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Division of American Home Products Corporation

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NUTRITIONAL REGULATORY AFFAIRS

August 27, 1998

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20204

RE: Notice of a Claim For Exemption From Premarket Approval

To whom it may concern:

Wyeth Nutritionals International (WNI), a business unit of Wyeth Ayerst Global Pharmaceuticals, hereby submits notice of a claim for exemption from the requirement for premarket approval based on a determination of generally recognized as safe (GRAS) status. The ingredients subject to this notice include an arachidonic acid-rich triglyceride derived from fermentation of *Mortierella alpina* (hereafter referred to as ARASCO) and a docosahexaenoic acid-rich oil derived from fermentation of *Crypthecodinium cohnii* (hereafter referred to as DHASCO). The uses subject to the notice include specific and limited uses in infant formulas for low birth weight (LBW) and term infants.

For reasons described in this notice, the conditions of use for these ingredients include their use only in specific combinations with each other or with other appropriate sources of arachidonic or docosahexaenoic acids; it is primarily for this reason that we are submitting a single notice for both ingredients rather than separate notices specific to each ingredient. In organizing this notice, we have utilized the Agency's proposal of April 17, 1997 (62 FR 18937) as guidance.

This notice includes the following components:

- The required administrative and regulatory details;
- Specification of the levels and conditions of use that we have determined are generally recognized as safe;
- A description of the ingredients and their method of production;
- A summary of the technical basis for our finding of safety, including a discussion of the unique aspects of assessing the safety of infant formula ingredients.
- A summary of the basis under which we have determined that these uses are generally recognized as safe.

Because all uses that are subject to this notice are in foods that fall within the 21 U.S.C. 201(aa) definition of "infant formula," each new significantly modified formulation containing these oils is subject to mandatory premarket notification to FDA under U.S. Infant Formula Act requirements. Thus, the agency will have an opportunity to review the specifics of any formulations containing these oils prior to their being provided in the U.S. For this reason, we have not dealt with formulation-specific issues in this notice.

Please feel free to contact me if you have questions with any of the components of this notice.

Sincerely,

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Notice of a Claim of GRAS Status (Pursuant to Proposed 21 CFR 170.3(c)

Non-Standard Nomenclature and Abbreviations

The following non-standard nomenclature and abbreviations are used in this submission:

DHA: Docosahexaenoic acid. DHA is designated as 22:6 n-3 using standard fatty acid nomenclature. DHA occurs in nature principally as a fatty acid component of phospholipids and triglycerides and comprises one of the predominant fatty acids in vertebrate neuronal and retinal tissue.

ARA: Arachidonic acid. ARA is designated as 20:4 n-6 using standard fatty acid nomenclature. ARA occurs in nature principally as a fatty acid component of phospholipids and triglycerides and comprises one of the predominant fatty acids in vertebrate neuronal tissue. Arachidonic acid serves as a precursor to a large number of biologically active second messenger molecules, including eicosanoids.

LCPs: Long-chain polyunsaturated fatty acids. Those of principle interest in infant nutrition include ARA and DHA.

DHASCO: Refined, bleached, deodorized docosahexaenoic acid-rich single-cell oil from a non-toxigenic strain of the microalgal species *Crypthecodinium cohnii (C. cohnii).* *C. cohnii* is a non-pathogenic organism, but this fact is not relevant to the GRAS status of processed oil derived from it because such oils do not contain viable cells of the production organism. Appropriate common or usual names for DHASCO are discussed below.

ARASCO: Refined, bleached, deodorized arachidonic acid-rich single-cell oil derived from a non-toxigenic strain of the fungus *Mortierella alpina (M. alpina).* *M. alpina* is a non-pathogenic organism, but this fact is not relevant to the GRAS status of processed oils derived from it because these oils do not contain viable cells of the production organism. Appropriate common or usual names for ARASCO are discussed below.

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Term Formulas: Infant formulas intended for healthy full-term infants. Term formulas are subject to mandatory nutritional requirements in the U.S. under the Infant Formula Act.

Preterm/LBW Formulas: Infant formulas intended for prematurely-born and low birth-weight (defined as < 2500 grams) infants. Prematurely-born infants are most often of low birth weight (LBW), and products referred to as "preterm formulas" are generally formulated specifically for LBW infants. LBW formulas are exempt from certain nutritional and other requirements of the Infant Formula Act (21 U.S.C. 412(h)(1)(A) and 21 CFR 107.50) so long as any variances have a valid medical rationale.

Claim of Exemption From the Requirement for Premarket Approval (Pursuant to Proposed 21 CFR 170.36(c)(1))

We hereby provide notice that we have determined that ARASCO and DHASCO oils are generally recognized as safe, and therefore exempt from the need for premarket approval, for the specified uses described below. The basis for this finding is described in the sections below.

Signed,

8/27/98

F. Owen Fields, Ph.D. Date

Name and Address of Notifier (Pursuant to Proposed 21 CFR 170.36(c)(1)(i))

F. Owen Fields, Ph.D.
Assistant Director
Wyeth Nutritionals International
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Common or Usual Name of the Notified Substance(s) (Pursuant to Proposed 21 CFR 170.36(c)(1)(ii))

Two processed triglyceride oil preparations are the subject of the notice. One oil is an arachidonic acid-rich triglyceride derived from fermentation of *Mortierella alpina* (hereafter referred to as ARASCO). The second oil is a fermentation-derived docosahexaenoic acid-rich oil from *Crypthecodinium cohnii* (hereafter referred to as DHASCO).

Common Name for ARASCO

ARASCO[R] is a trademark of Martek Biosciences, Inc.; further, the name has no meaning to consumers. Given both of these factors, ARASCO is not an acceptable common name for the ingredient. The common name used by Wyeth and other manufacturers around the world varies from market to market based on differing consumer understandings of various possible descriptors for this oil. As far as we are aware, ARASCO is not yet in commercial distribution for food use in the U.S.

Given precedence for the naming of sources of fatty acids used in food (e.g. "high-oleic" oils), acceptable common or usual names for the ingredient would include "arachidonic acid-rich oil" and "ARA-rich oil."

Common Name for DHASCO

DHASCO[R] is a trademark of Martek Biosciences, Inc.; further, the name has no meaning to consumers. Given both of these factors, DHASCO is not an acceptable common name for this ingredient. As with ARASCO, the common name used by Wyeth around the world for DHASCO varies. Acceptable common or usual names for the ingredient would include "docosahexaenoic acid-rich oil" or "DHA-rich oil." DHASCO is currently available in capsules in the U.S. as a dietary supplement; in such products it is promoted as a vegetarian source of DHA and is referred to as "DHA-rich oil of vegetable origin".

Conditions of Use and Populations Expected to Consume the Substance (Pursuant to Proposed 21 CFR 170.36(c)(1)(iii))

The nutrient content of infant formulas is variously described in three different sets of units; these units are as follows:

- *Weight per liter* (e.g. mg/liter) is generally used in infant formula plants to express quality assurance (QA) release limits. Weight per liter is sometimes also used to express the nutrient content of a infant formulas and human milk in the scientific literature.

- *Weight or units per 100 kcal* is generally used by regulatory agencies to control nutrient levels in infant formulas. This is because infants' caloric intakes are relatively constant (see below). Such units of expression are also sometimes used in the scientific literature. Weight or units per 100 kcal are the units used in the FDA regulations which describe infant formula nutritional requirements.

- *Percentages of total fatty acids* are the units sometimes used in setting regulatory limits for fatty acids (for example, in the European Union), and these units are often how the fatty acid content of infant formulas and human milk are expressed in the scientific literature. The fat content of infant formulas is generally relatively standard, and so use of these units generally allows the absolute amount of a given fatty acid in various formulas to be compared.

For the purposes of this notice, conditions of use will be expressed as mg of target nutrient (ARA and DHA) per 100 kcal so as to conform with existing U.S. infant formula nutrient requirements. In addition, because the scientific literature and international regulations sometimes express values for DHA and ARA in units of mg/liter, or as a percentage of total fatty acids, equivalent amounts of DHA and ARA in these units will be provided so as to allow convenient comparison of the notified conditions of use with the published and unpublished information which was used to arrive at these conditions of use.

The conditions of use that are the subject of the notice are as follows:

1) ARASCO and DHASCO, for use ONLY in combination with each other or with other safe and suitable sources of ARA or DHA, so as not to provide more than 70 mg ARA and DHA combined per 100 kcal. This is equivalent to ARA and DHA levels (combined) of less than 1.4% of total fatty acids, with ARA and DHA combined falling below 570 mg per liter for a typical preterm formula and 470 mg per liter for a typical term formula; AND
2) with the use to be such that the final ratio of ARA to DHA in the infant formula falls between 1.0 and 2.0.

Under these conditions of use, ARASCO and DHASCO combined will generally comprise less than 3.5% of an infant formula fat blend.

These conditions of use have been arrived at based on: the publically available results of double-blind, placebo-controlled clinical studies; preclinical studies; other scientific procedures and methods; compositional comparisons with a food (human milk) with a significant pre-1958 history of safe use; and post-1958 safe use in infant formulas available internationally. These studies and information are described in the section below which is responsive to the proposed 21 CFR 170.36(c)(4).

The populations intended to consume these substances include all infant formula-fed infants, whether of low birth weight or whether born at term. Thus, the products in which ARASCO and DHASCO are to be used would include all products that fall within the definition of "Infant Formula" found at Title 21 U.S.C. 201(aa). Infant formula consumption generally ceases to make a significant contribution to an infant's nutrition by 1.0 year of age in the U.S.

There is ample reason to believe that consumption of these ingredients by older children and adults as part of a mixed diet, at similar relative exposures, is also generally recognized as safe, but such use is not the subject of this notice.

Analytical Methods

Validated analytical methods exist which allow the determination of ARA and DHA in infant formulas and fat blends (Wyeth General Technical Report (GTR) # 27877) and raw materials (Wyeth GTR # 26540).

Briefly, aliquots of fat, fat blends, or lipid extracts are mixed with an internal standard and solubilized. The samples are then saponified with sodium hydroxide in methanol and the fatty acids are esterified to methyl esters. The resulting fatty acid methyl esters are extracted with hexane and quantified by gas chromatography.

These methods are linear, reproducible, accurate, and rugged.

Basis for the GRAS Determination (Pursuant to Proposed 21 CFR 170.36(c)(1)(iv))

The basis for this GRAS determination is scientific procedures; these include double-blind, placebo-controlled clinical trials to investigate metabolism and safety, preclinical studies on absorption, metabolism, and safety, analytical and other related studies, compositional comparisons to normal components of a food with an extensive pre-1958 history of safe use (human milk), compositional comparisons to infant formulas containing LCPs from other sources, actual safe use experience with these ingredients in infant formulas in over 30 countries, and general scientific principles, information, and methods.

Statement of Availability (Pursuant to Proposed 21 CFR 170.36(c)(1)(v))

The data and information which are the basis for this GRAS determination will be available for inspection and copying by U.S. FDA officials at the Wyeth Nutritionals International location at 2621 Van Buren Avenue, Norristown, PA 19403 between the hours of 9:00 AM and 5:00 PM (note that this is not the notifier's official mailing address). Alternatively, copies of the data and information (in whole or in part) will be provided to FDA upon request.

The notifier can be reached at 610-650-4142 to arrange for inspection and copying.

Identity of the Notified Substances (Pursuant to Proposed 21 CFR 170.36(c)(2))

Identity

Both of the notified substances are triglyceride preparations derived from microorganisms grown in liquid culture. Standard oil extraction and processing techniques are used in their isolation (see below). As far as we are aware, these preparations have no CAS registry numbers.

Specifications for these oils preparations are provided below.

Specifications

Other than the fatty acid parameters, these specifications are typical of other edible oils, including the other oils now used in infant formulas.

Specifications for ARASCO are as follows:

Chemical description:	triglyceride oil
Arachidonic Acid content, mg/gm (using high oleic sunflower oil for dilution)	400 +/-20
Appearance:	free flowing, clear oil @ 40° C
color:	yellow to orange
Odor:	characteristic, free from rancidity
Free fatty acid, %	<0.4 (typically less than 0.07)
Peroxide value, meq/kg	<5 (typically <2.0)
Trans fatty acid (%)	<3.5 (typically <1.0)
Moisture and insoluble impurities	<0.05
Soap (ppm)	<5
Fe (ppm)	<1
Si (ppm)	<500
S (ppm)	<40
P (ppm)	<10
Cu (ppm)	<0.1
Pb (ppm)	<0.2 (typically <0.1)
Cd (ppm)	<0.2 (typically <0.02)
Hg (ppm)	<0.2 (typically < 0.04)
Microbiological:	
Standard Plate Count	<250 CFU/ml

Specifications for DHASCO are as follows:

Chemical description:	triglyceride oil
Docosahexaenoic Acid content, mg/gm (using high oleic sunflower oil for dilution)	400 +/-20
Appearance:	free flowing, clear oil @ 40° C
color:	yellow to orange
Odor:	characteristic, free from rancidity
Free fatty acid, %	<0.4 (typically less than 0.07)
Peroxide value, meq/kg	<5 (typically <2.0)
Trans fatty acid (%)	<3.5 (typically <1.0)
Moisture and insoluble impurities	<0.05
Soap (ppm)	<5
Fe (ppm)	<1
Si (ppm)	<500
S (ppm)	<40
P (ppm)	<10
Cu (ppm)	<0.1
Pb (ppm)	<0.2 (typically <0.1)
Cd (ppm)	<0.2 (typically <0.02)
Hg (ppm)	<0.2 (typically < 0.04)
Microbiological:	
Standard Plate Count	<250 CFU/ml

Other Components and Contaminants

ARASCO and DHASCO have also been characterized for the following components/contaminants and have been found to compare favorably with other edible oils:

- *residual extraction solvent*; hexane is typically absent at a detection limit of <0.3 ppm
- *cyclic fatty acids* are not detectable
- *pesticide residues*; a standard screen for 74 pesticides was conducted, and both oils were negative at the level of quantitation for all 74 analytes (Ref. 48).
- *other heavy metals*, including arsenic (typ. <0.1 ppm) and nickel (typ. <0.1 ppm). In each case (and in the case of toxic heavy metals controlled by the above specifications), the levels cited are the level of quantitation for the analytical methods used.

