reported to be within the range of historical laboratory control data. A significant increase in micronuclei was observed in the positive control group, thus confirming the validity of the assay. Therefore, DHASCO®-B obtained *via* aqueous extraction was considered to be non-genotoxic as assessed in the *in vivo* mammalian micronucleus test.

Conclusions of Genetic Toxicity Testing

Based on criteria recognized in the International Conference on Harmonization (ICH) Guideline S2(R1): Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, March 2008:

"Comparative trials have shown conclusively that each *in vitro* test system generates both false negative and false positive results in relation to predicting rodent carcinogenicity… The test battery approach is designed to reduce the risk of false negative results for compounds with genotoxic potential, whereas a positive result in any assay does not necessarily mean that the test compound poses a genotoxic hazard to humans. Although positive *in vitro* data may indicate intrinsic genotoxic properties of a compound, appropriate *in vivo* data determine the biological significance of these *in vitro* signals in most cases."

Therefore, despite the equivocal results of the *in vitro* Mammalian Chromosome Aberration Test, the negative results of the *in vivo* Mouse Micronucleus Test and the Bacterial Reverse Mutation test are sufficient to demonstrate absence of genotoxic activity of DHASCO®-B. In addition, previous studies have shown that the algal oil was non-genotoxic in all three above-mentioned tests, including the *in-vitro* Mammalian Chromosome Aberration Test (Schmitt et al., 2012). Moreover, DHASCO®-B manufactured using IPA recovery technology was also tested in the same battery of tests and found to be non-genotoxic in all of them (see below).

Additional Supporting Preclinical Safety Studies (IPA Process)

DHA Algal Oil from *Schizochytrium* sp. (DHASCO®-B) manufactured using IPA recovery technology was evaluated by testing for gene mutations, clastogenicity and aneugenicity, and in a 90-day with *in utero* phase Sprague-Dawley rat dietary study (Fedorova-Dahms et al, 2011). All studies were performed in accordance with Good Laboratory Practice.

1. 90-day toxicity study with in utero phase (IPA)

The potential toxicity of DHASCO®-B obtained *via* IPA extraction was investigated in an additional 90-day dietary toxicity study, preceded by an *in utero* phase, in Sprague-Dawley rats (Fedorova-Dahms *et al.,* 2011). This study was conducted in accordance with OECD Test Guideline No. 408 (OECD, 1998) and U.S. Redbook Guideline IV.C.4.a (U.S. FDA, 2003). During the *in utero* phase, DHASCO®-B was administered at dietary levels of 0.5% (low-dose), 1.5%

(mid-dose), or 5% (high-dose) to F_0 rats (13 males and 26 females/group). Two control groups also were included in the study, one that received a standard low fat basal diet and one a basal diet supplemented with 5% fish oil. Males were fed the test or control diets for 4 weeks before mating, and throughout the mating and gestation periods. Females were fed the test or control diets for 4 weeks before mating, and throughout the mating, gestation, and lactation periods. In the subsequent 90-day F_1 phase, the test diets were fed to randomly selected offspring from each litter (1 to 2 animals/sex/litter) according to their original *in utero* groups. Twenty F_1 animals/sex/group were selected to proceed to the 90-day dietary phase and half of them (10 animals/sex/group) were included in a 30-day recovery period. Parameters evaluated in the F_0 generations included viability, signs of gross toxicity, behavioural changes, body weights, food consumption, and fertility, reproductive, and developmental indices. Gross necropsies were performed on all post-partum F_0 dams, and the ovaries and uteri from all F_0 animals were removed and weighed. All unselected pups were macroscopically examined on Post-Natal Day (PND) 22. Parameters assessed in the F_1 generation included viability, signs of gross toxicity, behavioural changes, ophthalmology, body weights, food consumption, FOB, motor activity, haematology, clinical chemistry, urinalysis, organ weights, and gross pathology. Histopathological examination was performed on selected organs and tissues from the both control and high-dose groups.

Based on the mean daily food consumption and body weight values for males, the mean intake of DHASCO®-B in the 4 weeks of premating was 340, 1022 and 3466 mg/kg bw/day in the low-, mid- and high-dose groups, respectively. In females, these corresponded to 404, 1187 and 4013 mg/kg bw/day.

There were no test-article related effects on mortality, clinical observations, body weight, body weight gain, food consumption, or food efficiency observed in the F_0 generation. Dietary administration of DHASCO®-B was not reported to adversely affect the fertility or reproductive performance of the F_0 rats: fertility indices, gestation index, gestation length, mean number of implantation sites, corpora lutea, and pre-and post- implantation loss percentage were comparable between the control and DHASCO®-B treated groups. There were no significant differences in litter size, stillbirth index, sex ratio, pup viability, survival or mean pup weights reported among groups during the lactation period. The normal attainment of developmental landmarks (*i.e.,* hair growth, pinna detachment, incisor eruption, and eye opening) was reported to be comparable among the control and test groups.

There were no test article-related macroscopic findings observed in any of the post-natal dams or unselected pups. Significantly lower absolute ovarian and uterine weights were observed in the fish oil control and the high-dose dams, and significantly lower relative ovarian and uterine weights were noted in high-dose dams compared to those in the basal diet control; however, the investigators did not consider these findings to be toxicologically significant as the changes were small in magnitude and within historical control values.

In the subsequent F_1 90-day phase, no mortality, clinical observations, or ophthalmological abnormalities related to administration of DHASCO®-B were noted in any group. There were no test article-related changes in motor activity or behaviour assessed in the FOB battery observed in any test group. There were no significant differences in mean body weights observed in males administered DHASCO®-B compared to their respective basal diet controls. Mid- and high-dose females exhibited higher body weights during the latter portion of the administration period until the end of the recovery phase compared to the basal diet controls. This finding in DHASCO®-B-treated females seems to be related to the fact that they did not compensate for the increased fat in the DHASCO®-B diet (twice that of the basal diet) by changes in food consumption. Rodents normally reduce food intake in response to increased caloric density of the diet. There were no statistically significant differences in food consumption noted in the test groups compared to the basal diet control group.

There were no adverse changes in hematology, clinical chemistry, coagulation, or urinalysis parameters in male and female rats that were attributable to the administration of DHASCO®-B. Slightly lower values for red blood cell count and packed red cell volume observed in males fed the high dose DHASCO®-B were considered clinically insignificant due to the low magnitude of the effect and its reversibility.

Males and females in the fish oil control and high-dose DHASCO®-B groups exhibited significantly higher mean ALP activity and lower serum cholesterol levels compared to their respective basal diet controls. Significantly higher triglyceride levels also were observed in males and females in the fish oil control group, and the high-dose females compared to the basal diet control, and this effect was observed throughout the recovery phase. The investigators noted that the increase in ALP activity observed in the high-dose and fish oil control groups was consistent with the findings of other studies that evaluated the safety of LC-PUFA oils (Arterburn *et al.*, 2000; Lina *et al.*, 2006; Blum *et al.*, 2007; Casterton *et al.*,

2009; Fedorova-Dahms *et al.*, 2011). Given that an increase in mean ALP activity also was observed in the fish oil control group, the investigators attributed this finding to the dietary administration of high levels of LC-PUFA. The investigators further noted that the reductions in cholesterol levels observed in the high-dose DHASCO®-B and fish oil control groups were expected, and that this effect was due to the lipid-lowering action of LC-PUFA (Harris *et al.*, 1988; Hempenius *et al.*, 2000; Ryan *et al.*, 2009).

At necropsy, mottled discoloration was noted in several livers of mid-dose females, high-dose males and females, and fish oil control males and females; however, these were not accompanied by histopathological findings and were not observed at the end of the recovery. Therefore, the investigators did not consider these findings to be adverse.

Compared to the basal diet control, some changes in liver, kidney, heart, spleen, ovaries and adrenals weight were reported both in the high-dose DHASCO®-B females and the fish oil females; however, these were without histological correlates for all but spleen and adrenals, were not observed at the end of the recovery period, or were also observed in the fish oil control group, and thus were deemed to be toxicologically insignificant by the authors.

The increase in adrenal and splenic weights had histopathological correlates. Minimal to slight extramedullary hematopoiesis of the spleen was noted in the basal diet, the fish oil control, and the high-dose DHASCO®-B males and females. The intensity was slightly increased in the fish oil group. Therefore, this was not considered to be related to DHASCO®-B. The increased incidence of slight cytoplasmic vacuolation of adrenal cortical cells of the zona fasciculata was observed both in the high-dose DHASCO®-B and the fish oil control.

Given that the histopathological findings observed in the high-dose DHASCO®-B group also were observed in the fish oil control group, the investigators did not consider these changes to be test article-related, but instead suggested that they were physiological adaptations to accommodate the large LC-PUFA load in the diet.

Based on the results of the study, the authors determined the NOAEL of DHASCO®-B to be 5% in the diet or 50,000 mg/kg diet, the highest dose tested, for male and female rats over a 90-day post-natal period following pre-natal parental exposure and during maternal lactation.