The processing of ARASCO and DHASCO (as described below) is not meaningfully different than the processing of the other oils used in our products and in other food products, and therefore it was not expected that ARASCO and DHASCO would meaningfully differ from other edible oils in the above parameters. Given this, and given that the conditions of use are such that ARASCO and DHASCO will comprise less than 3.5% of an infant formula fat blend, their use does not meaningfully affect the overall content of heavy metals, pesticides, trans fatty acids, residual extraction solvent, or non-saponifiables in the infant formulas in which they are used.

Fatty Acids Other Than ARA and DHA

ARASCO and DHASCO do not contain significant amounts of any fatty acids (other than ARA and DHA) that are not found in the other oils used in infant formulas. The typical fatty acid compositions of ARASCO and DHASCO are provided below.

Typical ARASCO Fatty Acid Composition:

Carbon No.	Range (typical)
14	0-2%
16	7-15%
16:1	0-1%
18	9-12%
18:1	10-20%
18:2	5-7%
18:3(n-6)	2-4%
20:3(n-6)	2-5%
20:4(n-6)	38-42%

Typical DHASCO Fatty Acid Composition:

Carbon No.	Range (typical)
10	0-1%
12	2-6%
14	13-17%
16	12-16%
16:1	0-2%
18	0-1%
18:1	17-24%
18:2	0-1%
22:6(n-3)	38-42%

As can be judged by the fatty acid compositions provided above, the use of ARASCO and DHASCO under the conditions of use that are the subject of this notice (less than 3.5% of a fat blend) does not meaningfully affect the overall level of fatty acids other than ARA and DHA.

Fatty acid Positional Distribution of ARA and DHA in ARASCO and DHASCO; Comparison With Human Milk Triglycerides

In addition to fatty acid composition, various triglyceride preparations can also differ with respect to the positional distribution of the fatty acids on the triglyceride backbone. A discussion of the meaningfulness of the fatty acid positional distribution of various oils necessitates a brief review of lipid digestion, absorption, and distribution.

Fat digestion in infants involves primarily three enzymes: gastric lipase, pancreatic lipase, and bile-salt stimulated lipase. Gastric lipase and pancreatic lipase, the quantitatively most important enzymes in formula-fed infants, are primarily specific to the sn-1 and sn-3 positions of triglycerides; the combined activity of these enzymes therefore produces predominately sn-2 monoglycerides and free fatty acids as products. Bile-salt stimulated lipase, which is present in human milk and to a lesser extent in pancreatic secretions, is capable of further converting a proportion of sn-2 monoglycerides into free fatty acids and glycerol (Refs. 99 and 100). It is as sn-2 monoglycerides and free fatty acids that triglycerides are absorbed into the body. Free fatty acid absorption rates depend on chain length and extent of unsaturation. The major differences in the extent of absorption of various fats is largely accounted for by the extent of formation of calcium soaps of saturated fatty acids with 16 or more carbons (Ref. 112).

Once dietary triglycerides are absorbed as free fatty acids and monoglycerides, triglycerides (as carried by chylomicrons and other lipoproteins) are reconstructed from the resulting monoglycerides, glycerol, and free fatty acids by the enzymatic machinery present in the intestinal mucosa. Minor amounts of cholesterol esters and phospholipids are also synthesized in the intestinal mucosa and packaged into lipoprotein particles (Refs. 99 and 100).

A consideration of the above leads to the conclusion that the fatty acid positional distribution of lipoprotein particle triglycerides (the primary forms in which fatty acids are found after their initial absorption) is determined by

the total percentage of a given fatty acid in the sn-1 and sn-3 positions of a food oil triglyceride versus those in the sn-2 position. In other words, two food oils (or oil blends) with similar percentages of a given fatty acid in the sn-2 position and similar total percentages of a given fatty acid will result in similar fatty acid positional distributions after digestion, absorption, and triglyceride reassembly.

A comparison of published data on the *average* fatty acid distribution of ARA in human milk triglycerides from women in a region of France indicates that in human milk roughly 45% of ARA is found in the sn-2 position (Ref. 115). Similar data are available from elsewhere (Ref. 105). In ARASCO oil, roughly 29% of ARA is found in the sn-2 position (Ref. 116).

Data from the studies mentioned above indicate that the *average* fatty acid positional distribution of DHA-containing human milk triglycerides are such that approximately 58% of DHA is found in the sn-2 position (Refs. 115 and 105). Approximately 46% of the DHA in DHASCO is found in the sn-2 position (Ref. 116).

Thus, both human milk and ARASCO plus DHASCO, after digestion, will result in significant amounts of free ARA and DHA and significant amounts of ARA and DHA-containing sn-2 monoglycerides.

Having said the above, however, it must be recognized that even if the proportion of ARA and DHA in the sn-2 position of ARASCO and DHASCO were significantly different than in human milk, this fact alone would not mean a great deal. We say this for several reasons. First, none of the oils commonly used in U.S. infant formulas have fatty acid positional distributions that are identical to those found in human milk; this includes sources of the dietary essential (and legally mandatory) fatty acids that serve as precursors to ARA and DHA (Ref. 125).

Second, while the fatty acid positional distribution of certain fatty acids in oils can affect the extent to which they are absorbed (Ref. 112), the extent to which a given oil preparation is absorbed and provides for accretion of fatty acids into tissues is experimentally addressable in preclinical and clinical studies. As described below, preclinical and clinical studies indicate that ARASCO and DHASCO are efficiently absorbed and deliver the target fatty acids to tissues.

Finally, the literature reports cited above indicate that there is variance in the fatty acid positional distribution of human milk (even among individuals from a single region), as might be expected from the fact that dietary triglycerides contribute to tissue fatty acid positional distributions (Ref. 126). Thus, there exists a *range* of fatty acid positional distributions in human milk, not a single characteristic distribution. As it turns out, ARASCO and DHASCO contain ARA and DHA at the sn-2 position at a proportion which is well within the range found in human milk (Refs. 105, 115, and 116)

Fermentation and Processing of ARASCO and DHASCO

Mortiella alpina (the organism that produces ARASCO oil) is fermented in a Gist-Brocades fermentation plant in Italy, initial oil extraction is conducted in France, and final processing is conducted in Martek's Winchester, Kentucky facility. All steps in ARASCO production can also be conducted in Martek's facility. *Crypthecodinium cohnii* (the organism that produces DHASCO oil) is fermented in Martek's Winchester, Kentucky plant and DHASCO is also processed (post-fermentation) in the Winchester, Kentucky plant.

All facilities have restricted access, have undergone food-grade inspection by the U.S. FDA or by local regulatory authorities, and are operating in compliance with current Good Manufacturing Practices (cGMPs) and all other applicable local regulations. Consistent with cGMPs, formalized process documents and Standard Operating Procedures (SOPs) are used by trained operators throughout all of the manufacturing and processing operations. The algal strain used to produce DHASCO and the fungal strain used to produce ARASCO have been used by Martek and/or Gist-Brocades for several years. As outlined in other sections of this notice, neither organism has ever been reported to produce toxins under production conditions, and testing in animals and humans has not indicated any adverse findings. Nevertheless, specific steps are still necessary to ensure that these products are manufactured in such a way that safety is maintained. These steps are outlined below.

Maintenance of Cultural Purity and Consistency

Many specific measures are required to ensure that cultural purity is maintained and to ensure that these fermentations take place under consistent conditions. The seed stocks of the production organisms are stored at -80° C or in liquid nitrogen and examined for cultural purity prior to use. In order to expand the inoculum, seed stock is transferred by trained technicians under laminar flow conditions to steam-sterilized culture vessels in a microbiology laboratory.

The seed and production fermenters that are used are constructed of food-grade stainless steel and connected by stainless steel transfer lines. All vessels and lines are cleaned and sterilized in place. Cleaning is

accomplished with caustic and acid solutions pumped from a central system using fixed cycles. Subsequent to cleaning, vessels and lines are flushed with water until neutral pH readings are obtained. Steam is supplied by two central boilers; the steam from these boilers is monitored for quality. Water for Martek's plant is supplied by the city of Winchester, Kentucky, is softened, and is monitored for quality on a regular basis.

Raw materials used in the fermentations meet established specifications and are purchased only from approved vendors. A manufacturer's Certificate of Analysis is provided with each lot and is reviewed prior to release of the product for use. Laboratory identity and quantitative analyses are conducted on selected raw materials where required. Raw materials and supplies are stored segregated by lot in a controlled warehouse. Inventories of raw materials are utilized on a first-in-first-out basis to ensure that storage times are minimized.

Vessels and lines are sterilized according to SOPs which clearly indicate proper valve positions as well as time and temperature requirements. Operators use temperature-sensitive indicators at specific sites to confirm the progress of sterilization. Raw materials are weighed and checked by a second operator prior to mixing and sterilization. Solutions added to the vessels are sterilized in excess of minimum sterilization parameters.

All fermenter operations are tested daily for sterility and/or freedom from contaminating organisms. Any confirmed contaminants result in immediate discard of the fermenter batch. When cultures are actively growing, cell counts and other analytical assays showing the progress of the culture are routinely conducted and compared to historical standards. Any deviations from established standards is investigated and, if outside of established guidelines, result in discard of the batch. Fermenters are continuously monitored for pH, dissolved oxygen, temperature, agitation RPMs, air flow, and back pressure. All tanks are visually monitored by trained operators for any culture changes.

All data generated during the fermentation process are permanently recorded. A final decision on whether or not to harvest is based on a review of all of these data.

All employees are trained by following SOPs during actual operations under the continuous supervision of a trained operator. Both trainer and trainee

initial and date documents to indicate satisfactory completion of each operation. Trainees must demonstrate competency without aid before they conduct independent operations.

All operating procedures and process documents are maintained in a computer file in such a way that only the most current documents are accessible to the operators. Any changes to a process or procedure must be approved. All changes to procedural or process documents are listed by revision number and date.

A number of published studies have described fermentation processes using *C. cohnii* and *M. alpina* (Refs. 69, 85, 87, 88, 89, 109, and 110)

Post-Fermentation Processing

Fermenter contents are chilled prior to harvest by centrifugation or similar separation procedures. Harvested product is kept cold until spray drying (or alternative drying operations) are conducted. Spray drying equipment is cleaned in place using operations which are similar to those used for the fermenters. Dried biomass is collected in sequentially numbered and labeled food grade plastic lined boxes or totes, nitrogen blanketed to ensure oxidative stability, and stored below freezing until ready for hexane extraction.

Oil processing is completed using conventional vegetable oil processing technology (oil is accumulated by both production organisms intracellularly). Briefly, biomass is hexane extracted in an extractor followed by standard winterization, refining, bleaching, and deodorization processes. These are all standard oil processing procedures, as described in a widely used textbook on oil processing (Ref. 40).

All operations are monitored and data are compared to historical values. Product is collected from the deodorizer in a clean environment and stored below freezing or under vacuum until used.

It is important to note that the oil processing steps mentioned above result in a highly purified product. High molecular weight substances (proteins, carbohydrates, and nucleic acids) and low molecular weight polar compounds (amino acids, nucleotides, etc) will be separated from the oil by the hexane extraction. In addition, subsequent processing steps, including winterization and steam stripping (which have been designed to remove any non-

triglyceride compounds that might result in off-flavors and reduced quality), will remove all but the most nonpolar substances (e.g. triglycerides, carotenoids, phytosterols).

Of course, both the organic extraction steps (cells must be disrupted in order for the oil to be extracted) and the very high temperatures used in the process (230 degrees Celsius is typically used in steam stripping) will ensure that viable cells of the production organism will not survive processing.

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Taxonomy of the Production Organisms

Crypthecodinium cohnii (Used in the DHASCO Process)

The taxonomic classification of *Crypthecodinium cohnii (C. cohnii)* places it in the eukaryotic microalgal phylum (or division) Pyrrophyta (or Dinophyta):

Phylum: Pyrrophyta
Class: Dinophyceae
Order: Peridiniales
Family: Cyrpthecodiniaceae
Genus: *Crypthecodinium*
Species: *cohnii*

The Martek strain of *C.cohnii* (MK8840) used in the production of DHASCO has been selected for rapid growth under high density, high shear environments. It has been deposited at the American Type Culture Collection (Rockville, MD) and has been assigned the ATCC accession number 40750. The culture is preserved under liquid nitrogen conditions at several locations and random genetic drift is monitored by assessing morphological and growth characteristics.

The major taxonomic grouping to which dinoflagellates belong is called the phylum or division Pyrrophyta or Dinophyta. Within this phylum there are four Classes (Dinophyceae, Ebriophyceae, Ellobiophyceae and Syndiniophyceae). Within the Class Dinophyceae there are 12 Orders. Within the Order Peridiniales there are 13 Families. Within the Family *Crypthecodinium* is found the species *cohnii.*

Dinoflagellates are an evolutionarily ancient group of microalgae, with fossil records dating back at least to the late Triassic period. The first written description of dinoflagellates was in 1753 when Baker described the bioluminescent organism *Noctiluca* (Ref. 63). Although the first genus name that is still valid (*Ceratium*) was coined by Schrank in 1793, dinoflagellate taxonomy really didn't start as a science until the 1830's and rapidly expanded with the development of better microscopy in the 1880's (Ref. 63). A taxonomic review of the dinoflagellates can be found in Ref. 58.