This dose level corresponds to 4,122 and 4,399 mg DHASCO®-B/kg body weight/day for male and female rats, respectively, or an average of 4,260 mg/kg body/day for both sexes.

2. Genotoxicity Studies (IPA Extraction)

Reverse Mutation (Ames) Assay

A reverse mutation assay in *Salmonella typhimurium* and *Escherichia coli* was conducted in accordance with "Bacterial Reverse Mutation Test": OECD Guideline for the Testing of Chemicals, Test Guideline 471 (OECD, 1997a; BSL, 2010a) at BSL Bioservice GmbH, Planegg, Germany.

No biologically relevant increases in revertant colony numbers of any of the 5 tester strains were observed following treatment with DHA Algal Oil or at any concentration level, neither in the presence or absence of metabolic activation. The study authors concluded that DHASCO®-B did not induce gene mutations by base-pair changes or frameshifts in the genomes of the tester strains used and therefore was non-mutagenic.

In-vitro Mammalian Chromosome Aberration Test

An *in-vitro* mammalian chromosome aberration test in human lymphocytes was conducted to OECD Guideline No 473 "*In-vitro* Mammalian Chromosomal Aberration Test" (OECD, 1997b; BSL, 2010b). The genotoxicity was assessed in the presence and absence of metabolic activation by S-9 homogenate at concentrations up to 5,000 µg/mL. The chromosomes were prepared 24 h after the start of treatment with the test item. The treatment interval was 4 h with and without metabolic activation (experiment I) and 4 h with and 24 h without metabolic activation (experiment II). Two parallel cultures were used. One hundred (100) metaphases per culture were scored for structural chromosomal changes. Ethylmethanesulfonate (EMS) at 400 and 600 lg/mL and cyclophosphamide (CPA) at 5 lg/mL were used as positive controls.

No toxic effects of DHASCO®-B were noted with and without metabolic activation in any dose group evaluated in experiments I and II. No biologically relevant increase of aberration rates was noted after the treatment and incidences were within the historical control data of the negative control. No biologically relevant increase in the frequencies of polyploid cells was found after treatment with DHASCO®-B. Positive controls, EMS and CPA, induced distinct and

biologically relevant increases in cells with structural chromosomal aberrations indicating the validity of the experiments.

Thus, DHASCO®-B did not induce structural (clastogenicity) or numerical (aneugenicity) chromosomal aberrations in any of the doses tested.

In-vivo Mouse Micronucleus Test

An *in vivo* mouse micronucleus test was conducted in accordance with OECD Guideline No 474 "Mammalian Erythrocyte Micronucleus Test" (OECD, 1997c; BSL, 2010c). The micronucleus test was performed to investigate the potential of DHASCO®-B to induce micronuclei in polychromatic erythrocytes (PCE) in murine peripheral blood. The test item was diluted with corn oil to achieve an orally administered volume of 10 mL/kg. A dose of 2000 mg/kg of DHASCO®-B was selected as the maximum tolerable dose. Corn oil was administered at the same dose. Peripheral blood samples were collected for micronuclei analysis 44 and 68 h after a single administration of the test item. CPA (40 mg/kg i.p.) was used as a positive control. For all experimental groups, including positive and negative controls, 10,000 polychromatic erythrocytes per animal were scored by flow cytometry analysis for incidence of micronucleated immature erythrocytes.

No biologically relevant increase of micronuclei was found after treatment with DHASCO®-B compared with both the negative control and historical control values. CPA used as positive control induced a significant increase in micronucleus frequency, thus demonstrating the validity of the assay. Thus, DHASCO®-B did not induce structural or numerical chromosomal damage in the immature erythrocytes of the mouse.

Collectively, these results confirm that DHASCO®-B possesses a toxicity profile similar to other current marketed algal oils and support the safety of DHA-rich algal oil for its proposed use in food.

Safe Use in Infant Formula

Support for the safe history of use of DHA from algal oil in infant formula comes directly from the U.S. FDA. In September 2011 the FDA completed a comprehensive analysis of infant

studies published since 2001 and providing DHA/ARA supplementation that reported adverse events. The FDA further analyzed all infant formula adverse event reports, focusing in particular on gastrointestinal related (GI) adverse events, in the CFSAN Adverse Event Reporting System (CAERS) database from 2000, before the introduction of DHA and ARA oils, to 2009, when essentially all formulas contained these oils. The FDA concluded, "We found no statistically significant increases in the proportion of GI adverse events reports in CAERS when we looked over the time interval from when infant formulas containing DHA and ARA oils were first introduced until they essentially replaced non-supplemented formula in the market place."(U.S. FDA, 2011)

See also Part 3 Section A.2 of this application.

Acute toxicity studies, carcinogenicity studies, neurotoxicity studies, and immunotoxicity studies are not available.

3. *Safety assessment reports prepared by international agencies or other national government agencies, if available.*

Please refer to Section 1.3 of this application.

C. Information on dietary intake of nutritive substance

1. A detailed list of the food groups or foods proposed to contain the nutritive substance, or changes to currently permitted foods

DSM Nutritional Products intends to market DHA Algal Oil produced from a new production strain of *Schizochytrium* sp. in Australia and New Zealand for use as a direct ingredient in infant formula products [including preterm and term infant formulas (ages from birth to 12 months), as well as in follow on formulas]. DHA Algal Oil (DHASCO®-B) may be used as a replacement for or alternative to DHASCO®.

2. The maximum proposed level of the nutritive substance for each food group or food, or the proposed changes to the currently permitted levels

According to Standard 2.9.1 of the Food Standards Code, n-3 long chain polyunsaturated fatty acids may be present in an infant formula or follow-on formula at a maximum 1% of the total fatty acid content. DHA Algal Oil (DHASCO®-B) may be used according to this Standard.

Docosahexaenoic acid (DHA) occurs naturally in human milk.

3. For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

Infant formula is a food group listed in the 2010 Australian National Infant Feed Survey (ANIFS) and the Australian National Children's Nutrition and 2007 Physical Activity Survey (ANCNPAS). Data regarding infant formula use among children living in southern Australia is available from the 2009/2010 South Australian Infant Dietary Intake (SAIDI) survey (Byrne et al., 2014).

4. The percentage of the food group in which the nutritive substance is proposed to be used or the percentage of the market likely to use the nutritive substance

There are only a few manufacturers of infant formula who currently include an algal source of DHA in their products sold in Australia and New Zealand. These are primarily Wyeth and Aspen, who now owns the Wyeth brand in Australia. The current stage-1 Wyeth products in New Zealand and the Aspen products in Australia contain 55 mg DHA (137.5 mg DHA Algal oil) per 100 g of reconstituted infant formula powder.

5. Information relating to the use of the nutritive substance in other countries

Historically, since the mid-1990s Martek's DHA-rich oil from *Crypthecodinium cohnii* (DHASCO®) has been used safely in infant (term and preterm) and follow-on formula. DHASCO® is now approved throughout the world as a source of DHA for infant (term and preterm) and follow-on formula and is used by nearly all manufacturers in the United States. DHASCO has been added to infant formula products in Australia and New Zealand since 1998.

Infant formulas containing DHASCO® are now marketed in over 75 countries worldwide. It is estimated that over 50 million infants have received formulas containing DHA from algal oil, and no safety concerns have arisen in connection with their commercial use. The following (Table 7) lists the commercial introductions of fortified formulas in various countries worldwide.

Table 7*Countries with commercial infant formulas containing algal oil as a source of DHA

Country	Manufacturer	Country	Manufacturer
Argentina	Wyeth	Latvia	Semper
Austria	Nestle	Lebanon	Wyeth
Australia	Wyeth	Lithuania	Semper
Bahamas	Wyeth	Malaysia	Wyeth
Bahrain	Wyeth	Malta	Wyeth
Barbados	Wyeth	Mauritius	Wyeth
Belgium	Mead	Mexico	Wyeth
Belize	Mead	Myanmar	Numico
Brazil	Nestle	Netherlands	Friesland
Brunei	Mead	New Zealand	Wyeth
Cambodia	Numico	Norway	Semper
Canada	Mead	Oman	Wyeth
Cayman Island	Wyeth	Pakistan	Nestle
Chile	Wyeth	Peru	Wyeth
China	Mead	Philippines	Wyeth
Colombia	Wyeth	Poland	Mead
Costa Rica	Mead	Portugal	Numico
Curacao	Wyeth	Puerto Rico	Abbott