Structure, Chemical Composition, and Consumption

Dinoflagellates represent a very large and diverse grouping of biflagellate, coccoid, filamentous, palmelloid and amoeboid forms that range in size from 0.01 mm to 2.0 mm in diameter. There is a tremendously wide diversity in morphology, nutrition, and habitat among dinoflagellates. Dinoflagellates are thought to be the most important members of the phytoplankton in marine and freshwater ecosystems alike (phytoplankton are the base of the entire marine food chain and of most freshwater food chains; Ref. 63). Consequently, they are a major constituent of many food webs.

Dinoflagellates are eukaryotes with organellar structures characteristic of all other eukaryotes, although the nucleus appears to be devoid of histones. Like most microbes, the most common form of reproduction is asexual (Ref. 63).

Toxic Dinoflagellates

Of the many characterized species of dinoflagellates, only about 1% have been associated with certain toxins. Interestingly, all of the species that have been found to be capable of toxin production are photosynthetic. There are no known nonphotosynthetic (heterotrophic) dinoflagellates that produce or are associated with toxin production. Those dinoflagellates that are associated with toxins are capable of producing monospecific blooms that concentrate on the surface waters by phototaxis. Because filter feeding shellfish depend on these organisms for food, a bloom of such dinoflagellates can severely affect shellfish and can have a major economic impact on the shellfish industry. Not all isolates of known toxic species produce the toxin. Because of this observation, and because some dinoflagellates have been shown to harbor endosymbiotic bacteria, the ability of some toxic dinoflagellates themselves to produce the toxin had recently been questioned. For example, researchers have shown that a bacteria (*Moraxella sp.*) isolated from a toxic strain of *Protogonyaulax tamarensis* is responsible for toxin production (Ref. 41).

Although Kuster in 1908 was the first to report the culturing of any dinoflagellate species *in vitro* with *Gymnodinium fucoru*, many workers later believed this to actually have been the first reported cultivation of *Crypthecodinium cohnii* (Ref. 60). Since that time, *C. cohnii* has been one of

the best studied of all the dinoflagellates because, unlike many species of dinoflagellates, it is easily cultured in the laboratory. *C. cohnii* is nonphotosynthetic (heterotrophic) and therefore its culture requires the input of energy sources (Ref. 60). Of the dinoflagellate species which have compelling evidence for a sexual stage, only *Crypthecodinium cohnii, Ceratrium cornatum,* and *Peridinium volzii* have been subjected to any form of detailed genetic analysis. Beam and Himes created a series of mutants and employed microdissection methods for zygotes to undertake genetic analyses of *C. cohnii* (Refs. 43 and 56).

The molecular genetics of *C. cohnii* has also been studied more than any other dinoflagellate. Comparisons of sibling strains of *C. cohnii* either by RFLP mapping or by comparisons of soluble protein isozymes (e.g. leucine aminopeptidase, acid phosphatases and proprionyl esterase) have indicated that individual stain variations can be detected and potentially used as genetic markers (Ref. 56). Ribosomal RNA sequencing of members of the species has also been conducted (Ref . 42)

In the many reports on the use of *C. cohnii* grown under a wide variety of culture conditions over the last 80 years, there have never been any indications that *C. cohnii* is capable of producing toxins, nor is *C. cohnii* closely related to any of the toxin-associated dinoflagellate species (Ref. 44).

In summary, *C. cohnii* is not closely related to any toxin-associated organisms and in its 80 year history of cultivation there has been no reported association with any toxicity or pathogenicity. In addition, as described in the preclinical safety assessment section of this submission, products of *C. cohnii* have been tested in toxicology studies, and we have taken the additional precautionary step of specifically testing for the presence of dinoflagellate toxins in both crude biomass and in processed oil from *C. cohnii.* These analyses further confirm that this organism is incapable of producing toxins.

Mortierella alpina (Used in the ARASCO Process).

Mortierella alpina (*M. alpina*) is a common soil fungus that is widely distributed in nature (Ref. 59). It is among the dominant fungal species in some soils (Ref. 45). A review of fungal taxonomy can be found in Ref. 57. The taxonomic classification of *M. alpina* places it in the eukaryotic Kingdom Fungi:

Phylum: Eumycota
Class: Phycomyceteae
Order: Mucorales
Family: Mortierellaceae
Genus: *Mortierella*
Species: *alpina*

The strain of *M. alpina* used in the production of ARASCO has been selected for rapid growth under the high density, high shear environments typical of commercial fermentation conditions. The culture is preserved under liquid nitrogen conditions at several locations and random genetic drift is monitored by assessing morphological and growth characteristic changes. To date no identifiable genetic drift has been seen in the organism.

As stated above, *M. alpina* is a common soil fungus that is widespread in nature. Because of this, human exposure to *M. alpina* is fairly common. A literature search failed to locate any reports of *M. alpina* being reliably associated with pathogenic or toxicologic activity (Ref. 64). There is an abstract from 1966 which reported that *M. alpina* was isolated from liver lesions in a calf in New Zealand (Ref. 62). A more recent opinion, however, indicates that the organism identified in this abstract was likely *Mortiella wolfii*, a fungus that is pathogenic for cattle but not humans, and *M. alpina* has not been accepted as a pathogenic species (Ref. 61). This is consistent with the fact that *M. alpina* has a maximum temperature of growth of 30-32° C (Ref. 61).

Of course, given the nature of the processing of ARASCO oil and the rather harsh conditions involved, viable cells of the production organism will not be present in the final processed oil intended for food use. In addition, the safety testing outlined in this submission confirms that *M. alpina* is not capable of producing harmful substances.

Summary

The production organisms involved in the production of ARASCO and DHASCO are widespread in the human environment. In the long history of mankind's accidental or purposeful interactions with these organisms, there have been no reports that either organism is capable of producing harmful substances under the conditions used for production of ARASCO and DHASCO oils, and neither organism is currently believed to be capable of displaying pathogenic activity. In addition, it has been confirmed (by assessing the toxicological profile of the final processed products) that these organisms are suitable for use in producing food ingredients (see summaries below).

Information on Self-Limiting Levels of Use (Pursuant to Proposed 21 CFR 170.36(c)(3))

Use of these oils in infant formulas is self-limited by their very high cost versus commodity oils and by the need to maintain an appropriate level of other fatty acids (linoleic and linolenic acids, etc.) in infant formula fat blends.

Detailed Summary of the Basis for the GRAS Determination (Pursuant to Proposed 21 CFR 170.36(c)(4)(i)(A))

Overview

In this section, the detailed basis for our GRAS determination is summarized. Included in this summary are the following:

- A discussion of the unique aspects of assessing the safety of new infant formula ingredients.
- A discussion of the safety of ARA and DHA as nutrients (that is, not considering issues raised by the *source* of these nutrients) for use in infant formulas.
- A discussion of the basis for concluding that the specific sources of ARA and DHA that are subject to this notice (ARASCO and DHASCO), under the conditions of use described in this notice, present a reasonable certainty of no harm (i.e., meet the technical requirements for safety).
- A discussion of the general recognition of the safety of ARASCO and DHASCO under the specified conditions of use.

Considerations Unique to Assessing the Safety of Infant Formula Ingredients

The assessment of the safety of infant formula ingredients presents some unique issues. These unique issues can be described as follows:

1. Infant formulas may constitute the sole source of nutrition for the first few months of an infant's life. This results in relatively consistent individual daily exposures to infant formula ingredients. For example, 90th percentile formula intakes for both male and female infants at various ages is generally less than 20% greater than 50th percentile intakes (Ref. 127). This contrasts markedly with adults, whose mixed diets result in highly variable individual daily exposures to food ingredients.
2. Following the first few months of life, an infant's diet typically includes an increasing percentage of non-infant formula food products, so that the contribution of formula to the total nutrient intake of the infant gradually decreases. Further, an infant's intake of calories expressed on a kcal/kg bodyweight/day basis generally decreases with time. Combined, these

factors result in exposures to infant formula ingredients which gradually decrease with time. In the U.S., infant formula consumption generally ceases to make a meaningful contribution to infant's nutrient intakes by 1 year of age.

3. An infant's total intake of food components in the first few months of life, expressed on a gram per kg bodyweight per day basis, is markedly higher than a typical adult's intake; this is due to infants' rapid growth rate. Expressed on a kcal per kg bodyweight per day basis, the average intake of a 4 kg, one-month old male infant is approximately three times (energy), two times (protein), 4.4 times (fat), and 2.8 times (carbohydrate) as high as a 70 kg adult male (Ref. 127). This is one of the reasons that clinical studies, in addition to preclinical studies, must often be relied upon in assessing new infant formula ingredients.

4. Unlike foods consumed by adults, a fully natural model (human milk) is available to guide infant formula compositional decisions. Given this, infant formulas are generally formulated to be compositionally and/or physiologically equivalent to human milk (Ref. 46). The presence of a nutrient in human milk (although such presence alone is not sufficient to support safety) is universally considered as important evidence which should be used in assessing the safety of new infant formula ingredients. This concept has been formally recognized in authoritative guidelines and in international infant formula regulations (Refs. 46 and 47).

5. Under the Infant Formula Act, FDA must be notified, prior to marketing, of all "new" infant formulas, including infant formulas containing new physiologically important ingredients. This provides the agency with information on each separate new use of each new infant formula ingredient. This is in obvious contrast with uses of new ingredients in foods for adults, where all uses of a new ingredient need not be brought to the agency's attention.

6. Two related questions must be answered in turn when considering whether a given nutrient addition to infant formula is safe. First, it must be determined what amount and chemical form of the nutrient(s) (considered separately from issues raised by its *source*) is safe, and second, it must be determined if a *particular source* of the nutrient(s) is safe (e.g., does it contain harmful innate or introduced constituents)? In the remainder of this notice, we have endeavored to keep these two questions separate whenever practical.

Long-Chain Polyunsaturated Fatty Acids (LCPs)

This section provides information on, and the rationale for the addition of, ARA and DHA to infant formulas. Information on ARASCO and DHASCO (the specific sources of ARA and DHA that are the subject of this notice), including data from preclinical safety, preclinical nutritional, and clinical assessments, is included in later sections of this notice.

Purpose of LCP Addition to Infant Formulas

Although significant improvements in the composition of infant formulas have been achieved, infant formulas do not perfectly mimic the nutritional support provided by human milk. This fact has led Wyeth and others to focus on further possible improvements to formulas. One of these improvements involves the addition of the LCPs ARA and DHA to selected products.

Infants require both n-3 and n-6 fatty acids for normal growth and development. Human milk contains both the n-3 and n-6 families of essential fatty acids; the typical milk fat of European women contains approximately 1.0-1.5 weight % alpha-linolenic acid (18:3n-3; a metabolic precursor to DHA) and 10-15 weight % linoleic acid (18:2n-6; a metabolic precursor to ARA; Refs. 2 and 49). Currently marketed infant formulas generally contain linoleic acid in levels equal to or greater than the levels present in human milk, and must meet the mandatory lower limits set by numerous national and international regulatory agencies (Ref. 1). Many infant formulas also contain levels of alpha-linolenic acid similar to those found in human milk; in the European Union (EU), minimum levels of alpha-linolenic acid are mandated (Refs. 2 and 65).

However, long chain polyunsaturated fatty acids (C20 and C22) are also present in human milk, at 0.3-1.2% and 0.5-1.5% for the n-3 (DHA) and n-6 (ARA) families, respectively (Ref. 49 and references therein). In contrast to the C18 essential fatty acids, many infant formulas do not presently contain longer-chain n-3 or n-6 fatty acids (LCPs), despite the recognition that these fatty acids serve important biological functions as structural components of cellular membranes and as precursors to biologically active secondary messenger molecules (Ref. 3).

Structural Rationale for LCP Addition

In adults, C18 essential fatty acid precursors can be converted to LCPs via enzymatic elongation and desaturation. However, there is reason to believe that the ability of preterm and term infants to elongate and desaturate C18 essential fatty acid precursors to LCPs is not adequate to match the tissue accretion rates observed in breast-fed infants. Plasma and red blood cell membrane levels of n-3 and n-6 LCPs fall rapidly following birth in preterm infants, probably due to the termination of maternal (placental) provisions and the very low stores of these fatty acids in premature infants. In preterm infants fed human milk this decline stabilizes, presumably due to the preformed ARA and DHA present in human milk. In preterm infants fed infant formulas lacking preformed LCPs, circulating LCP levels decline following birth and continue to fall (see below and Refs. 4 and 9). Similar observations have been made in term infants (see below and Refs. 16 and 20).

Tissue depletion in infants fed formulas lacking LCPs is not limited to those tissues (blood fractions) that can easily be sampled in clinical studies. Autopsy studies and studies on surgical samples have shown that tissue levels of LCPs in breast-fed vs. formula-fed infants differ (Refs. 23-28, 31, and 96). These studies, considered in total, indicate that brain cortex, liver, muscle, and adipose tissue in infants fed formulas lacking DHA are clearly lower in DHA (and most often ARA) when compared to tissues from infants fed breast milk. In most cases the observed differences are structurally significant (generally a 15-35 % decrease is seen).

The manipulation of absolute levels and ratios of linoleic acid to alpha-linolenic acid (metabolic precursors to ARA and DHA) in order to provide for ARA and DHA accretion into tissues similar to that seen in breast-fed infants has been attempted. Such attempts have failed in both preterm and term infants, and in addition it was found that a very low ratio of linoleic to linolenic acid resulted in growth deficits (Refs. 20 and 21). Thus, the only way to allow formula-fed infants to attain tissue LCP levels equivalent to those of human milk-fed infants is to provide preformed ARA and DHA in the diet.

Functional Rationale for LCP Addition

Given that the structure of biological membranes influences their function, it could rationally be hypothesized that the *structural* differences evident between infants fed LCPs and those not fed LCPs might result in differences in *function*. As might be expected, this hypothesis has initially been tested in those organs in which LCPs are the most abundant, namely, the brain and retina.

Using preterm infants (whose tissue LCPs deficits could be predicted to be more dramatic than seen in healthy term infants) at least four double-blind, placebo-controlled studies that have appeared in the peer-reviewed literature have tested this hypothesis by assessing differences (using a number of different methods) between the visual and neuronal maturation of infants fed formulas with and without added preformed LCPs. All four studies have shown that there are differences in the rate of maturation of these functions between infants receiving dietary DHA and those not receiving dietary DHA (Refs. 5, 6, 32, 33, 35, 36, 37, 70, 82, and 91).

A number of such studies have also now been conducted in healthy term infants, which are born with tissue stores of LCPs greater than those of premature infants. These studies indicate that whether differences in neuronal and/or visual maturation can be detected in term infants appears to depend on the specific methodology used in the testing (sweep visual evoked potentials (VEP), other types of VEP tests, acuity card procedures, etc.), the dosage of LCPs fed (the dose of DHA used in the different studies has varied by nearly four-fold), and/or the base formula used in the studies (Refs. 16, 32, 34, 38, 39, 54, 67, 68, 72, 79, and 92). The most recent study on term infants provides a useful discussion of the differences among the various studies (Ref. 54).

Balanced Addition of DHA and ARA

The addition of DHA to infant formula may be achieved by the addition of marine fish oil alone. However, this is an unbalanced addition of LCPs, in that most marine oils contain relatively high levels of DHA and much lower levels of ARA. *In vitro*, the presence of n-3 LCPs such as DHA down-regulates the chain elongation/desaturation of linoleic acid to n-6 LCPs (Ref. 7). This effect has also been demonstrated *in vivo* in an animal model; addition of n-3 LCPs with the only source of n-6 fatty acids being linoleic acid

produces an undesirable reduction in tissue ARA and a concomitant elevation of n-3 LCPs in piglets (Ref. 8). Clinical studies have demonstrated that this phenomenon occurs in human infants as well; such studies have documented a reduction in plasma and red blood cell ARA status after administration of a formula supplemented with marine oils rich in eicosapentaenoic acid (EPA; an n-3 fatty acid abundant in some fish oils but which is not found in DHASCO) and DHA (Ref. 9). There are indications that such reductions in ARA status may result in decreased growth (Refs. 18 and 21), and it has further been demonstrated that plasma ARA levels are positively correlated with the growth of premature infants (Refs. 19 and 124).

Finally, although alterations in clotting parameters are not evident in infants fed n-3 fatty acids in the form of fish oil in the absence of preformed ARA (Ref. 66), consumption of very high levels of n-3 fatty acids concomitant with a constant intake of n-6 fatty acids in adults has been shown to increase bleeding time (Federal Register of June 5, 1998 at page 30751; 62 FR 30751). Therefore, given all of the above, an important consideration for infant formula companies was the identification of sources of DHA and ARA that would allow a properly balanced addition (where "balanced" means at the approximate ratios at which they are found in human milk) of these fatty acids to infant formulas.

Expert Recommendations

A number of expert groups have considered the safety of, and the physiological need for, the addition of preformed LCPs to infant formulas. As early as 1991 the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) recommended addition of DHA and ARA to formulas intended for preterm infants (Ref. 10). This was followed in 1992 by a similar recommendation from the British Nutrition Foundation (Ref. 11).

In 1993 an expert committee was assembled under the auspices of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations and tasked with drafting a report on the role of fats and oils in human nutrition; as part of their considerations they surveyed the role of lipids in early development. As a result, this committee recommended the addition of preformed ARA and DHA to both term and preterm infant formulas (Ref. 12). In addition, the Bureau of Nutritional Science of the Canadian Health Protection Branch assembled an expert

committee to address the nutritional needs of the preterm infant; this expert group also recommended that preformed ARA and DHA be added to preterm infant formulas, so long as the safety of the LCP source(s) used had been demonstrated (Ref. 13). An expert panel assembled by the International Society for the Study of Fatty Acids and Lipids (ISSFAL) has also recommended the addition of DHA and ARA to infant formulas (Ref. 14).

Regulatory Status of LCPs Worldwide

Based on the existing expert recommendations, there have been efforts to amend existing infant formula standards to explicitly allow for the addition of LCPs to infant formulas. Within the European Union (EU), the addition of LCPs has been considered and approved by the European Commission's Scientific Committee for Food (SCF) and the Standing Committee for Food. Based on these findings, addition of preformed LCPs to infant formulas (at total levels of up to 2.0% of total fatty acids) has been permitted throughout the EU for approximately two and a half years (Ref. 65).

The Australia/New Zealand National Food Authority has also proposed explicitly permitting the addition of LCPs to both preterm and term infant formulas (Ref. 50). In addition, the Australian Therapeutic Goods Administration (the agency that regulates therapeutics) has approved a therapeutic claim to the effect that LCPs "may assist normal neurological development in infants whose diets are deficient in long chain polyunsaturated fatty acids" (Ref. 22).

Current Use of LCPs in Infant Formulas

All of the world's major infant formula producers have either added or are in the process of studying the addition of LCPs to infant formulas. Given this, it is not surprising that LCPs derived from fish oil, egg lipid, ARASCO and DHASCO, and/or nontraditional vegetable oils are currently in use in Japan, Australia, throughout Europe, and elsewhere. The then-current use of LCPs in preterm and term formulas in Europe was cataloged several years ago (Ref. 17). This use has since expanded, and LCPs are now in use in the nearly all European preterm formulas and a significant percentage of term formulas. In at least one European country (the U.K.), it is no longer possible to procure a formula for preterm infants that does not contain added DHA.

Outside of Europe use of LCPs has been common in Japan, Korea and much of Asia for several years; Wyeth Nutritionals International (WNI) was the first to introduce LCPs in infant formulas over much of the rest of the world (see list of countries below). At the time of writing, the U.S., Canada, and a few developing Asian countries and African nations are the only countries in the world in which LCPs are not currently being added to infant formulas.

Published Clinical Studies on LCP Addition to Infant Formulas

A number of clinical studies have been conducted with sources of LCPs other than ARASCO and DHASCO. These studies will be summarized in this section; the clinical studies that have been conducted using ARASCO and DHASCO as sources of LCPs will be discussed in a later section of this notice.

In clinical studies on infants, two outcomes which are relevant to safety are generally assessed. First, the growth of infants fed a test formula(s) is assessed. Secondly, elements other than growth which may indicate safety (adverse events, tolerance, etc.) are assessed.

A number of clinical studies have addressed the effects of LCP supplementation on the growth of preterm and term infants. Among these are the studies of Carlson (Refs. 9, 18, 19, 35). The first of these employed a high-EPA fish oil as a source of DHA (ARA was not added to the test formula because a practical source was not available at the time the study was initiated). In this study, tissue levels of ARA in the fish oil-fed infants were reduced (presumably by the predictable metabolic competition with n-3 LCPs), and this group was also observed to grow more slowly than the group receiving an unsupplemented control formula. A second study by Carlson using a low EPA fish oil (which would be expected to have less of an effect on ARA status) showed that the effects of such supplementation on growth were less but still detectable.

In contrast, other studies (Refs. 5, 37, 66, 77, and 82) have found no such effect on growth when DHA was supplemented in the absence of ARA; this is despite the fact that these studies were of a similar design, involved similar doses of DHA, and were powered similarly to those of Carlson.

Thus, the data on preterm infants with respect to the effects of DHA alone on growth is mixed; in addition, prior to the studies conducted on ARASCO and DHASCO (described below) most of the existing studies in preterm infants did

not involve balanced addition of ARA and DHA, and most therefore involved infants whose ARA status was compromised (as could have been predicted) by n-3 supplementation in the absence of n-6 supplementation. Because the association of ARA status with growth has been known for several decades, this latter factor was an obvious possible confounder in some of the studies.

Using term infants, a number of studies (Refs. 16, 34, 38, 54, 67, 68, 75, 76, 78, 83, 86, and 92) have shown that LCP addition, whether ARA status is compromised or not, has no effect on growth. The largest such study on term infants, and the study that involved the longest period of feeding, was recently published in a peer-reviewed journal (Ref. 34). In this adequately-powered study, which involved a full year of feeding of term infants with formulas supplemented with DHA alone (from fish oil) or in combination with ARA (from egg lipids), normal growth was documented and the incidence of adverse events was similar in all study groups, despite the fact that the ARA status of the group supplemented with only DHA was reduced compared to the control formula group. The authors concluded that "Based on the normal growth, the control formula and the formulas supplemented with fish oil (DHA alone) or egg yolk phospholipid ([ARA] + DHA) appear suitable for feeding to healthy, term infants" (Ref. 34). Thus, the growth data from clinical studies on term infants is uniformly reassuring.

Elements of Safety Other Than Growth

By this point at least 20 clinical studies (not counting those studies on ARASCO and DHASCO which are summarized later in this notice) have been published in which LCP-supplemented formulas have been fed to preterm and term infants (Refs. 5, 9, 16, 18, 19, 34, 35, 37, 38, 66, 67, 68, 73, 75, 76, 77, 82, 83, 86, and 92). Among all of these studies, no significant differences in the incidence of adverse events between LCP-supplemented and non-LCP supplemented infants were reported.

Specific Sources of ARA and DHA; ARASCO and DHASCO

Above the safety of ARA and DHA (as separated from the issues raised by the *sources* of these fatty acids) was discussed. In this section the safety of ARASCO and DHASCO, the specific sources of ARA and DHA that are the subject of this notice, are discussed. Because the use of ARASCO and DHASCO allows flexibility in designing supplementation levels (some other sources of LCPs do not allow such flexibility), the studies described below have added significantly to the body of information in this area.

Overview; Safety and Physiological Considerations

The safety and bioavailability of the ARASCO and DHASCO triglyceride preparations has been established by a number of different methodologies. First, although triglycerides have no toxic potential *per se*, the preclinical safety studies described below were used to confirm that the production systems do not result in harmful contaminants which copurify with the oils. Second, preclinical nutrition studies, as described below, have been conducted to confirm that these triglyceride preparations are digestible and support tissue fatty acid accretion in animal models. Third, clinical absorption/metabolism studies in infants were conducted to confirm that these triglyceride preparations, when incorporated into our formula systems, support accretion of the target fatty acids into term and preterm infant's tissues. Fourth, a large growth and safety clinical study in the most vulnerable population of infants (those born prematurely) has confirmed that these oils are safe.

Studies by others have also been conducted, and those that have been published are also discussed in a later section of this notice.

Intake Estimates for ARASCO and DHASCO

We have determined the estimated intake of ARASCO and DHASCO by term and LBW (preterm) infants based on the notified use levels in term and preterm formulas.

A number of different intake estimates are calculated below; in each case the estimates provided are probable average intakes (in keeping with standard

practice in the infant formula industry) calculated using the reported 50th percentile caloric intakes of infants and using the "target levels" we currently add to our infant formulas. "Target levels" are the levels we attempt to achieve in commercial practice, but due to the inevitability of analytical and manufacturing variance under commercial production conditions the levels of use that are the subject of this notice are somewhat broader than are our target levels.

For the purposes of conducting safety assessments on new chemical entities, it is common to introduce a number of conservatisms (e.g., use of 90th percentile exposure estimates, use of maximal rather than probable levels of use, etc.). Although the usefulness of these now-standard safety assessment paradigms for assessing the safety of traditional food ingredients (such as triglyceride preparations) has been recognized to be limited, we nevertheless have provided factors immediately below which could be used, if desired, to build such conservatisms into the intake estimates.

- 90th percentile caloric intakes of infants are generally less than 20% greater (a factor of 1.2 times) than 50th percentile intakes (Ref. 127). Thus, the application of a factor of 1.2 to the 50% intakes provided below will allow calculation of approximate 90th percentile intakes.
- The maximal levels of use provided pursuant to 21 CFR 170.36(c)(1)(iii) are 40% greater than probable levels of use. Thus, the application of a factor of 1.4 to the probable levels of use identified below will allow calculation of maximal levels of use.
- The calculations provided below account for the fact that infant formula intake gradually decreases over time and also account for the typical gradual replacement of infant formula by other foods. If maximum conservatism is desired (that is, if it is desired to assume that intake on a bodyweight basis does not decrease and that other foods are not introduced into infants' diets), then intake estimates for one-month old infants can be used to estimate maximal exposures for older infants.

Projected Intake by Preterm Infants

In order to calculate the projected 50th percentile intakes of premature infants at the target levels at which these fatty acids are used in our formulas, we have used the following facts: 1) preterm infants require approximately 120 kcal/kg/day in the diet; 2) infant formulas intended for use by premature infants contain approximately 5.2 grams of fat per 100 kcal; 3) undiluted ARASCO is approximately 42% percent by weight ARA; 4) undiluted DHASCO is approximately 43% by weight DHA; and 5) we target ARA at 0.6% of fatty acids and DHA at 0.4% of fatty acids. As discussed elsewhere, the levels we target are partially based on compositional comparisons to human milk and are partly derived from clinical data.