Table 7*Countries with commercial infant formulas containing algal oil as a source of DHA			
Country	**Manufacturer**	**Country**	**Manufacturer**
Cypress	Wyeth	Qatar	Wyeth
Czech Republic	Nestle	Russia	Semper
Dominican Republic	Mead	Saudi Arabia	Wyeth
Ecuador	Wyeth	Singapore	Wyeth
Egypt	Wyeth	Slovakia	Nestle
Finland	Semper	South Africa	Wyeth
France	Mead	South Korea	Abbott
Germany	Nestle	Spain	Mead
Gibralter	Wyeth	Sweden	Semper
Greece	Wyeth	Switzerland	Nestle
Guam	Abbott		Wyeth
Guyana	Wyeth	Thailand	Wyeth
Hong Kong	Wyeth	Trinidad	Wyeth
Iceland	Semper	Turkey	Wyeth
India	Nestle	UK	Wyeth
Indonesia	Wyeth	UAE	Wyeth
Ireland	Wyeth	Ukraine	Nestle
Israel	Maabarot	United States	Mead
Italy	Mead	Uruguay	Wyeth
Jamaica	Wyeth	Venezuela	Wyeth
Jordan	Wyeth	Vietnam	Mead
Kuwait	Wyeth	Yemen	Wyeth

**In combination with ARASCO®*

6. For foods where consumption has changed in recent years, information on likely current food consumption

The 2010 ANIFS survey indicates that 34% of Australian infants less than 1 month of age are consuming at least some infant formula with 26.5% consuming infant formula in the 24 hours prior to participating in the survey. By 6 months of age nearly 70% of infants are consuming at least some infant formula although 60% continue to receive breast milk as well. So for most young infants, infant formula contributes at least part of their daily caloric intake. The 2009/2010 SAIDI survey provides insight regarding older infants and young children and suggests that as complementary feeding begins the proportion of children aged 12-16 months consuming any formula drops to 32% and to 23% for breast milk (Bryne et al., 2014). The 2007 ANCNPAS survey suggests that young children ages 2-3 years consume little if any formula with only 0.5% of total daily energy intake supplied by formula. Based on available consumption data, it is likely that most of the DHA from DHA Algal Oil will be consumed by infants 1-12 months of age with a much smaller percentage of young children 12-24 months consuming formula with DHA Algal Oil. Only a fraction of young children 24-36 months will consume these products.

D. Information related to the nutritional impact of a nutritive substance other than vitamins and minerals (for vitamins and minerals see Part E)

1. *Information related to the nutritional purpose of adding the nutritive substance to each food*

(a) data to demonstrate that specific food(s) containing the form and amount of the nutritive substance can contribute to the nutritional purpose in the target population group at the anticipated level of intake; or

While the content of n-3 LCPUFA varies slightly between algal oils, the bioequivalence of oil derived from the algal strains Crypthecodinium cohnii (DHASCO®) and Schizochytrium sp. (DHA-S; DHA-O; DHA-B) has been confirmed (Arterburn et al., 2007). The bioavailability of DHA from algal sources used in infant formula has been demonstrated by Birch et al. (2010)

and others. Specifically, Birch and co-workers demonstrated a dose-response increase in red blood cell (RBC) DHA concentrations when infants were supplemented with 0.32 (n=64 infants), 0.64 (n=59 infants), and 0.96% DHA (n=65 infants) from algal oil along with a fixed level of ARA. DHA RBC levels increased as the percentage of DHA in the formula increased with DHA status being statistically different between formula groups at 4 and 12 months of age. The bioavailability of DHA from algal oil in young children (~4 years) has also been demonstrated with Ryan and Nelson (2008) reporting a 300% increase in capillary whole blood content of DHA after 4 months of 400 mg DHA supplementation versus placebo.

(b) data to demonstrate that the nutritional composition of the specified substitute food can be aligned with the reference food.

Not applicable.

E. Information related to the nutritional impact of a vitamin or mineral

Not applicable.

F. Information related to potential impact on consumer understanding and behavior

1. Information to demonstrate the level of consumer awareness and understanding of the nutritive substances in the food(s)

DHASCO® has been added to infant formula products in Australia and New Zealand since 1998 so consumer awareness of DHA is expected to be high. The 2013 Australian Dietary Guidelines (NHMRC, 2013) recognizes fish as a nutritious source of omega-3 LCPUFAs and encourages consumption of low mercury fish to promote "…a number of health benefits for women and their children." With this recent advice, understanding of the role of DHA in infant formula is likely to be high among purchasers of this product.

2. Information on the actual and/or potential behavior of consumers in response to proposed food(s)

DHASCO®-B is proposed as an alternative to or replacement for DHASCO® as a source of docosahexaenoic acid (DHA) in infant formula (term or preterm) or follow on formula, so changes in consumer behavior are not expected.

3. Information to demonstrate that the consumption of food(s) containing the nutritive substance will not adversely affect any population groups (e.g. particular age or cultural groups).

Human milk represents the optimal form of infant nutrition and DSM Nutritional Products agrees that breastfeeding is the best method of feeding infants. Some components of human breast milk composition, such as DHA, have been shown to vary widely based on composition of maternal diet and also to change over the course of lactation from birth to weaning. Infant formulas are intended to serve as a substitute for breast milk for infants who cannot be breast fed, should not receive breast milk, or for those whom breast milk is not available. Those parents who need or choose formula have the right to the most nutritionally optimal formula available. The composition of infant formula should serve to meet the particular nutritional requirements of infants and to promote normal growth and development.

Further support for the safe history of use of DHA from algal oil in infant formula comes from the U.S. FDA. In September 2011 the FDA completed a comprehensive analysis of infant studies published since 2001 and providing DHA/ARA supplementation that reported adverse events. The FDA further analyzed all infant formula adverse event reports, focusing in particular on gastrointestinal related (GI) adverse events, in the CFSAN Adverse Event Reporting System (CAERS) database from 2000, before the introduction of DHA and ARA oils, to 2009, when essentially all formulas contained these oils. The FDA concluded, "We found no statistically significant increases in the proportion of GI adverse events reports in CAERS when we looked over the time interval from when infant formulas containing DHA and ARA oils were first introduced until they essentially replaced non-supplemented formula in the market place."(U.S. FDA, 2011)

See also Part 3 Section A.2 of this application.

PART 3 STANDARDS RELATED TO SPECIAL PURPOSE FOODS AND STANDARDISED FOODS

INFANT FORMULA PRODUCTS

A. Information related to composition

1. Purpose of the compositional change

DHASCO®-B provides an alternative/additional source of docosahexaenoic acid (DHA) in infant formula products [including infant formula for term infants (0-12 months), infant formula for pre-term infants (0-12 months), and follow-on formula (6-12 months)].

Fish oil is a traditional source of DHA for infant formula products. For consumers who have concerns with exposure to environmental contaminants from fish, fish allergy or lifestyle choices such as vegetarians/vegans, DHA Algal Oil provides a consistent, sustainable, vegetarian source of DHA.

2. Supporting evidence for the nutritional safety, tolerance and efficacy of the proposed compositional change

(I) Nutritive substance (including energy or macronutrient), novel food, or novel food ingredient

(a) Characterisation of proposed substance or the comparable substances in breast milk

Fatty Acid Profile-(Table 8) –There was no new fatty acid methyl ester peaks (≥ 4mg/g) identified in any of the lots of DHA Algal Oil. The fatty acid profile is consistent and reproducible in all lots. The major fatty acids (>1% of total fatty acids) are DHA (22:6 n-3), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n-9), linoleic acid (18:2 n-6), eicosapentaenoic acid (20:5 n-3) and docosapentaenoic acids (22:5 n-6). All of the fatty acids detected are well known and already present in the diet from a variety of vegetable and animal sources.

Table 8. Fatty acid profile (GC area %) for DHA Algal Oil

FAMEs (%)	08-6530	08-6586	08-6585	08-6592	08-6643

FAMEs (%)	08-6530	08-6586	08-6585	08-6592	08-6643
% C12:0	<0.1	<0.1	<0.1	<0.1	<0.1
% C14:0	1.30	1.15	1.09	1.04	1.31
% C14:1	<0.1	<0.1	<0.1	<0.1	<0.1
% C15:0	0.24	0.23	0.24	0.23	0.26
% C16:0	13.95	13.06	14.13	13.43	14.59
% C17:0	<0.1	<0.1	<0.1	<0.1	<0.1
% C16:1	<0.1	<0.1	<0.1	<0.1	<0.1
% C18:0	1.64	1.69	1.70	1.72	1.52
% C18:1 n-9	24.52	26.47	24.85	27.96	21.46
% C18:1 n-7	0.22	0.27	0.25	0.28	0.28
% C18:2 n-6	2.05	2.15	2.04	2.01	1.82
% C20:0	0.31	0.32	0.33	0.33	0.32
% C18:3 n-3	0.10	<0.1	<0.1	<0.1	<0.1
% C20:1 n-9	<0.1	0.14	0.13	0.14	0.12
% C20:2 n-6	0.12	0.14	0.13	0.12	0.14
% C20:3 n-6	<0.1	<0.1	<0.1	<0.1	<0.1
% C22:0	0.32	0.38	0.36	0.39	0.32
% C20:3 n-3	<0.1	<0.1	<0.1	<0.1	<0.1
% C20:4 n-6	0.67	0.70	0.66	0.63	0.77
% C20:5 n-3	5.90	6.10	6.43	6.10	6.61
% C24:0	0.12	0.14	0.14	0.15	0.13
% C22:2 n-6	0.54	0.53	0.51	0.49	0.57
% C24:1 n-9	<0.1	<0.1	<0.1	<0.1	<0.1
% C22:4 n-3	<0.1	<0.1	0.10	<0.1	<0.1
% C22:5 n-6	2.63	2.65	2.33	2.26	2.78
% C22:5 n-3	0.55	0.63	1.00	0.95	0.68
% C22:6 n-3	44.35	42.65	42.96	41.23	45.71
%	0.47	0.60	0.62	0.54	0.61

These analyses were conducted at Eurofins Central Analytical Laboratories (Kingstree, SC). Certificates of Analysis are provided in Appendix 2.