It is necessary to note here that after birth, preterm infants generally receive their initial nutritional support via parenteral routes and gradually go through a transition to enteral feeds; this transition requires a variable period of time which depends on the infant's health status. The calculations below assume that the infants have achieved full enteral feeds (i.e., are getting all of their nutrition orally).

Intake factors:

- Preterm infants consume 120 kcal/kg/day
- Preterm formula contains 5.2 g fat/100 kcal
- Preterm infants consume 5.2 x 1.2 = 6.24 g fat/kg/day
- 0.6% of fatty acids as ARA is targeted
- 6.24 grams/kg/day x 0.006 = 0.037 g or 37 mg ARA/kg/day
- 37 mg/0.42 = 88.1 mg ARASCO/kg/day (undiluted ARASCO is approximately 42% by weight ARA).

Similarly:

- 0.4% of fatty acids as DHA is targeted
- 6.24 grams/kg/day x 0.004 = 0.025 g or 25 mg DHA/kg/day
- 25 mg/0.43 = 58.1 mg DHASCO/kg/day (undiluted DHASCO is approximately 43% DHA by weight)

Projected Intake by Term Infants

Term infants generally have a caloric intake in their first month within the range of, or slightly less than, preterm intakes; however, energy intake on a body weight basis decreases from that point at least until 6 months, at which time diet recall surveys indicate that caloric intake remains relatively steady for a few months (Ref. 127). In addition, as infants grow older they rely less and less on infant formula and more on other foods (Refs. 98 and 127). Thus, a term infant's intake of ARASCO and DHASCO in their first month of life (on a bodyweight basis) will be equal to that estimated for preterm infants, but intake will then decrease over time. To illustrate this phenomenon, consider the estimated intake of an infant consuming a term infant formula at a 50th percentile intake with DHA and ARA at the targeted level:

1 month of age: 120 kcal/kg/day, 100% of calories from formula:

Intake of ARASCO = 88.1 mg/kg/day (as derived above)
Intake of DHASCO = 58.1 mg/kg/day (as derived above)

6 months of age: 92 kcal/kg/day, 80% of calories from formula (Refs. 98 and 127):

Intake of ARASCO = (88.1)(92/120)(0.8) = 54 mg/kg/day
Intake of DHASCO = (58.1)(92/120)(0.8) = 36 mg/kg/day

10 months: assuming 92 kcal/kg/day, 50% of calories from formula (Ref. Fomon):

Intake of ARASCO = (88.1)(92/120)(0.5) = 34 mg/kg/day
Intake of DHASCO = (58.1)(92/120)(0.5) = 22 mg/kg/day

Of course, because the levels of use that are subject of this notice provide ARA and DHA levels which are within the range of levels of ARA and DHA in human milk (Ref. 49), the intake of ARA and DHA from infant formulas containing ARASCO and DHASCO at this level will be within the range of intake of ARA and DHA by infants consuming human milk.

Safety of ARASCO and DHASCO (Pursuant to Proposed 21 CFR 170.36(c)(4)(i)(A))

The technical safety of ARASCO and DHASCO under the conditions of use outlined in this notice has been established by preclinical (toxicology) and clinical studies; these studies have been conducted by Wyeth and others. In addition, preclinical and clinical studies have established that ARASCO and DHASCO are efficiently absorbed and provide for accretion of ARA and DHA into the tissues of animals and human infants; such studies provide information which is relevant to nutritional safety. Preclinical safety (toxicology) studies are described immediately below, preclinical nutritional studies are described immediately thereafter, and clinical studies are described at the end of this section.

Summaries of the Preclinical Safety Studies on ARASCO and DHASCO Oils

Wyeth Nutritionals International, other infant formula companies, and the manufacturers of ARASCO and DHASCO have carried out a number of *in vitro* and *in vivo* toxicology studies on ARASCO and DHASCO oils. These studies were generally conducted according to protocols that are now standard in major contract laboratories. Note that many of the studies abstracted below are identified by a Wyeth General Technical Report number (Wyeth GTR#). Unless specifically noted, all studies described below were conducted under Good Laboratory Practice (GLP) conditions. Studies that have been published are designated as such. Finally, note that doses are either expressed as mg/kg/day or as a percentage of fat in the diet. In each case, approximate multiples of the projected 50th percentile intakes by preterm and one-month old term infants are provided.

Because refined, bleached, deodorized (RBD) oils are used in infant formula, almost all of the studies below were conducted on refined, bleached, deodorized oils. The exception to this is the study on dinoflagellate toxins, in which both RBD DHASCO oil and a crude extract from the production organism were tested.

Mutagenicity Test on RBD-ARASCO in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test) (Wyeth GTR# 26197; sponsored by Martek Biosciences)

The results of the Ames Test indicate that under the conditions of this study, refined, bleached, deodorized ARASCO (RBD-ARASCO) did not cause a positive increase in the number of histidine revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9), indicating that ARASCO preparations lack mutagenic activity. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were used. Six doses of test article and three plates per dose were used.

Mutagenicity Test on RBD-DHASCO in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test) (Wyeth GTR# 26198; sponsored by Martek Biosciences)

The results of the Ames Test indicate that under the conditions of this study, RBD-DHASCO did not cause a positive increase in the number of histidine revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9), indicating that DHASCO preparations lack mutagenic activity.

Mutagenicity Test on RBD-ARASCO in the L5178Y TK+/-Mouse Lymphoma Forward Mutation Assay (Wyeth GTR# 26199; sponsored by Martek Biosciences)

The test material RBD-ARASCO was evaluated as negative for inducing forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells under the nonactivation and S9 metabolic activation conditions used in this study, indicating that it lacks mutagenic activity.

Mutagenicity Test on RBD-DHASCO in the L5178Y TK+/-Mouse Lymphoma Forward Mutation Assay (Wyeth GTR# 26200; sponsored by Martek Biosciences)

The test material RBD-DHASCO was evaluated as negative for inducing forward mutations at the TK locus in L5178Y mouse

lymphoma cells under the nonactivation and S9 metabolic activation conditions used in this study, indicating that it lacks mutagenic activity (Ref. Martek paper).

Mutagenicity Test on RBD-ARASCO Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells (Wyeth GTR# 26201; sponsored by Martek Biosciences)

The test article RBD-ARASCO was considered negative for inducing chromosomal aberrations in CHO cells under both the nonactivation and activation conditions of this assay, indicating that it lacks mutagenic activity.

Mutagenicity Test on RBD-DHASCO Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells (Wyeth GTR# 26202; sponsored by Martek Biosciences)

The test article RBD-DHASCO was considered negative for inducing chromosomal aberrations in Chinese hamster ovary cells under both the nonactivation and activation conditions of this assay, indicating that it lacks mutagenic activity.

Acute Oral Toxicity Study of DHASCO in Rats (Wyeth GTR# 26203; sponsored by Martek Biosciences)

The acute oral toxicity of DHASCO was evaluated in male and female albino rats when administered at a level of 20.0 g/kg of body weight (approximately 345 times 50th percentile infant intakes). The estimated LD_{50} for males and females was determined to be greater than 20.0 g/kg, the highest dose tested. The gross necropsy at termination revealed no visible lesions (Ref. 97).

Acute Oral Toxicity Study of ARASCO in Rats (Wyeth GTR# 26204; sponsored by Martek Biosciences)

The acute oral toxicity of ARASCO was evaluated in male and female albino rats when administered as a single gavage dose at a level of 20.0 g/kg of body weight (approximately 227 times 50th percentile

infant intakes). The estimated LD_{50} for males and females was determined to be greater than 20.0 g/kg, the highest dose tested. The gross necropsy at termination revealed no visible lesions (Ref. 97).

4-Week Oral Gavage Toxicity Study with ARASCO, DHASCO, and Formulaid (ARASCO and DHASCO) in Rats (Wyeth GTR# 26205; sponsored by Martek Biosciences)

ARASCO and DHASCO oils were administered by oral gavage to male and female rats for four weeks. The study groups were: (Group 1) 3,750 mg/kg High Oleic Sunflower Oil, (Group 2) 50 mg/kg ARASCO, (Group 3) 1,000 mg/kg ARASCO, (Group 4) 2,500 mg/kg ARASCO, (Group 5) 25 mg/kg DHASCO, (Group 6) 500 mg/kg DHASCO, (Group 7) 1,250 mg/kg DHASCO, (Group 8) 1,500 mg/kg "Formulaid" ("Formulaid" is a Martek trademark for a 2:1 mixture of ARASCO:DHASCO), (Group 9) 3,750 mg/kg Formulaid. These levels of exposure range between approximately 0.6 to 28 times 50th percentile infant intakes.

At dose levels of up to 2,500 mg/kg ARASCO, or up to 1,250 mg/kg DHASCO, or up to 3,750 mg/kg Formulaid (the highest doses tested) there was no evidence of toxicity as based on antemortem observations, body weight, body weight gain, or clinical or anatomical pathology (Ref. 97).

Nine Week Oral (Diet) Safety Study in Rats (Wyeth GTR# 24227; conducted in Wyeth-Ayerst Research's safety testing laboratories)

ARASCO and DHASCO oils were administered orally in the diet to male and female rats (15/sex/group) continuously for nine consecutive weeks. The study groups were: (Group I; chow control) Certified Rodent Chow #5002 Meal, (Group II; synthetic diet control) Soybean Oil Basal Diet, (Group III) 2.9% ARASCO, 2.0% DHASCO, (Group IV) 23.2% ARASCO, 16.0% DHASCO. The diets of the animals in Groups II, III, and IV were standard synthetic diets. Percentages are expressed as a percent of the fat blend (50 grams per kg) used in standard synthetic diets. Total fat in the diets was kept constant.

No treatment-related changes in mortality, clinical observations or ophthalmology occurred. There were no biologically significant treatment related organ weight changes. The 23.2% ARASCO, 16.0% DHASCO level (the highest dose tested) was considered the no toxicologic effect level (NTEL). The levels of exposure in this study ranged between approximately 1.45 to 11.6 times 50th percentile infant intakes.

Subchronic (3-Month) Combined Neurotoxicity and Toxicity Study of ARASCO and DHASCO in the Rat Via Oral Gavage Administration (Wyeth GTR# 26206; sponsored by Martek Biosciences)

This study was conducted to assess the toxicity of ARASCO and DHASCO when administered separately via oral gavage to male and female rats (20/sex/group) at dose levels of 1.0 and 2.5 g/kg/day of ARASCO and 0.5 and 1.25 g/kg/day of DHASCO for 90 days. Two groups of control animals (20/sex) received untreated standard laboratory diet or the vehicle (high oleic sunflower oil) at the same dose volume as administered to the treated animals.

No significant differences were observed between groups in mortality or in physical observations, body weights and food consumption values, organ weights, neurobehavioral evaluations, terminal organ and body weights, or macroscopic and microscopic evaluations. Based on the absence of toxic effects in all treated groups, the no observable adverse effect level (NOAEL) was 2.5 g/kg/day for ARASCO and 1.25 g/kg/day for DHASCO when administered via oral gavage to rats for 90 days under the conditions of this study. The levels of exposure in this study ranged between approximately 9-28 times estimated infant 50th percentile infant intakes. The results of this study have been published (Ref. 108).

Developmental Toxicity Study with ARASCO and DHASCO in Rats (Wyeth GTR# 26207; sponsored by Martek Biosciences)

This study assessed the developmental toxicity, including teratogenic potential, of ARASCO and DHASCO when administered by oral gavage to pregnant rats during the period of organogenesis (Days 6 through 15 of gestation). Four groups were administered the test material in a carrier (high oleic sunflower oil) as a single daily dose.

Low and high-dose concentrations of ARASCO and DHASCO were prepared and administered separately at 1,000 and 2,500 and 500 and 1,250 mg/kg, respectively, in the appropriate concentration of high-oleic sunflower oil to achieve a total oil administration of 2,500 mg/kg.

There were no test material-related necropsy findings for the maternal animals. There were no significant differences in mean preimplantation loss, postimplantation loss, percent live fetuses (male, female, and total), resorptions (early, late, and total) or sex ratio for any test material-treated groups. Fetal external, soft tissue, or skeletal abnormalities were present in control and treated groups in a non-dose related pattern. Administration of ARASCO and DHASCO to rats during organogenesis of the conceptus did not produce developmental toxicity at any of the dose levels tested in this study. The levels of exposure in this study range between approximately 9 and 28 times estimated 50th percentile infant intakes.

Thirteen Week Oral Safety Study in Rats (Wyeth GTR# 26043; conducted in Wyeth-Ayerst Research's safety testing laboratories)

ARASCO and DHASCO oils were administered orally in the diet to male and female rats continuously for 13 weeks. The study groups were: Group I: soybean oil basal diet; Group II: 2.0% ARASCO, 1.3% DHASCO; Group III: 10% ARASCO, 6.5% DHASCO; Group IV: 40% ARASCO, 26% DHASCO; Group V: Rodent Chow (#5002 meal). Percentages are expressed as a percentage of the fat blend (totalling 50 g/kg) used in standard synthetic diets. Total fat in the diets was kept constant.

Analysis of this study indicates that no significant differences were seen in mortality, body weight, food consumption, clinical observations, or ophthalmologic observations. The levels of exposure in this study ranged between approximately 1 to 20 times estimated 50th percentile intakes. These doses were as high as could be administered in the diet (in the highest dose group, ARASCO and DHASCO combined made up 66% of the total fat blend).