Unsaponifiable Matter and Sterol Profile – An independent laboratory analyzed the sterols of the unsaponifiable fraction and results are shown in Table 9. DHA Algal Oil contained approximately 1.0 wt% of unsaponifiable matter. On average, 0.8 wt% of the total oil as sterols was characterized and showed a consistent profile throughout all five runs by GC/MS. The major sterols in DHA Algal Oil, above 10 GC area %, were identified as ß-sitosterol, cholesterol, and stigmasterol. All of the sterols detected are well known and already present in the diet from a variety of vegetable and animal sources.

Table 9: Sterol content of DHASCO®-B					
Sterol	**08-9530**	**08-9586**	**08-9585**	**08-6592**	**08-6643**
24-methylene-cholesterol	1.3	2.0	1.2	1.1	1.9
beta-sitosterol	10.2	11.4	10.7	14.6	9.7
Brassicasterol	1.3	1.7	1.0	0.9	1.5
Campestanol	0.1	0.1	0.1	0.1	0.1
Campesterol	2.0	1.7	1.8	2.2	1.5
Chlerosterol	1.6	1.6	1.6	1.6	1.6
Cholesterol	13.3	14.4	11.2	9.8	12.8
delta-5,23-stigmastadienol	1.0	0.8	0.8	0.8	0.8
delta-5,24-stigmastadienol	0.4	0.5	0.4	0.5	0.4
delta-5-avenasterol	1.7	1.8	2.9	0.9	1.6
delta-7-avenasterol	0.3	0.4	0.3	3.2	0.3
delta-7-campesterol	0.4	0.4	0.4	0.6	0.4
delta-7-stigmastenol	1.7	2.0	1.8	2.5	1.6
Sitostanol	0.5	0.6	0.5	0.5	0.5
Stigmasterol	64.20	60.6	65.2	60.7	65.3
Total sterols in fat (wt%)	0.56	0.51	0.55	0.51	0.55

This testing was also carried out at Eurofins Central Analytical Laboratories and the Certificates of Analysis are presented in Appendix 2.

The major sterols (≥10 wt% total sterols in fat) of DHASCO®-B, which include stigmasterol, beta-sitosterol, and cholesterol, are all found in human breast milk and commercially

available infant formula (Table 10). Stigmasterol and beta-sitosterol are plant sterols found in oils ubiquitous in the food supply and commonly used as sources of essential fatty acids in infant formula including corn, palm, safflower, soybean, and sunflower oil. As such, the plant sterols stigmasterol and beta-sitosterol have been reported in infant plasma at levels up to 4 times higher than that reported for infants fed human or cow's milk (Mellies et al., 1976).

The minor sterols (~2.0 wt% total sterols in fat) of DHASCO®-B, which include brassicasterol, campesterol, delta-5-avenasterol, delta-7-stigmastenol, chlerosterol, 24-methylene-cholesterol, and delta-5,23-stigmastadienol, are also reported in human milk, infant formula, or common foods and dietary oils. Due to the availability of campesterol from commercially available infant formula, plasma campesterol has been reported to be up to 4 times higher in infants fed infant formula vs. human or cow's milk (Mellies et al., 1976).

Finally, several sterols and stanols are found at trace levels (less than 0.5 wt% total sterols in fat) in DHASCO®-B including, campestanol, delta-5, 24-stigmastadienol, delta-7-avenasterol, delta-7-campesterol, and sitostanol. Various plant stanols have been evaluated by competent authorities world-wide and approved for use in a variety of foods, beverages and dietary supplements (Cantrill and Kawamura, 2008). Campestanol is one of the most common plant stanols among those approved for use in food and beverage (Cantrill and Kawamura, 2008). Sitostanol and delta-7-avenasterol have been reported in human milk and the remaining trace sterols are found in common dietary oils including olive and sunflower (Table 10).

Table 10: Sterols present in DHASCO®-B compared to human milk, infant formula, or the human diet

DHASCO®-B Sterol	Human Milk	Infant Formula	Human Diet	Citations
Stigmasterol	**x**	**x**	**Plant oils**[1]	Christie, 2012 Benoit et al., 2010 Huisman et al., 1996 Haug and Harzer, 1984
beta-sitosterol	**x**	**x**	**Plant oils**	Christie, 2012 Benoit et al., 2010 Huisman et al., 1996 Haug and Harzer, 1984
Cholesterol	**x**	**x**	**Animal Products**	Laitinen et al., 2009

				Huisman et al., 1996 Haug and Harzer, 1984
Brassicasterol		x[2]	**Canola Oil/Shellfish**	FDA, 2012 Phillips et al., 2012 Connor and Lin, 1981
Campesterol	**x**	**x**	**Plant oils**	Christie, 2012 Laitinen et al., 2009 Huisman et al., 1996 Haug and Harzer, 1984
delta-5-avenasterol	**x**		**Plant oils**	Christie, 2012 Laitinen et al., 2009
delta-7-stigmastenol			**Plant oils**	Christie, 2012
Chlerosterol			**Olive Oil**	Boskou, 2006 Codex Stan 33-1981
24-methylene-cholesterol			**Shellfish**	Phillips et al., 2012 Connor and Lin, 1981
delta-5,23-stigmastadienol			**Olive Oil**	Codex Stan 33-1981
Campestanol			**Plant Oils**	Cantrill and Kawamura for JECFA, 2008
delta-5,24-stigmastadienol			**Olive Oil**	Codex Stan 33-1981
delta-7-avenasterol	**x**		**Plant oils**	Christie, 2012 Laitinen et al., 2009
delta-7-campesterol			**Sunflower oil**	Johansson et al., 1979
Sitostanol	**x**		**Olive Oil**	Laitinen et al., 2009 Codex Stan 33-1981

[1]Plant oils include – corn, palm, safflower, soybean, and sunflower oil.
[2]GRAS notification for use in commercial infant formula, commercial availability of infant formulas containing canola oil not determined.

Lipid Class Profile – DHA Algal Oil was found to contain 91 to 95% triacylglycerols (TAG), about 2% DAG, and 2% MAG.

Testing was also carried out at Eurofins Central Analytical Laboratories and the Certificates of Analysis are presented in Appendix 2.

(i) The mean amount and range of the proposed or comparable substance in breast milk.

Human milk is a rich source of n-3 and n-6 LCPUFA, including DHA (22:6 n-3), arachidonic acid (ARA; 20:4 n-6); eicosapentaenoic acid (EPA; 20:5 n-3); docosapentaenoic acid (DPA n-3; 22:5 n-3) and the omega-6 fatty acid docosapentaenoic acid (DPA n-6; 22:5 n-6). Levels of these fatty acids in human milk from various countries are summarized in Table 11.

Table 11: Levels of Long-chain Polyunsaturated Fatty Acids in Human Milk

	DHA n-3 mean (% total fatty acids)	ARA n-6 mean (% total fatty acids)	EPA n-3 mean (%total fatty acids)	DPA n-3/n-6 mean (% total fatty acids)
World wide	**0.32[1]**	**0.47[1]**	**0.09[2]**	**0.19/0.06[3]**
Japan[3]	0.99	0.40	0.26	0.29/.05
Philippines[3]	0.74	0.39	0.15	0.23/.08
Chile[3]	0.43	0.42	0.09	0.22/.09
China [3]	0.25 [4]	0.49	0.06	.18/.06
Mexico[3]	0.26	0.42	0.07	0.16/.05
UK[3]	0.24	0.36	0.11	0.18/.06
Australia[3]	0.23	0.38	0.10	0.18/.04
U.S.	0.21[6]	0.45 [3]	0.06[7]	0.14/.06[3]
Canada[3]	0.17	0.37	.08	0.17/.04

[1]Brenna et al. *Am J Clin Nutr* 2007; 85:1457-64; [2]Yuhas et al. *Lipids* 2006; 41:851-858 (excluding Japan);[3] Yuhas et al. *Lipids* 2006; 41:851-858; [4]Mean calculated from Peng et al., 2007; Yuhas et al., 2006; Xiang et al., 1999; 2005; Dodge et al., 1999; [5] Mean calculated from Yuhas et al., 2006 and Peng et al. 2007; [6]Mean calculated from Yuhas et al., 2006; Bopp et al., 2005; Auestad et al., 2001; Jensen et al., 2000; 2005; Francois et al., 1998; Henderson et al., 1992; Carlson et al., 1986; [76]Mean calculated from Yuhas et al., 2006; Auestad et al., 2001; Jensen et al., 2000; 2005; Francois et al., 2003.