Analysis of Dinoflagellate Extract (DHASCO - Oil and Spray-Dried Biomass) (Wyeth GTR# 26219; sponsored by Wyeth; conducted in Dr. Daniel Baden's laboratory)

In order to both confirm that the final processed product is free of toxins as well as to confirm that the production organism is incapable of producing such toxins, we have had both crude biomass as well as the processed product (RBD DHASCO) analyzed for known dinoflagellate toxins by standard procedures. A series of analyses were conducted on dinoflagellate *(Crypthecodinium cohnii)* extract (DHASCO oil and spray dried biomass). The analyses of the DHASCO oil and spray-dried biomass included an intraperitoneal mouse bioassay (non-specific for lethal substances), a radioimmunoassay (specific for brevetoxin and ciguatoxin structures), a synaptosome binding assay (specific for Site 5 Toxins, including brevetoxin and ciguatoxin structures), an HPLC analysis with ADAM derivatizing reagent (specific for okadaic acid and derivatives), a microtiter plate protein phosphatase inhibition assay (specific for okadaic acid and mycrocystin), a high performance liquid chromatography assay (for saxitoxin and derivatives), and an enzyme-linked immunoassay (RidaScreen assay for saxitoxin). In addition, capillary electrophoresis (specific for saxitoxin and derivatives, used as a check on the HPLC and ELISA assays) was conducted on the DHASCO spray-dried biomass. These analyses were generally conducted using published, peer-reviewed methods (Refs. 93, 94, 95, 101, 111, 114, 122, and 123), but were not conducted under GLP conditions.

The bioassay used (the intraperitoneal injection of mice) is an AOAC-recognized method and is a non-specific and sensitive test capable of detecting the presence of a wide variety of dinoflagellate toxins; the largest injected dose in this bioassay exceeded 10% of the animal's bodyweights. The analytical techniques used (HPLC, ELISA, radioimmunoassay, and capillary electrophoresis) are relatively specific for a given class of toxins. The biochemical assays (the phosphatase inhibition and synaptosome binding assays) are specific for given functional classes of dinoflagellate toxins, but should also be able to detect functionally-related molecules (i.e., those with related biochemical activities). All tests were negative (at the limits of detection) and the materials were considered non-toxic at the levels tested.

Evaluation of single cell sources of docosahexaenoic acid and arachidonic acid: a 4-week oral safety study in rats (sponsored by the Mead Johnson Research Center).

ARASCO and DHASCO were administered together at an approximately two-to-one ratio in the diet of rats for four weeks. Doses ranged from approximately 11 to 22 times the anticipated 50th percentile infant exposures. Control animals were fed either a high-fat diet (13.1%, w/w; equivalent to the fat content of the treated groups) or a low-fat diet (5%, w/w). In this study, there were no reported treatment-related differences in body weight, food intake, organ weights, hematology or clinical chemistry. This study has been published (Ref. 51).

Safety assessment of an arachidonic acid-enriched oil derived from *Mortierella alpina*: summary of toxicological data. (Studies sponsored by Gist-Brocades B.V., Delft, The Netherlands).

In this report, several studies were summarized. First, HPLC analyses of metabolites produced by the production strain under various fermentation conditions were conducted. Second, mutagenicity studies were conducted using gene mutation assays on bacteria and mammalian cells. Third, chromosome aberration assays (both *in vitro* and *in vivo)* were completed. Fourth, an acute toxicity study was conducted in rats. Fifth, a four-week oral toxicity study was conducted in rats. The findings of this series of studies were reported as follows: 1) No known mycotoxins were found to be produced by the production strains under the production conditions; 2) ARASCO did not show mutagenic or clastogenic activity; 3) The acute oral toxicity of ARASCO, expressed as the LD50 value, exceeded 18.2 g/kg body weight; 4) ARASCO, tested alone and in combination with a docosahexaenoic-rich fish oil (DHA-oil) derived from fish oil (at a ratio of 2:1 AA:DHA), resulted in no signs of toxicity. In this study, levels of serum phospholipids and triglycerides tended to be decreased in the highest dose ARASCO groups. The no observed adverse effect level of ARASCO in this study was designated as 3000 mg ARASCO/kg body weight/day, both when fed alone and when fed combined with the DHA-rich fish oil. This dose level correlates to approximately 37 times

the 50th percentile infant intakes. This series of studies has been published (Ref. 52).

Summary of Preclinical Safety Studies

The following table summarizes to results and the doses used in the various acute and subchronic studies summarized above and expresses these doses as a multiple of the estimated 50th percentile infant intakes.

Study	Dose (mg/kg or % fat)	X 50% Intake
Acute Oral Toxicity in Rats (Martek)	ARASCO: 20,000 DHASCO: 20,000 Results : No lethality	227 345
Four Week Oral Gavage Toxicity Study in Rats (Martek)	ARASCO: 50;1000;2500 DHASCO: 25;500;1250 Combination: 1500;3750 Results: No effect	0.6; 11.3; 28 0.4; 8.6; 21 11.3; 28
Nine Week Diet Study in Rats (Wyeth)	2.9%ARASCO/ 2.0%DHASCO 23%ARASCO/ 16%DHASCO Results: No effect	1.45 11.6
Three Month Combined Neurotoxicity and Toxicity Study via Oral Gavage in Rats (Martek)	Control: Sunflower Oil ARASCO: 1000;2500 DHASCO: 500;1250 Results: No toxicity	 11.4; 28 8.6; 21
Teratogenicity Study via Oral Gavage in Rats (Martek)	Control: Sunflower Oil ARASCO: 1000;2500 DHASCO: 500;1250 Results: No toxicity	 11.4; 28 8.6; 21
13 Week Oral Study in Rats (Wyeth)	2.0% ARASCO/1.3% DHASCO 10% ARASCO/6.5% DHASCO 40% ARASCO/26% DHASCO Results: No toxicity	1.0 5.0 20.0
Mouse interperitoneal injection (Wyeth)	2 grams per mouse > 100,000 (>10 % of body weight)	 1135
4-Week study (Mead Johnson)	1000/2000 ARASCO 500/1000 DHASCO Results = no effects	11/22
Acute study (Gist-Brocades)	18.2 g/kg ARASCO	206
4-Week study (Gist-Brocades)	3.0 g/kg/day ARASCO	34.1

In summary, the mutagenic potential of ARASCO and DHASCO have been examined in various studies. In all such studies, ARASCO and DHASCO were found to be without mutagenic potential. In addition, the toxicologic potential of ARASCO and/or DHASCO have been assessed in 2 acute, 3 four-week, and four subchronic toxicology tests. The doses used in these studies ranged up to 345 times the estimated 50th percentile intake of infants and were as high as could be delivered without potentially disrupting the diets of the animals. No toxicologic effects were seen, for either of these oils, singly or in combination, at any of the doses tested, in any of the studies, indicating that ARASCO and DHASCO have no toxicologic potential under the conditions of the studies. Finally, as an additional confirmatory step, the potential of *Crypthecodinium cohnii* to produce known dinoflagellate toxins or other toxins has been assessed using analytical and biochemical techniques and a standard bioassay. As expected from the history of the organism and the toxicological studies summarized above, no such toxins were detected.

Preclinical Nutritional Assessment Of ARASCO And DHASCO; Absorption and Distribution Studies in Animals

Dietary fat availability is determined primarily by two factors: the solubility of constituent fatty acids once the fatty acids are enzymatically freed from the triglyceride; and the position of attachment of the fatty acids to the glycerol backbone. Availability of fatty acids from different dietary sources can be estimated fairly accurately from consideration of these two factors. Generally, free fatty acids have greater solubility when the chain length of the fatty acid is short than when it is long. In addition, solubility increases as the number of unsaturations increases. The highly unsaturated nature of the fatty acids of interest here, ARA and DHA, results in their being efficiently absorbed when they are in free form in the intestinal lumen (Refs. 99, 100, and 112).

Therefore, based on what is known about the structure of these oils and the mechanisms of fat absorption, we expected that they would demonstrate acceptable bioavailability. In order to confirm this expectation, extensive preclinical evaluation of ARASCO and DHASCO oils has been undertaken. We have chosen to assess bioavailability and fatty acid accretion preclinically in rodents and in swine. As described below, these studies have confirmed that these oils are as bioavailable as traditional food oils and allow for the expected accretion of ARA and DHA into animals' tissues. Studies which have been published are designated as such.

Fatty acid absorption of ARA and DHA in rats (Wyeth GTR# 24449; conducted by Wyeth)

The results of this study indicate that in rats both of these fatty acids, when provided in the form of ARASCO and DHASCO, are very well absorbed, with a fecal loss of less than 5% for both ARA and DHA. This compares very well with traditional food oils.

Tissue accretion of fatty acids in rat pups (Wyeth GTR# 24592; conducted by Wyeth).

The tissue accretion (liver, brain and plasma) of fatty acids in 14 day old rat pups whose dams were fed DHA as DHASCO and increasing doses of either linoleic acid (LA) or ARA was assessed in this study.

These data demonstrate that preformed dietary ARA is a much more specific means of ensuring ARA accretion in developing mammals than is dietary LA. This study has been published (Ref. 128).

Brain fatty acid levels in rats fed ARASCO and DHASCO (Wyeth GTR# 26223; sponsored by Wyeth)

Brain fatty acid levels of rat pups were measured at 1, 3, and 6 weeks of age when their dams were fed diets (and the rats weaned to the same diet) containing no LCP, ARA alone, DHA alone, or a combination of ARA and DHA. Neural and glial cells of three brain subregions were examined.

The results of the study demonstrated that the combination of ARA and DHA (as provided in the form of ARASCO and DHASCO) supports brain accretion of these fatty acids. Various regions of the brain and different cell types were found to accumulate LCPs at different rates and to different levels. This study has been published (Ref. 106).

Retinal Fatty Acid Composition of Rat Pups (Wyeth GTR# 26222; sponsored by Wyeth)

Retinal fatty acid composition from the rats of the study described above has also been examined. Retinal rod outer segment fatty acid profiles were found to be responsive to dietary ARA and DHA (i.e., retinal ARA and DHA increased in response to increased dietary ARA and DHA) when these fatty acids were fed in the form of ARASCO and DHASCO. These data suggest that the dietary content of LCPs is an important factor influencing membrane fatty acid composition of visual cells during development. These data have been published (Ref. 129).

Retinal Fatty Acids of Piglets Fed Microbial Sources of DHA and ARA (Wyeth GTR# 26221; sponsored by Wyeth)

Newborn piglets received diets for 28 days containing a standard infant formula fat blend, the standard fat blend with added ARA alone, standard fat blend with DHA alone, or the standard fat blend with a combination of ARA and DHA added. ARA and DHA were provided as ARASCO and DHASCO. Retinal ARA and DHA levels were found to reflect dietary LCP, with optimal tissue levels being achieved by

provision of a combination of ARA and DHA in the diet. These data have been published (Ref. 130).

Plasma and Erythrocyte Fatty Acids of Piglets Fed Microbial Sources of DHA and ARA (Wyeth GTR# 26532; sponsored by Wyeth)

A neonatal pig model was used to determine if dietary supplementation with microbial sources of DHA and ARA at slightly greater concentrations than normally found in human milk would influence fatty acid accretion in phospholipids of plasma and erythrocytes. It was found that the combined addition of ARA and DHA (as ARASCO and DHASCO) provides balanced circulating levels of ARA and DHA.

Thromboxane and Prostacyclin Generation in Piglets Fed ARASCO and DHASCO (Sponsored by Wyeth)

In addition to neural development, LCPs play an important role in many cellular signaling mechanisms. ARA is the primary precursor to prostaglandins, leukotrienes, thromboxanes, and prostacyclins, while n-3 LCPs may modulate ARA-mediated effects. Thromboxane and prostacyclin generation in lung tissue were evaluated following 28 days of feeding of ARA, DHA, or a combination at physiologically relevant doses to neonatal piglets. ARA and DHA were provided as ARASCO and DHASCO. Thromboxane and prostacyclin production in piglets receiving ARA was higher than in control piglets, while the presence of dietary DHA alone significantly suppressed the production of these prostaglandins. The balanced feeding of ARA and DHA together resulted in prostaglandin production intermediate between the control and ARA alone groups. These data further support the combined addition of ARA and DHA to formula and should discourage the use of DHA alone. These data have been published (Ref. 30).

In summary, the above results indicate that ARASCO and DHASCO are efficiently absorbed, accreted into tissues, and serve their expected physiological roles in animal models.

Clinical Safety And Nutritional Assessment Of ARASCO and DHASCO

At least 6 clinical studies have been conducted on infant formulas containing ARASCO and DHASCO; three have been conducted by Wyeth and the remainder by other infant formula companies. Wyeth's studies are described immediately below; studies conducted by others that have appeared in the peer-reviewed literature will be described thereafter.

Wyeth's Clinical Program

The goals of our clinical program were:

1) to ensure a proper dosage and ratio of ARA and DHA. Human milk has long been safely fed to preterm and term infants. Hence, if tissue LCP levels typical of human milk-fed infants can be reproduced, concerns over possible deleterious effects due to imbalances of LCP levels can be eliminated.

2) to confirm that the ARA and DHA levels chosen support normal growth and are safe.

Preterm Dose-Response Study

The preterm dose-response study we carried out was conducted to confirm the bioavailability and appropriate tissue accretion of arachidonic (ARA) and docosahexaenoic (DHA) acids when added at escalating doses as ARASCO and DHASCO to our preterm formula (Preemie SMA). The study was conducted as an open, sequential, prospective, inpatient study in preterm infants; feedings were conducted for 4-5 weeks. Doses of 0.32% ARA/0.24% DHA (low dose), 0.5% ARA/0.35% DHA (mid dose), and 1.1% ARA/0.76% DHA (high dose) were fed. This study demonstrated that the addition of the mid dose of ARA and DHA (as ARASCO and DHASCO) to our preterm formula produces ARA and DHA profiles which consistently match those of a group of human milk-fed preterm infants in total plasma phospholipids and red cell phospholipid subclasses.