(ii) The variability of the levels of the proposed or comparable substance and consideration of the influence of maternal diet or other physiological factors (e.g. hormones, biochemical processes)

Breast milk always contains DHA, but the amount of this, and other n-3 LCPUFA, is largely dependent on maternal intake. Common maternal food sources of DHA include fatty fish with trace amounts available from eggs and chicken meat. Reported averages for human milk DHA of Australian mothers consuming typical diets, i.e. not supplemented with DHA, range from 0.14-0.28% of total fatty acids (Makrides et al., 1996; Yuhas et al., 2006). To support brain and eye development during pregnancy and early post-natal life numerous government authorities and expert groups have recommended that pregnant and nursing women consume up to 450 mg DHA per day (Table 12).

Table 12 World-wide LCPUFA Intake Recommendations and Guidelines: Women, Infants, and Children

Organization	Amount of DHA (and ARA)	Reference
EFSA	0-12 mo - 20-50 mg DHA/100kcal	EFSA, 2014. Scientific Opinion on the essential composition of infant and follow-on formulae. EFSA Journal 2014;12(7):3760, 106 pp. doi:10.2903/j.efsa.2014.3760
EFSA	100 mg/day to 24 months; 24-36 months - 250 mg EPA+DHA/day	EFSA NDA Panel, 2013. Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. EFSA Journal 2013;11(10):3408, 103 pp. doi:10.2903/j.efsa.2013.3408
Early Nutrition Academy	6-36 mo - not to exceed 1.0% of total fatty acids	Koletzko B, et al., 2013. Compositional Requirements of Follow-Up Formula for Use in Infancy: Recommendations of an International Expert Group Coordinated by the Early Nutrition Academy ENA cited substantiation: 1. Glaser C, Lattka E, Rzehak P, Steer C, Koletzko. Matern Child Nutr 2011;7(suppl 2):27-40.
European Society of Pediatric Gastroenterology, Hepatology, and Nutrition	Preterm infants to a target weight of 1800 g - 11-27 mg DHA/100kcal	Agostoni C, Buonocore G, Carnielli VP, et al. 2010. Enteral nutrient supply for preterm infants: commentary from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition. J Pedia Gastro Nutr 50:1-9.
FAO/WHO Expert Consultation	0-6 mo - 0.1-0.18 %E DHA/day 0-6 mo - 0.2-0.3 %E ARA/day	FAO, 2010. Fats and Fatty Acids in Human Nutrition. FAO Food and Nutrition Paper 91. ISSN 0254-4725. Table 2.2

	6-24 mo – 10-12 mg DHA/kg 2-4 yr – 100-150 mg/day 4-6 yr – 150-200 mg/day 6-10 yr – 200-250 mg/day	
FAO/WHO Expert Consultation	Pregnancy and nursing - An intake of 300 mg EPA+DHA of which at least 200 mg should be DHA	FAO, 2010. Fats and Fatty Acids in Human Nutrition. FAO Food and Nutrition Paper 91. ISSN 0254-4725. Pg. 16
Dietary Guidelines for Americans, 2010	Pregnancy and nursing - Moderate evidence indicates that intake of omega-3 fatty acids, in particular DHA, from *at least* 8 ounces of seafood per week for women who are pregnant or breastfeeding is associated with improved infant health outcomes, such as visual and cognitive development. Therefore, it is recommended that women who are pregnant or breast-feeding consume at least 8 and up to 12 ounces of a variety of seafood per week, from choices that are lower in methyl mercury.	*Dietary Guidelines for Americans, 2010.* 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010
U.S. 2010 Dietary Guidelines Advisory Committee (DGAC)	Pregnancy and nursing – Two servings of seafood/wk to provide at least 250 mg DHA+EPA, primarily as DHA	Report of the DGAC on the Dietary Guidelines for Americans, 2010 http://www.cnpp.usda.gov/DGAs2010-DGACReport.htm

Agence Français de Sécurité Sanitaire des Aliments	250 mg DHA/d for pregnant women 250 mg DHA/day for breastfeeding women	AFSSA Opinion Regarding the Update of the Recommended Dietary Intake for Fatty Acids. AFSSA-Hearing n2006-SA-0359. 2010.
Agence Français de Sécurité Sanitaire des Aliments	70 mg DHA/d for children 1-3 years 125 mg DHA/d for children 3-9 years 250 mg DHA/day for children 10-18 years	AFSSA Opinion Regarding the Update of the Recommended Dietary Intake for Fatty Acids. AFSSA-Hearing n2006-SA-0359. 2010.
Belgian Superior Health Council	0.1-0.4 E% DHA/d for children ages 12-36 months 0.1-0.25 E% ARA/d for children ages 12-36 months	Superior Health Council. Recommendations Nutritionnelles Pour La Belgique. CSS No. 8309. Revision 2009.
European Food Safety Authority (EFSA)	Pregnancy and nursing – Up to 450 mg/day - 250 mg DHA+EPA/d for all adult women plus an additional 100-200 mg DHA/d for pregnant and nursing women	Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. EFSA Journal 2010; 8:1461. http://www.efsa.europa.eu/en/scdocs/doc/1461.pdf
EFSA	100 mg DHA/d for children ages 7-24 months 250 mg DHA+EPA/d for children 2-18 years	Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. EFSA Journal 2010; 8:1461. http://www.efsa.europa.eu/en/scdocs/doc/1461.pdf
International Society for the Study of Fats and	At least 200 mg DHA/d during pregnancy and nursing	ISSFAL Policy Statement 4: Recommendations for intake of polyunsaturated fatty acids by pregnant and lactating women. 2009.

Lipids		
March of Dimes	At least 200 mg DHA/d during pregnancy and nursing	http://www.hotindienews.com/2009/04/06/1223#comments , 2009.
Perinatal Lipid Intake Working Group	At least 200 mg DHA/d during pregnancy and nursing	Koletzko B, Cetin I, and Brenna TJ. Perinatal Lipid Intake Working Group Consensus Statement: *Dietary fat intakes for pregnant and lactating women*. Brit J Nutr 98:873-7, 2007.
Australia New Zealand National Health and Medical Research Council	Children ages 1-3 years 40 mg/d DHA+EPA+DPAn-3 Children ages 4-8 years 55 mg/d DHA+EPA+DPAn-3 Adolescents ages 9-13 years 70 mg/d DHA+EPA+DPAn-3 Teens ages 14-18 years 126 mg/d DHA+EPA+DPAn-3 Pregnancy 110- 115 mg/day DHA+EPA+DPAn-3 Lactation 140-145 mg/day DHA+EPA+DPAn-3	Nutrient reference values for Australia and New Zealand including recommended dietary intakes. 2006. www.nhmrc.gov.au/publications/synopses/_files/n35.pdf
Superior Health Council of Belgium	Pregnancy and nursing – 200-300 mg DHA per day	Hoge Gezondheidsraad Superior Health Council, Advisory Report, Recommendations and claims made on omega-3 fatty Acids (SHC 7945). 2004. https://portal.health.fgov.be/pls/portal/docs/PAGE/INTERNET_PG/HOMEPAGE_MENU/ABOUTUS1_MENU/INSTITUTIONSAPPARENTEES1_MENU/HOGEGEZONDHEIDSRAAD1_MENU/ADVIEZENENAANBEVELINGEN1_MENU/ADVIEZENENAANBEVELINGEN1_DOCS/OMEGA-3%20ENGLISH.PDF
UK Scientific Advisory	Pregnancy and nursing - Long-chain n-3 LCPUFA	Scientific Advisory Committee on Nutrition. Advice on fish consumption, paragraph 5.18.

Committee on Nutrition (SCAN)	(EPA+DHA+DPA) – 450 mg/day	ISBN 0 11 243083. The Stationary Office. London. http://www.sacn.gov.uk/pdfs/fics_sacn_advice_fish.pdf
Health Council of the Netherlands	0-5 months – 20 mg DHA/kg/day; 40 mg/kg/day ARA 6-11 months – 150-200 mg fatty acids from fish/day 1-18 years – 150 – 200 mg fatty acids from fish/day Pregnancy and lactation – 200 mg fatty acids from fish/day	Health Council of the Netherlands. Dietary reference intakes: energy, proteins, fats, and digestible carbohydrates. The Hague: Health Council of the Netherlands, 2001; publication no. 2001/19ER (corrected edition: June 2002).