In this study, there was no difference observed between the various experimental formula groups in weight, length and head circumference,

formula intake and tolerance, hematologic measures, routine urinalysis and renal function.

The results of this study have been published (Ref. 74).

Preterm Growth and Safety Study

As the second part of our clinical development program, we designed a long-term growth and safety trial of adequate duration and with a sufficient power to detect relatively subtle effects on infant growth. This clinical study was of a randomized, prospective, double-blinded (formula groups), parallel, and multiclinic design; 16 clinical centers in the U.S. and Canada were involved with the study. It was designed to have a minimum statistical power to be able to detect weight differences of 400 grams (roughly 10% of bodyweight) between the study groups with a 90% probability. In actuality, we significantly exceeded our minimum enrollment goal (44 per group), and thus the sensitivity of the study was greater than predicted by the above power calculations.

The study had three feeding groups of appropriate for gestational age (AGA) premature infants: human milk fortified (where indicated) with a standard human milk fortifier (to provide adequate protein and calcium), Wyeth premature formula (control), and Wyeth premature formula supplemented with the levels of ARA (approximately 0.6%) and DHA (approximately 0.4%) that were determined in our dose-response study to allow support of tissue levels of ARA and DHA at human-milk like levels (Ref. 74). Anthropometric (growth) measures, blood fatty acid composition, and routine hematologic and clinical chemistry tests were performed at baseline.

During Phase I of the study, which extended from enrollment to 40 weeks post-conceptional age (PCA), infants were fed either their randomly assigned study formula (24 kcal/oz, with or without LCPs) or human milk (fortified as appropriate with a standard human milk fortifier) for 3 to 12 weeks. During Phase II (40 to 48 weeks PCA), infants were fed an otherwise identical 20 kcal/oz formulation (in keeping with the normal caloric density of term formulas) of their assigned study formula (with or without LCPs) or human milk. During the follow-up portion of this study (from 48 to 92 weeks PCA - to roughly one year corrected age), infants in both formula groups were fed standard Wyeth term formula (which was not supplemented with LCPs) and beikost (solid foods, juices, etc.); the diets of both formula groups were therefore

equivalent during the follow-up portion of the study. The human milk fed infants continued on human milk supplemented (based on the decision of the caregiver) with non LCP-supplemented term formula and/or beikost during the follow-up phase of the study.

Results at 40 and 48-week Timepoints (Phases I and II)

77 infants completed Phases I and II of the study in the LCP-supplemented group, 78 in the control group, and 132 in the human milk-fed group; among the human milk-fed infants, only 10 were exclusively human milk-fed by 48 weeks. The study demonstrated that SMA Preemie with and without added ARASCO and DHASCO supported growth at equal rates at both the 40 and the 48 week timepoints. Both formula groups grew significantly better than the human milk-fed group, an expected observation given the higher nutrient density of the preterm infant formulas as compared with human milk.

The study also demonstrated the safety and tolerance of the ARASCO and DHASCO-supplemented formula. Clinical chemistry/hematology measurements were generally the same among the two formula groups and the human milk group at both time points. The primary exceptions to this were among the human milk group, which (expectedly) had lower indices of protein intake (serum albumin and urea nitrogen were lower than in the formula groups) and calcium/phosphorus status (serum calcium and phosphorus were lowered and alkaline phosphatase was elevated as compared to the formula-fed groups). In addition, LCP-fed infants had higher serum cholesterol than did the control group, but were found to be equivalent to the exclusively human milk-fed group in this parameter.

The COSTART adverse events dictionary, a standard adverse events dictionary used in clinical research, was used in tabulating adverse events (AEs). Except for the nervous system (in which the LCP-supplemented infants had significantly fewer adverse events - at both 40 and 48 week timepoints - than the control formula group) there were no significant differences in the number of adverse events grouped by body system at 40 or 48 weeks between the formula groups. The human milk group had significantly fewer GI events than the formula groups (an expected observation - human milk is generally better tolerated than are infant formulas) and had significantly fewer nervous system events than the control formula group. Nervous system events were similar in the LCP-supplemented and human milk-fed groups. There was no difference among

the groups in the number of infants who were dropped from the study due to study events.

Regarding individual adverse events, given the large number of statistical comparisons (approximately 113), some significance at the $p<0.05$ level was expected due to random chance. The observed statistically significant differences between the infant formula groups in individual adverse events were as follows:

- at 40 weeks, the incidence of anemia and "nervousness" (generally reported on case report forms as "irritability") were significantly lower in the LCP-supplemented group;
- at 48 weeks, the incidence of anemia and nervousness (irritability) remained significantly lower in the LCP-supplemented group, and the level of flatulence was increased in the LCP-supplemented group.

The differences between formula and human milk-fed groups were as expected: both the control and LCP-supplemented formula groups generally had a lower incidence of jaundice and a higher incidence of GI events than did the human milk-fed group.

Tissue levels of LCPs in the control group, as expected, were generally lower than in the human milk-fed and LCP-supplemented groups. Plasma and RBC levels of ARA and DHA in the LCP-supplemented groups were significantly higher than in the control group and were generally not significantly different than levels in the exclusively human milk-fed sub-group.

Therefore, the study demonstrated that the addition of LCPs as provided by ARASCO and DHASCO does not affect the growth of preterm infants at up to 48 weeks corrected age (over roughly twice the period of time premature infants are generally fed such formulas). The study also re-confirmed that infants fed (as a percent of fatty acids) approximately 0.6% ARA (as ARASCO) and approximately 0.4% DHA (as DHASCO) had plasma and RBC levels of ARA and DHA that were generally equivalent to those of the subgroup of exclusively human milk-fed infants. The study further established that ARASCO and DHASCO addition is safe as judged by adverse events, hematology, clinical chemistry, and urinalysis.

This portion of the study has been published in abstract form (Refs. 80 and 90).

Follow-up Phase of the Study; One Year Time Point

As stated above, we also incorporated a follow-up phase into this study which extended to 1 year corrected age (that is, one year after term for these prematurely born infants). With respect to plasma and red blood cell (RBC) ARA, the levels among the three groups of infants (control formula, LCP-supplemented formula, and human milk-fed groups) were similar by the age of 92 weeks (1 year corrected age). With respect to plasma and RBC DHA, however, it was evident that even at 92 weeks PCA (44 weeks after DHA supplementation had ceased) there was still a significant difference in tissue DHA levels in four out of 5 phospholipid subfractions between those infants who had received DHA supplementation early in life (until 48 weeks PCA) and those that had not. In general, DHA levels in the supplemented group remained similar to those of the breast-fed group, despite the fact that LCP supplementation had ceased 44 weeks earlier. This observation was surprising given that the diets received by both formula-fed groups from 48 to 92 weeks were similar, given that these diets contained adequate and balanced levels of LCP precursors, and given that the infants had received other foods for several months. Other investigators have made similar observations in a study on term infants (see summary below and Ref. 54).

This portion of the study has also been published in abstract form (Ref. 131).

The complete results of all phases of this study have been submitted to Docket No. 96N-0391 in response to a request from FDA and FASEB for comments on the subject of preterm infant nutrition.

Term Dose-Response and Safety Study

Wyeth has also conducted a study in Adelaide, Australia to assess the proper dose of LCPs for term infants; in addition, this study was intended to further confirm the safety of LCPs for term infants.

The study was a six-week, double-blind, randomized, parallel, outpatient study at three clinical centers in Australia. The primary investigator was Dr. Robert Gibson, a well-known expert on LCPs and infant feeding. Four different study formulas were evaluated as well as a human milk-fed group. The 4 study formulas were Wyeth term infant formulas with ARA and DHA added (as ARASCO and DHASCO) at the concentrations listed below (expressed as percent of total fatty acids):

- Formula 1 - no added LCPs
- Formula 2 - 0.2% ARA and 0.2% DHA
- Formula 3 - 0.32% ARA and 0.2% DHA
- Formula 4 - 0.4% ARA and 0.25% DHA

Formula Groups 3 and 4 had ARA and DHA levels similar to average human milk levels. The nutrient profiles of the study formulas were identical and varied only with respect to the level of ARA and DHA added to the formula. The base formula used in this study, our Australian SMA formula, is essentially the same formula we previously provided in the U.S. under the SMA brand and now manufacture in the U.S. for private-label distribution and in Canada under our own brand.

22, 21, 22, 21, and 26 infants completed the study in Groups 1, 2, 3, 4, and the human milk-fed group, respectively. As expected, infants fed human milk had higher levels of ARA and DHA after 6 weeks of feedings than did infants fed unsupplemented formula (Formula 1). Also as expected, infants fed supplemented formulas (Formulas 2, 3, and 4) had significantly higher levels of ARA and DHA after 6 weeks of feedings than infants fed unsupplemented formula. There were generally no significant differences between the ARA and DHA content of the various blood fractions of the human milk group and those formula groups that had ARA and DHA supplemented at levels that were similar to those found in human milk (Formula Groups 3 and 4). Therefore, as would be expected, the infants' levels of ARA and DHA generally corresponded with their intake of these fatty acids, indicating that these sources of LCPs are bioavailable in term infants.

There were no significant differences between any of the feeding groups with regard to weight, length, or head circumference at the end of the study. The average weight and height of infants in all feeding groups fell within the ranges set for infants by the U.S. National Center for Health Statistics.

Therefore, as expected, LCP supplementation, and the level of LCP supplementation, did not affect growth. All of the study formulas, regardless of their LCP content, were well-tolerated, and there were no differences among the formula groups in tolerance or acceptance. There was also no relationship between the LCP content of the formulas and adverse events.

In conclusion, this study confirmed the safety of ARASCO and DHASCO addition to term infant formula. Further, the study established a dose of ARASCO and DHASCO that allows a term formula to maintain infants' tissue ARA and DHA levels equivalent to those of a group of breast-fed infants.

This study has also been published in abstract form (Ref. 78).

Clinical Studies on ARASCO and DHASCO Conducted by Others

Two other major infant formula companies have conducted clinical studies in which ARASCO and DHASCO were used as sources of ARA and DHA in infant formulas.

The first two such studies to be published were supported by Numico (formerly Nutricia), a major European infant formula manufacturer. The first of these studies confirmed that ARASCO and DHASCO support RBC and plasma ARA and DHA at levels typical of those found in human milk-fed infants (Ref. 71).

The second such study (Ref. 53) involved a comparison of the efficiency of absorption (by preterm infants) of ARA and DHA provided by ARASCO and DHASCO oils, ARA and DHA provided by human milk, and ARA and DHA provided by egg phospholipids. The authors of this study concluded that intestinal absorption of ARA and DHA, when provided as ARASCO and DHASCO, was equivalent to the absorption of ARA and DHA when provided in breast milk. Thus, the study established that ARASCO and DHASCO are as efficiently absorbed as are the ARA and DHA-containing triglycerides in human milk.

The most recently published clinical study on ARASCO and DHASCO was sponsored jointly by NIH and Mead Johnson Nutritional Research (Ref. 54). This study involved the feeding of term infants with human milk (n=29), a standard term infant control formula (n = 26), a formula supplemented with DHA alone (0.35% of fatty acids) as DHASCO (n = 26), and the same formula supplemented with ARA and DHA (0.72 and 0.36%, respectively) as ARASCO and DHASCO (n = 27). The study involved 17 weeks (4 months) of feedings with the test formulas described above followed by a follow-up phase (which extended to one year) in which formula-fed infants were fed formula unsupplemented with DHA and/ or ARA.

The results of this study were reported as follows:

- As expected: infants whose formulas were supplemented with both DHA and ARA had RBC levels of ARA and DHA similar to human milk-fed infants; those infants that were supplemented with DHA only had human milk-like tissue levels of DHA but levels of ARA that were depressed to levels lower than found in control infants; and those infants fed control formula had lower blood levels of both DHA and ARA as compared with either human milk-fed infants or with DHA and ARA supplemented infants. Despite the fact that supplementation extended only to 17 weeks, RBC DHA levels remained similar to those of breast-fed infants up to the 52-week (one-year) time point. This longer-term effect of DHA supplementation was similar to that seen in the one-year follow-up phase of our preterm growth and safety study.

- Growth among the various groups was similar, as were adverse events, confirming the safety of supplementing infant formulas with ARASCO and DHASCO.

- Visual acuities at 17 and 52 weeks in the groups fed preformed DHA (whether as human milk lipids or as DHASCO) were superior to those of infants fed control formula, indicating that preformed DHA in the diet is essential for the optimal development of term infants' visual systems.

Studies on ARASCO and DHASCO have also been conducted in adults; while these studies uncovered nothing surprising, they are difficult to apply to the notified use conditions (Refs. 104, 117, 118, 119, 120, and 121).

Regulatory Status of ARASCO and DHASCO Globally

The views of the experts working in various regulatory authorities globally towards a given food ingredient(s) can be judged by considering the global regulatory status of the ingredients(s) in question. Detailed regulatory reviews of ARASCO and DHASCO have been completed in the Netherlands and in the U.K. Several years ago, the Netherlands Ministry of Welfare, Public Health, and Culture, in response to a request from another infant formula manufacturer, considered the use of ARASCO and DHASCO in both preterm and term infant formulas. After review by two regulatory/scientific bodies, the Advisory Commission on the Commodities Act and the Provisional Commission on the Safety of Novel Foods, they have determined that these sources are safe and suitable for these uses and granted an exemption from their now-defunct national Novel Foods regulation (Ref. 15).