[1]LCP=long-chain (20 and 22 carbon atoms) polyunsaturated fatty acids

Unfortunately, evidence indicates that DHA intake is lower than recommendations among Australian women who are pregnant or who have given birth in the past 12 months (Taylor et al., 2014). Young women (n=7486) participating in the Australian Longitudinal Study on Women's Health, whose pregnancy status could be determined, participated in an assessment of fish consumption. Likely due to concerns regarding mercury exposure from fish, women who were currently or recently pregnant consumed significantly less fish compared to women not currently pregnant. Specifically, reported fish intake ranged from 0-691 g per day with pregnant or recently pregnant women consuming an average of 28 g per day vs. non-pregnant women which consumed 31-33 g per day. The estimated amount of total long-chain n-3 LCPUFA consumed by pregnant and recently pregnant women was almost 100 mg/day lower than national recommendations. DHA food supplements and pre-natal vitamins with DHA are a common and effective means to bridge the gap between the low DHA intake provided by the habitual diet of most women and the recommendations for increased DHA intake. Makrides and co-workers (1996) supplemented nursing Australian women with DHA doses ranging from 0-1.3 g DHA/day and reported a "strong, specific, and dose-dependent effect" of supplemental DHA on DHA in human milk.

(iii) Comparison of relevant biochemical, physiological and/or functional endpoints between breastfed infants and infants fed the infant formula product containing the proposed composition change.

Relevant biochemical endpoint - Blood DHA Status of infants fed formula with added DHA vs. those fed human milk

Fetal accumulation of LCPUFA during gestation and by early provision of human milk significantly promotes infant LCPUFA status as measured in various tissues. Infant LCPUFA stores are quickly eroded, however, if human milk feeding is abbreviated and a source of LCPUFA, such as enriched infant formula, is not provided (Birch et al., 2002; Hoffman et al., 2003; Hoffman et al., 2004; Makrides et al., 2002). Infant tissue LCPUFA content can further decline as LCPUFA formula/human milk is replaced by solid foods. Typical early weaning foods are low in or devoid of DHA and thus fail to supplement the gap left by the discontinuation or reduction of LCPUFA supplemented formula or human milk (Luukkainen et al., 1996). For example, Birch et al. (2010) report significant declines in red blood cell DHA and arachidonic acid (ARA; 20:4 n-6) concentrations at 12 months of age in infants introduced to solid foods beginning at 4 months despite continued provision of DHA and ARA-rich infant formula and despite 92% of infants reported as consuming fish/poultry/red meat by 9 months. Similarly, Schwartz and co-workers (2009) report a precipitous decline in DHA blood levels despite supplementation with an alpha-linolenic acid (ALA; 18:3 n-3)-fortified vegetable/potato/meat jarred food designed for older infants. ALA is the metabolic precursor to DHA but *in vivo* conversion of ALA to DHA is limited and insufficient to support DHA blood levels comparable to direct DHA consumption from human milk (Brenna et al., 2009). Parental concerns regarding allergens associated with fish and seafood appear to further limit the availability of DHA in the weaning diet (ESPGHAN Committee on Nutrition, 2008). In fact, even if human milk supplementation is available ad libitum throughout the first year, the LCPUFA status of non-LCPUFA supplemented infants declines and is significantly depleted at 12 months (Groh-Wargo et al, 2005).

The need for DHA supplementation is particularly apparent in preterm infants where even human milk appears inadequate to increase DHA status to levels seen in term infants (Smithers et al., 2008). Despite feeding a combination of DHA-enriched human milk and preterm infant formula to provide a DHA level comparable to that found in Japanese breast milk (1.0%), Smithers and co-workers failed to observe saturation of preterm infant

erythrocyte DHA content. The author's concluded that these results together with those recently published by Henriksen et al., (2008) suggest "that higher levels of dietary DHA may be required to meet the nutritional needs of preterm infants." The author's further suggest the level of DHA needed to meet preterm infant DHA needs may be as high as 2.0% of total dietary fat (Smithers et al., 2008).

Relevant functional endpoints – Brain and eye development of infants fed formula with added DHA vs. those fed human milk

Eye/Visual Development

The FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition (FAO, 2010) has recognized the evidence supporting a critical role of DHA in retinal development for infants and children 0-24 months old as "convincing" and recommends 10-12 mg/kg DHA daily to meet these needs of 6-24 month old children and up to 0.36% of total fatty acids from human milk to meet the needs of infants 0-6 months. Due to the evidence in support of maternal DHA intake and improved infant visual function, related health claims are now approved for use in the European Union. The European Commission (EU Community Register of Health Claims, 2011) has authorized the use of the following claim:

Docosahexaenoic acid (DHA) maternal intake contributes to the normal development of the eye of the foetus and breastfed infants

Similarly, evidence supports a role of DHA from infant formula in the visual development of infants and young children. Most recently, Birch and co-workers confirmed not only the importance of DHA for visual acuity, but confirmed the continuation of this benefit to 4 years of age (Birch et al., 2007) and a threshold of benefit at 0.30% of total fatty acids (Birch et al., 2010). Birch and co-workers (2010) supplemented full-term infants (n=343) with formula containing 0.32% DHA, 0.64% DHA or 0.96% DHA in combination with 0.64% ARA and reported significantly improved visual acuity with 0.32% DHA + ARA but no additional improvements in response to higher levels of DHA. The European Food Safety Authority (EFSA), as part of the authorization procedure for health claims directed toward children, recently conducted an evidence-based review of 12 randomized controlled trials (RCTs) and one meta-analysis of RCTs supplementing DHA and ARA from various sources via infant formula, follow-on formula, and weaning foods on infant visual outcomes. EFSA concluded that "A cause and effect

relationship has been established between the intake of infant and follow-on formula supplemented with DHA at levels around 0.3% of total fatty acids and visual function at 12 months in formula-fed infants born at term from birth up to 12 months and in breastfed infants after weaning up to 12 months (EFSA, 2009). Based on the preponderance of evidence supporting DHA for visual function the European Commission (EU Community Register of Health Claims, 2011) has authorized the use of the following claim on labels of foods supporting a daily intake of 100 mg DHA or, if used on follow-on formula, containing 0.3% of fatty acids as DHA:

- *Docosahexaenoic acid (DHA) intake contributes to the normal visual development of infants up to 12 months of age*

Brain Development

The most significant period of neural growth and development occurs during the brain growth spurt which begins during the final trimester of pregnancy and continues throughout the first few years of life (Dobbing and Sands, 1973). It is during this time period that LCPUFA supplementation of infants and young children via maternal supplementation, human milk, infant formula, and a variety of DHA-rich weaning foods can most impact brain and eye development and function. Studies of post-natal LCPUFA supplementation of term infants during early life via LCPUFA infant formula (Willatts et al., 1998; Birch et al., 2000; Agostoni et al., 2000; Drover et al., 2009) or maternal DHA supplementation during lactation (Jensen et al., 2005; Jensen et al., 2010) report cognitive and behavioral (Kohlboeck et al., 2011) advantages of LCPUFA supplementation that are often sustained in later life. Most recently, Drover and co-workers (2011) found that term infants supplemented with LCPUFA from infant formula for the first 12 months of life scored higher on tests of cognitive development at 18 months of age. Five-year-old children whose mothers were supplemented with DHA during the first 4 months of lactation have been reported to perform better on tests of sustained attention than children of non-supplemented mothers (Jensen et al., 2010). Even infants fed breast milk throughout the first year of life benefit from additional DHA supplementation with earlier achievement of developmental milestones reported (Agostoni et al., 2009). Evidence in support of a role of maternal DHA supplementation during pregnancy and/or lactation and childhood neurodevelopment is sufficient to suggest an important role for DHA (Ryan et al., 2010). The 2010 Dietary Guidelines for Americans note that “moderate evidence indicates

that intake of omega-3 fatty acids, in particular DHA, from *at least* 8 ounces of seafood per week for women who are pregnant or breastfeeding is associated with improved infant health outcomes, such as visual and cognitive development." (USDA and DHHS, 2010) The FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition (FAO, 2010) recognized the evidence supporting a critical role of DHA in brain development for infants and children 0-24 months old as "convincing" and recommends 10-12 mg/kg DHA daily to meet the needs of 6-24 month old children and up to 0.36% of total fatty acids in human milk to meet the needs of infants 0-6 months. In fact, based on the available evidence the European Commission (EU Community Register of Health Claims) has authorized the use of the following claim on labels of foods contributing to a minimum daily intake of 200 mg DHA in addition to the adult recommended intake of 250 mg EPA+DHA:

- *Docosahexaenoic acid (DHA) maternal intake contributes to the normal development of the brain of the foetus and breastfed infants*

Similarly, a recent EFSA Opinion (2014) regarding DHA and brain development concludes the following regarding DHA from infant formula and dietary supplements, "…a cause and effects relationship has been established between the consumption of DHA and contribution to normal brain development". In order to bear this claim , food for older infants and young children should provide a daily intake of 100 mg DHA in one or more servings, and for children 2-18 years, foods should provide a daily intake of 250 mg DHA. The following claim wording has been proposed:

"DHA contributes to normal brain development"

(b) Nutritional safety and tolerance of the proposed compositional change

The nutritional safety and tolerance of the addition of DHA Algal Oil to infant formula comes from a comprehensive analysis conducted by the U.S. FDA of infant studies published since 2001 and providing DHA/ARA supplementation that reported adverse events (U.S. FDA, 2011). The FDA further analyzed all infant formula adverse event reports, focusing in particular on gastrointestinal related (GI) adverse events, in the Center for Food Safety and Nutrition's Adverse Event Reporting System (CAERS) database from 2000, before the introduction of DHA and ARA oils in U.S. infant formula, to 2009, when essentially all U.S formulas contained

these oils. The FDA concludes, "We found no statistically significant increases in the proportion of GI adverse events reports in CAERS when we looked over the time interval from when infant formulas containing DHA and ARA oils were first introduced until they essentially replaced non-supplemented formula in the market place."(U.S. FDA, 2011)

(i) Human infant studies demonstrating that the infant formula products containing the substance at the proposed level, will support normal infant growth and/or development over a minimum interval of 3 to 4 months beginning no later than 1 month of age.

Table 13 summarizes the studies of infants supplemented with DHA (and ARA) from a variety of sources (algal oil, fish oil, egg phospholipids) and followed for growth. In all cases, supplementation was initiated within the first month of life and continued at least 3-4 months. Three studies are of full-term infants and 2 are of pre-term, all were double-blind randomized, controlled trials and included a reference group consuming human milk. All studies maintained at least 20 infants per group throughout the duration of supplementation; several ended with over 50 per group.

Strength and Quality of Evidence and Studies - Given the study design, duration, and sample size of the studies in Table 13 the strength of the evidence supporting the maintenance of normal growth by DHA (combined with ARA) levels ranging from 0.12-0.35% of total fatty acids in infant formula is strong and supported by n=6 studies of mostly moderate to high quality conducted in over 1000 infant (mixed term and preterm) participants. Specifically, studies of full-term infants reported no differences between DHA+ARA supplemented groups vs. control or human milk fed groups. Studies of preterm infants reported enhanced growth outcomes in response to DHA+ARA supplementation compared to control and human milk-fed infants.

Table 13. Randomised Controlled Trials with DHA from various sources (and ARA) Reporting Growth Outcomes[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/ DHA+ARA)**	**Age at Start/Duration of Supplementation**	**Outcome Summary**	**Study Quality and Limitations**
Auestad *et al.* (1997) (2003) DB RCT	0.43	0.12	63/45/46	~ 7 days/12 mo	No difference in growth outcomes (weight, length, head circumference) between groups No difference in growth between groups 27 mo post-supplementation	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured. Limitations - high drop-out rate (23%); limited follow-up (80%) at 27 mo post supplementation; sufficient statistical power not verified (i.e. no power calculation provided) Overall - moderate quality
Auestad *et al.* (2001) DB RCT	0.45	0.14	82/77/162	~9 days/12 mo	No difference in growth (weight, length, head circumference) between groups	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study population. Limitations - high drop-out rate (27%) Overall - moderate-high quality

Table 13. Randomised Controlled Trials with DHA from various sources (and ARA) Reporting Growth Outcomes[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/ DHA+ARA)**	**Age at Start/Duration of Supplementation**	**Outcome Summary**	**Study Quality and Limitations**
Clandinin *et al.* (2005)* DB RCT	0.64	0.32	76/62/117	14 days/~12 mo	Feeding formulas with DHA and ARA from algal and fungal oils resulted in enhanced growth, i.e. weight and length significantly (P<.05) greater in response to DHA+ARA formula.	Quality - selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study population. Limitations - Groups differed somewhat at baseline; Drop-out rate 14% Overall - moderate quality
Makrides *et al.* (1999) DB RCT	0.34	0.34	33/21/21	5-7 days/12 mo	No difference in growth (weight, length, head circumference) between groups	Quality - selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured. Limitations - Groups differed somewhat at baseline; Drop-out rate 18%; statistical power limited, i.e. n=25 per group required, n=21 completed. Overall - limited quality

Table 13. Randomised Controlled Trials with DHA from various sources (and ARA) Reporting Growth Outcomes[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/ DHA+ARA)**	**Age at Start/Duration of Supplementation**	**Outcome Summary**	**Study Quality and Limitations**
O'Connor et al. (2001)* DB RCT	0.41-0.43	0.15-.0.27	43/91/180	~3 days/12 mo	No difference in growth (weight, length, head circumference) between groups	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study population. Limitations - drop-out rate (20%) Overall - moderate-high quality
Vanderhoof et al (1999)* DB RCT	0.50	0.35	133/78/77	~21 days/6 mo	DHA and ARA from algal and fungal oils promoted length and wt at 40 wk PCA *vs.* BF (P<.05).	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study Limitations - none Overall - high quality

[1]While in excess of 20 studies have investigated the role of DHA in infant formula for growth outcomes, as requested by FSANZ, this table includes only studies meeting the following criteria: LCPUFA supplementation started within 1 month of age and supplementation provided for a minimum of 3-4 months. Study included a human milk fed control group.

Abbreviations: DB RCT - double-blind, randomized controlled trial; DHA - docosahexaenoic acid; ARA - arachidonic acid; CA - corrected age; mo - months; %FA = percent of fatty acids; BF = breast-fed; C = control; PCA = post-conceptual age; wk = weeks; wt = weight

*Preterm infant study

(ii) Evidence to demonstrate there is no risk of nutrient imbalances as a result of infants fed the IF product containing the proposed compositional change must be provided.

Strength and Quality of Evidence and Studies - Given the study design, duration, and sample size of the studies in Table 14 the strength of the evidence confirming the maintenance of balanced DHA and ARA blood levels in response to LCPUFA formula is strong and supported by n=8 studies of mostly moderate to moderate-high quality conducted in over 1700 infant (mixed term and preterm) participants. Specifically, there is no evidence to suggest that a risk of nutrient imbalance exists as a result of feeding infants infant formula containing DHA along with ARA. Feeding these LCPUFA in conjunction with each other supports blood levels of these fatty acids similar to that found in breast-fed infants while supplementation of DHA alone typically depresses ARA tissue levels. No studies of DHA+ARA supplementation have reported reduced ARA blood levels when providing DHA+ARA at levels consistent with current commercial practice (Table 14).

Table 14. Randomised Controlled Trials with DHA+ARA Intervention Reporting Blood Fatty Acid Status[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/DHA)**	**Duration of Supplementation (months)**	**Outcome Summary**	**Study Quality and Limitations**
Auestad *et al.* (1997) (2003) DB RCT	0.43	0.12	63/45/46	12	ARA levels supported. DHA+ARA maintained DHA and ARA blood levels similar to BF and higher than C at 4 and 12 mo (P<.001). No difference 27 mo post-supplementation	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured. Limitations - high drop-out rate (23%); limited follow-up (80%) at 27 mo post supplementation; sufficient statistical power not verified (i.e. no power calculation provided) Overall - moderate quality

Table 14. Randomised Controlled Trials with DHA+ARA Intervention Reporting Blood Fatty Acid Status[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/DHA)**	**Duration of Supplementation (months)**	**Outcome Summary**	**Study Quality and Limitations**
Auestad *et al.* (2001) DB RCT	0.45	0.14	82/77/162	12	ARA levels supported. DHA+ARA formula maintained DHA and ARA blood levels similar to BF and higher than C (P<.01).	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study population. Limitations - high drop-out rate (27%) Overall - moderate-high quality
Ben *et al.* (2004)	NR	NR	26/52/69	6	ARA levels supported. ARA levels similar to BF at 3 and 6 mo.	Quality - Groups comparable at baseline; appropriate outcomes, outcomes reliably measured. Limitations - Limited description of selection and allocation procedures described; disposition of subjects not provided; no power calculation provided. Overall - limited quality

Table 14. Randomised Controlled Trials with DHA+ARA Intervention Reporting Blood Fatty Acid Status[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/DHA)**	**Duration of Supplementation (months)**	**Outcome Summary**	**Study Quality and Limitations**
Hoffman *et al.* (2000) DB RCT	0.72	0.36	20/20/18	4	ARA levels supported. DHA+ARA maintained ARA levels comparable to BF infants and significantly higher than C (P<.05).	Quality - Appropriate outcomes, outcomes reliably measured. Limitations - Limited description of selection and allocation procedures described; disposition of subjects not provided; no power calculation provided. Overall - limited quality
Makrides *et al.* (1999) DB RCT	0.34	0.34	33/21/21	12	ARA levels supported. At 34 and 52 wk DHA+ARA same as BF and significantly higher ARA than C or DHA-alone (P<.01).	Quality - selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured. Limitations - Groups differed somewhat at baseline; Drop-out rate 18%; statistical power limited, i.e. n=25 per group required, n=21 completed. Overall - limited quality

Table 14. Randomised Controlled Trials with DHA+ARA Intervention Reporting Blood Fatty Acid Status[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/DHA)**	**Duration of Supplementation (months)**	**Outcome Summary**	**Study Quality and Limitations**
O'Connor *et al.* (2001)* DB RCT	0.42	0.26	40/120/253	12	ARA levels supported. ARA levels were similar to BF and higher or the same as C.	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study population. Limitations - high drop-out rate (20%) Overall - moderate-high quality
Vanderhoof *et al.* (1999)* DB RCT	0.50	0.35	133/78/77	6	ARA levels supported. ARA levels similar to BF and significantly higher than C (P<.05).	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study Limitations - none Overall - high quality

[1]This table includes only studies meeting the following criteria: LCPUFA supplementation started within 1 month of age and supplementation provided for a minimum of 3-4 months. Study included a human milk fed control group.