ARASCO and DHASCO were first used in Europe several years ago and are thus not considered "novel" within the European Union (EU) regulatory definition. Nevertheless, Wyeth voluntarily submitted a dossier requesting an opinion on these sources of LCPs from the U.K. Advisory Committee on Novel Foods and Processes (ACNFP) in 1995. After a review by the Committee on Toxicity (COT), we received a favorable opinion on the general food safety of the oils from the ACNFP (Ref. 29), and following this we introduced preterm formulas containing ARASCO and DHASCO oils in the U.K., Ireland, and elsewhere. We were still waiting for an additional opinion from the U.K. COMA Panel on Maternal and Child Nutrition (which was waiting for the longer term clinical data summarized above) when the EU Novel Foods Regulation was finalized, and at that point (May of 1997) the U.K. ceased all work, and ceased providing all opinions, on voluntary ACNFP applications.

Data on these sources was also presented in a series of meetings with the Japanese Ministry of Health and Welfare (Koseisho) in Tokyo in the Fall of 1996; they voiced no objection to the use of these oils.

Current Use of ARASCO and DHASCO in Infant Formulas

Wyeth has introduced ARASCO and DHASCO into almost all of the markets in which we provide preterm formulas. In addition, we have introduced these oils into term formulas in Australia and New Zealand. In a significant number of these countries, formal approvals or informal regulatory opinions were granted prior to introducing the product. In addition, other companies now provide infant formulas containing ARASCO and DHASCO. A partial list of the countries in which ARASCO and DHASCO are now in use in infant formulas would include those in the following table:

Country	*Company*	*Trademark*
Netherlands *	Numico (Nutricia)	Nenatal (preterm)
Belgium	Numico	Nenatal (preterm)
Finland	Numico	Nenatal (preterm)
Spain	Novartis	Adapta PEG (term)
United Kingdom*	Wyeth	SMA LBW
Ireland	Wyeth	SMA LBW
France	Wyeth	Modilac LBW
Israel	Maabalot	Trade Name Unknown
Australia	Wyeth	SMA LBW
		SMA Gold (term)
New Zealand	Wyeth	SMA LBW
		SMA Gold (term)
South Africa	Wyeth	S-26 LBW
Turkey	Wyeth	S-26 LBW
Malta	Wyeth	S-26 LBW
Egypt *	Wyeth	S-26 LBW
Saudi Arabia	Wyeth	S-26 LBW
U.A.E.	Wyeth	S-26 LBW
Bahrain	Wyeth	S-26 LBW
Taiwan*	Wyeth	S-26 LBW
Singapore*	Wyeth	S-26 LBW
Hong Kong#	Wyeth	S-26 LBW
China (P.R.C.)*	Wyeth	S-26 LBW
Philippines*	Wyeth	S-26 LBW
Indonesia*	Wyeth	S-26 LBW
Malaysia*	Wyeth	S-26 LBW
Mexico*	Wyeth	S-26 LBW
Venezuela*	Wyeth	S-26 LBW
Argentina*	Wyeth	S-26 LBW
Colombia*	Wyeth	S-26 LBW
Panama*	Wyeth	S-26 LBW
Guatemala*	Wyeth	S-26 LBW
Honduras*	Wyeth	S-26 LBW
Trinidad	Wyeth	S-26 LBW

*Formal approval necessary or informal opinion granted prior to product introduction

Food control regulations in Hong Kong and mainland China (P.R.C.) remain separate after reunification.

Conclusions Regarding Evidence of Safety

Based on the chemical structure of these oils, the source organisms' inability to produce harmful substances, the controlled methods of manufacture and processing, and the intended use (to compositionally mimic human milk), we expected these oils to be safe. These expectations have been confirmed by the preclinical and clinical studies summarized above.

Based on consideration of the structure of these oils, we anticipated that they would be as bioavailable as, and metabolized in a manner similar to, other food oils. These expectations have been confirmed by the preclinical and clinical studies summarized above.

Therefore, based on the information and data summarized above, based on the cited publications and expert recommendations, based on current regulatory approvals, based on compositional comparability to human milk, and based on the current safe use of these oils in infant formulas in at least 32 countries globally, we have concluded that ARASCO and DHASCO present a reasonable certainty of no harm under the conditions of use specified in this notice.

Reports Inconsistent with GRAS Status (Pursuant to Proposed 21 CFR 170.36(c)(4)(i)(B))

There are no reports of which we are aware that are inconsistent with the GRAS status of ARASCO and DHASCO under the conditions of use that are the subject of this notice.

There are reports which are inconsistent with the GRAS status of the addition of DHA only (in the absence of preformed ARA, and regardless of the source from which DHA is derived) to infant formulas intended for preterm infants. Despite the fact that such use is not the subject of this notice, we have summarized these reports below:

- There are peer-reviewed literature reports which indicate that the reductions in tissue ARA levels seen when n-3 LCPs are fed in the absence of preformed ARA are undesirable. These reports have previously been mentioned in this notice. Specifically, there are indications that such reductions in ARA status result in decreased growth (Ref. 18), and it has further been demonstrated that plasma ARA levels are positively correlated with the growth of premature infants (Refs. 19 and 124). However, such affects have not been evident in all studies in which n-3 LCPs were fed to preterm infants in the absence of preformed ARA (Refs. 5, 37, and 66).
- There is a single report available (in abstract form only) which indicates that feeding DHA alone (in the absence of preformed ARA) affected the language development of infants. In the same study, feeding DHA with preformed ARA resulted in no effect on language development (Ref. 55).
- There are reports that very high levels of n-3 LCPs (greater than 50 mg/kg/day) in the presence of constant levels of n-6 LCPs can affect clotting parameters (62 FR 30751). No effects on clotting parameters have been seen in infants fed roughly 25 mg/kg/day n-3 LCPs (approximately the level which is subject to this notice) in the absence of ARA supplementation (Ref. 66), and this phenomenon is not relevant to a situation in which ARA and DHA are fed together.

Again, under the specified conditions of use that are the subject of this notice (addition of ARASCO and DHASCO together at specified ratios, or in combination with other safe sources of ARA or DHA), the above reports are not relevant.

General Recognition of the Safety of ARASCO and DHASCO (Pursuant to Proposed 21 CFR 170.36(c)(4)(i)(C))

Just as there are unique aspects of assessing the technical safety of infant formula ingredients, there are unique aspects when considering the general recognition of the safety of infant formula ingredients. These factors are as follows:

1. The focus of reviews by expert groups considering the desired nutrient composition of infant formulas and the standards applied when an expert group considers the GRAS status of a new food ingredient are different in scope. When expert groups consider the use of a nutrient in infant formulas, they generally consider two separable but related questions: a) what level of the nutrient(s) is safe; and b) should the nutrient's addition be "recommended" (by academic groups) or "mandated" (by groups with a regulatory purpose, e.g. FASEB's current review). Because the need - or lack of need - for a nutrient is irrelevant to determining its GRAS status (GRAS status requires only, and is affected only by, a showing of technical safety and the general recognition of this safety), for regulatory purposes it is necessary to keep discussions of dietary need separate from discussions of GRAS status.

2. Compositional comparison of a food (or food ingredient) to food(s) with a history of safe use has been recognized as a valid factor which can contribute to GRAS status as determined by scientific procedures (see 21 CFR 170.30(f) and proposal of April 17, 1997 at page 18937 "Substantial Equivalence to a GRAS Substance"). As stated previously, infant formula manufacturers have available a fully natural model (human milk) with which to conduct such compositional comparisons. This is in obvious contrast to most other foods, for which the history of use may span only a few hundred years. FDA has generally not objected to self-determinations of GRAS status for nutrients found in human milk, so long as they are used at levels similar to human milk levels.

3. Infant nutrition is a specialized area of research and development, and the group of individuals who are considered experts qualified by scientific training and experience to evaluate the safety of infant formula ingredients (i.e. "qualified experts" as described in the Agency's proposal) is rather small. Given this, once information is presented at a major scientific conference it will be generally known among the majority of the expert community. Further, the small size of the expert community

makes it possible to directly involve a significant percentage of the expert community in the safety assessment of a new ingredient.

Our finding that the specified uses of ARASCO and DHASCO in preterm and term infant formulas are generally recognized as safe is based on the following:

- Recommendations that ARA and DHA should be added to infant formulas have been made by various expert groups (including those assembled by the World Health Organization, the European Union, the British Nutrition Foundation, and ISSFAL (Refs. 10, 11, 12, 14). A necessary component of these recommendations was a finding of safety.
- ARA and DHA are explicitly approved for use in infant formula in the European Union and in many other countries. Regulatory officials in these countries are often experts qualified by training and experience to judge the safety of food ingredients.
- ARA and DHA are present (at levels consistent with those that are the subject of this notice) in a food - human milk - with a significant pre-1958 history of use (Ref. 49).
- ARASCO and DHASCO are currently in safe use as sources of ARA and DHA in more than 32 countries.
- In roughly 16 of these countries, infant formulas containing ARASCO and DHASCO were subject to premarket approval by the relevant national regulatory authorities or were granted informal regulatory opinions.
- Significant preclinical research (both toxicological and nutritional studies) has been completed which confirms the safety of ARASCO and DHASCO. Many of these studies have been published (Refs. 30, 51, 52, 64, 97, 103, 108, 113, 128, 129, 130), and these published studies are supported by the unpublished data and information summarized in this notice.
- Significant clinical research has been conducted on ARA and DHA, both as supplied by ARASCO and DHASCO and as supplied by other sources (Refs. 4, 5, 6, 9, 16, 18, 19, 32, 33, 34, 35, 36, 37, 38, 39, 53, 54, 55, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 86, 90, 91, 92, and 102).

- A significant percentage of the expert community in infant nutrition was involved in the clinical safety assessment of ARASCO and DHASCO, either on Wyeth's behalf or on the behalf of other infant formula companies. For example, Wyeth's studies have involved at least 23 clinical investigators qualified by training and experience to evaluate the safety of infant formula ingredients, and all of these investigators have concluded, based on the clinical data, that ARASCO and DHASCO are safe for use in infant formula (Refs. 78, 80, 90, and 131). Studies on ARASCO and DHASCO funded by other infant formula companies have involved at least 25 clinical experts qualified by training and experience (Refs. 53, 54, 71, and 102).
- In addition to the above, two expert panels (one assembled by Martek and one by Wyeth) have considered the GRAS status of ARASCO and DHASCO. Both panels have concluded that ARASCO and DHASCO are generally recognized as safe for use in infant formulas. These expert panels' findings are summarized below.

Expert Panel Opinions

Two expert panels have considered the GRAS status of ARASCO and DHASCO for use in infant formula.

The first expert panel to consider the GRAS status of ARASCO and DHASCO for use in infant formula was assembled by Martek Biosciences, the manufacturer of these oils. This panel consisted of Joseph Borzelleca, Ph.D. (an expert in toxicology), Gary Flamm, Ph.D (also an expert in toxicology and formerly with FDA), and Allan Forbes, M.D. (an expert in nutrition and also formerly with FDA). These experts reviewed the toxicology and other data which were available in the Fall of 1995 and concluded that "Based on the history and experience with the consumption of infant food containing DHA and ARA, the addition of these fatty acids to milk-based infant formula at levels corresponding to those found in human milk is Generally Recognized as Safe (GRAS) for the above intended conditions of use. Preparation of the oils, DHASCO and ARASCO, which contain DHA and ARA, respectively, have been shown to be of suitable purity and safety by adequate toxicological studies. We, therefore, consider levels of DHASCO and ARASCO which do not exceed 100 mg DHASCO per kg body weight per day or 150 mg ARASCO per kg per day (used alone or in combination) to be GRAS when used in

human pre-term and term infant formulas providing the preparations meet established specifications of suitable purity." (Ref. 132).

The second panel of experts was assembled by Wyeth Nutritionals International earlier in 1998; given that LCP addition to preterm formulas is now the standard of care over much of the world, this panel's deliberations were specific to term infant formulas. This panel included an international selection of experts in infant nutrition, including Professor R. Uauy-Dagach of Chile, Dr. M.T. Clandinin of Canada, Dr. C. Dupont of France, Dr. R. Gibson of Australia, and Dr. J. Vanderhoof of the U.S. All of these individuals are well-known experts in infant nutrition, and Drs. Uauy-Dagach, Clandinin, and Gibson are experts in the area of LCPs in infant development and have conducted many of the seminal studies in the area.

After considering the data and information summarized in this notice, this group came to the following conclusion "On the basis of preclinical and clinical research to date, it is now generally recognized that it is safe to supplement term infant formulas with AA [arachidonic acid] (derived by fermentation) and DHA (derived either by fermentation or from specialized low EPA [eicosapentaenoic acid] fish oils) at levels which approximate those found in human milk" (Ref. 133).

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END SUBMISSION

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AM

Wyeth Nutritionals International

DATE: August 27, 1998

TO: Whoever gets stuck with this.

FROM: F. Owen Fields

RE: Enclosed Materials

Note the following:

- I was unable to save the files in WP format, and so had to save them in Word format. You should nevertheless be able to open them using Wordperfect. See the enclosed floppy.
- I enclosed 5 copies - docket, chem, tox, admin, and OSN (due to the fact that the notice involves infant formula use).
- The FASEB report (done under contract to FDA) on term infant formulas is reportedly nearly done. One of the items it deals with is the subject of this notice.

I would very much welcome discussing the possible contents of the response letter, if this option is available to us. I am concerned about the language primarily due to the fact that we are a global company that deals with many languages, and it is easy - and common - for shades of meaning to get garbled during translation. For example: "FDA has not determined it is GRAS" can easily become "FDA has determined it is not GRAS."

Owen

1998 SEP -1 A 2:22