Abbreviations: DB RCT - double-blind, randomized controlled trial; DHA - docosahexaenoic acid; ARA - arachidonic acid; mo - months; %FA = percent of fatty acids; BF = breast-fed; C = control;

*Preterm infant study

(c) Efficacy of the proposed compositional change

This application should not be assessed for any type of health claim.

(i) description and measures of the physiological, biochemical and/or functional effect(s) of the substance.

Table 15 summarizes the studies of infants supplemented with DHA (and ARA) from a variety of sources (algal oil, fish oil, egg phospholipids) and followed for visual development. In all cases, supplementation was initiated within the first month of life and continued at least 3-4 months. Six studies are of full-term infants and 1 of pre-term, all were double-blind randomized, controlled trials and included a reference group consuming human milk. Studies of full-term infants supplementing 0.34% or less of total fatty acids as DHA (n=5 of 6 studies) failed to find a benefit of DHA supplementation to visual outcomes. The remaining studies providing a range of 0.26 (pre-term)-0.36% (full-term) of total fatty acids as DHA in both term and preterm infants reported improvements in at least one measure of visual function.

Strength and Quality of Evidence and Studies - Given the study design, duration, and sample size of the studies in Table 15 the strength of the evidence confirming a benefit of visual function to term and preterm infants consuming LCPUFA is limited and supported by studies of mixed quality. Based on a broader evidence base than that meeting FSANZ criteria for relevant infant studies the European Commission (EU Community Register of Health Claims, 2011) has authorized the use of the following claim on labels of foods supporting a daily intake of 100 mg DHA or, if used on follow-on formula, containing 0.3% of fatty acids as DHA:

- *Docosahexaenoic acid (DHA) intake contributes to the normal visual development of infants up to 12 months of age*

Table 15. Randomised Controlled Trials with DHA (and ARA) Intervention Reporting Vision-related Outcomes[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/DHA+ARA)**	**Age at initiation/ Duration of Supplementation**	**Outcome Summary**	**Study Quality and Limitations**
Auestad *et al.* (1997) (2003) DB RCT	0.43	0.12	63/45/46	~7 days/12 mo	No visual benefits, no difference in visual outcomes between groups. No difference in visual outcomes 27 mo post-supplementation	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured. Limitations - high drop-out rate (23%); limited follow-up (80%) at 27 mo post supplementation; sufficient statistical power not verified (i.e. no power calculation provided) Overall - moderate quality
Auestad *et al.* (2001) DB RCT	0.45	0.14	82/77/162	~9 days/12 mo	No visual benefits, no difference in visual outcomes between groups	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study population. Limitations - high drop-out rate (27%) Overall - moderate-high quality

Table 15. Randomised Controlled Trials with DHA (and ARA) Intervention Reporting Vision-related Outcomes[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/DHA+ARA)**	**Age at initiation/ Duration of Supplementation**	**Outcome Summary**	**Study Quality and Limitations**
Hoffman *et al.* (2000) RCT	0.72	0.36	20/20/18	~4 days/4 mo	Visual outcomes improved (P<.05). DHA+ARA resulted in better VEP at 17 and 52 weeks.	Quality - Appropriate outcomes, outcomes reliably measured. Limitations - Limited description of selection and allocation procedures; disposition of subjects not provided; no power calculation provided. Overall - limited quality
Makrides *et al.* (2000) DB RCT	0.34	0.34	33/21/21	~ 7 days/12 mo	No effect on visual acuity	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study population. Limitations - none Overall - high quality

Table 15. Randomised Controlled Trials with DHA (and ARA) Intervention Reporting Vision-related Outcomes[1]						
Study/Study Design	**ARA (%FA)**	**DHA (%FA)**	**Number of Subjects at longest duration (BF/C/DHA+ARA)**	**Age at initiation/ Duration of Supplementation**	**Outcome Summary**	**Study Quality and Limitations**
O'Connor *et al.* (2001)* RCT	0.42	0.26	40/120/253	~ 3 days/12 mo	Visual outcomes improved (P<.05). DHA+ARA supplemented formula improved visual acuity at 6 mo of age	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power, large study population. Limitations - high drop-out rate (20%) Overall - moderate-high quality
Singhal *et al.* (2007) RCT	0.3	0.32	73/89/91	~ 7 days/6 mo	No effect on visual acuity	Quality - Groups comparable at baseline; selection and allocation procedures described; disposition of subjects provided; appropriate outcomes, outcomes reliably measured; adequate statistical power Limitations - high drop-out rate (~45%) Overall - moderate quality

[1]While numerous studies have investigated the role of DHA in infant formula for visual outcomes, this table includes only studies meeting the following criteria: LCPUFA supplementation started within 1 month of age and supplementation provided for a minimum of 3-4 months. Study included a human milk fed control group.

Abbreviations: DB RCT – double-blind, randomized controlled trial; DHA - docosahexaenoic acid; ARA - arachidonic acid; CA - corrected age; mo - months; %FA = percent of fatty acids; BF = breast-fed; C = control; VLBW – very low birth weight; VEP – visual evoked potential
*Preterm infant study

B. Information related to the dietary intake or dietary exposure

1. Data to enable the dietary intake or exposure of the target population to be estimated

Most studies included in the current application provide DHA up to 0.36% of total fatty acids in infant formula. This equates to about 19 mg DHA/100 kcal. Caloric intake and thus DHA intake will vary with age and introduction of complementary foods. At a level of 500 kcal per day, infant formulas containing 0.36% of total fatty acids as DHA would provide 95 mg of DHA daily. It is estimated that 100 mg DHA is the daily reference value for infants and young children ages 7-24 months (EFSA, 2010).

Use levels are expected to be similar to the current use of DHASCO® in infant formula products.

2. Data on the recommended level of formula consumption for the target population

This section is not applicable as DSM Nutritional Products is not the manufacturer of the final infant formula product.

(i) the capacity of the product scoop (in grams of product)

Not applicable.

(ii) the number of scoops required per feed

Not applicable.

(iii) the volume of water required per feed

Not applicable.

(iv) total volume of the made-up feed

Not applicable.

(v) recommended number of feeds per day relevant to each age group in the relevant target population.

Not applicable.

3. Information relating to the substance

Intake of docosahexaenoic acid is traditionally achieved through consumption of fatty fish. Recommended intake levels for DHA are not always practically achieved from fish consumption, owing to limited availability/consumption of fish in some geographic areas, traditional/cultural dislikes of seafoods, concerns with exposure to environmental contaminants from fish, seafood allergy and vegan diets. DHA Algal Oil provides a consistent, sustainable, vegetarian source of DHA.

C. Information related to labelling requirements under Part 2.9 of the Code

1. Information related to safety or nutritional impact of the proposed labelling change

A labelling change is not proposed. Current labelling of DHASCO® would also apply to the substance that is the subject of this application, DHASCO®-B.

2. Information to demonstrate that the proposed labelling change be will understood and assist consumers

A labelling change is not proposed.

D. Information related to internationally recognized standards, codes of practice, recommendations and guidelines

Please see Part 1 Section 1.8.

STATUTORY DECLARATION

Statutory Declarations Act 1959

I, *Erin Sylvester, 6480 Dobbin Road Columbia MD 21045 USA, Regulatory Affairs Manager*

make the following declaration under the *Statutory Declarations Act 1959*:

1. The information provided in this application fully sets out the matters required
2. The information provided in this application is true to the best of my knowledge and belief
3. No information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

_____
Signature of person making the declaration

Declared at _____ on _____ of _____
Place Day Month and Year

Before me,

_____
Signature of person before whom the declaration is made

_____

_____

Full name, qualification and address of person before whom the declaration is made (in printed letters)

*****A statutory declaration must be made before a prescribed person under the *Statutory Declarations Act 1959*, available online at http://www.frli.gov.au/ComLaw/Legislation/ActComplilation1.nsf/current/bytitle/7E3AE20F8329B422CA256F71004DB642?OpenDocument&mostrecent=1.

CHECKLIST

Please see Appendix 1.

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