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ORIGINAL SUBMISSION

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Mead Johnson® Nutritionals

2400 West Lloyd Expressway Evansville, IN 47721-0001 812-429-5000

August 3, 2001

Linda Kahl, Ph.D.
Office of Food Additive Safety (HFS-215)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

REC'D AUG 06 2001

Dear Dr. Kahl:

Re: GRAS Notice for ARASCO® (arachidonic acid-rich single-cell oil) Level in Term Infant Formula

The enclosed GRAS Notice follows a meeting with members of the Office of Food Additive Safety and the Office of Nutritional Products, Labeling and Dietary Supplements held on June 8, 2001. In this meeting, Mead Johnson Nutritionals (MJN) informed FDA that the generally recognized as safe (GRAS) level of ARASCO® added to infant formula as stated in the Martek GRAS Notice No. 000041 does not encompass the higher level of ARASCO used in MJN infant formula. Martek has informed MJN that the data and information contained in their GRAS notice support the higher level and that, in fact, they had intended to cover the level of ARASCO used in MJN formula. To address this issue, MJN, with Martek's support, is submitting the enclosed GRAS Notice to inform FDA that MJN has determined ARASCO is GRAS through scientific procedures for use in infant formulas at a level up to 1.88% of total dietary fat (an extension of the 1.25% level stated in the Martek GRAS Notice).

The enclosed GRAS Notice includes:

- A summary of relevant safety and use information previously reviewed by FDA (Agency Response Letter GRAS Notice No. GRN 000041 to Martek Biosciences Corporation, May 17, 2001).
- A discussion supporting the selection and safety of MJN's proposed level of ARASCO addition.
- A letter from Martek to MJN supporting the enclosed GRAS Notice and higher ARASCO level in infant formula.

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- The results from additional preclinical and clinical safety studies not previously reviewed by OFAS.

In accordance with the criteria set forth in the Federal Register 62 FR18938, 18961 (1997), MJN submits the following information as part of its GRAS exemption claim.

Name and Address of Notifier: Mead Johnson Nutritionals, 2400 W. Lloyd Expressway, Evansville, Indiana 47721.

Common or Usual Name of the Substance: The common or usual name for ARASCO is "arachidonic acid rich oil."

Applicable Conditions of Use: ARASCO is intended to be used as a source of arachidonic acid (ARA) in infant formulas for use by term infants. The maximum use level for this oil will be 1.88% of total dietary fat.

Basis for GRAS Determination: MJN has determined that ARASCO is a GRAS ingredient on the basis of scientific procedures.

Availability of Data: The data and information that are the basis for MJN's GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at reasonable times at the offices of Mead Johnson Nutritionals, 2400 W. Lloyd Expressway, Evansville, Indiana 47721 or will be sent to FDA upon request.

GRAS Exemption Claim: The use of ARASCO as a source of ARA in term infant formulas, at a maximum use level of 1.88% of total dietary fat, is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because MJN has determined that such use is GRAS.

An original and two copies of this notice are enclosed for your review. If you have any questions regarding this notice, please call me at (812) 429-7886.

Sincerely,

Thomas L. Ferguson
Director, Regulatory Affairs
Mead Johnson Nutritionals

Enclosures

Cc (w/o encl.): Paulette Gaynor
George Pauli
Alan Rulis
Laura Tarantino

GRAS Notice for Extending the Level of Use of ARASCO® (single cell oil) as a Source of Arachidonic Acid in Infant Formulas

August 3, 2001

Mead Johnson Nutritionals
Evansville, IN

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Executive Summary

Mead Johnson has determined that ARASCO® (single cell oil) as a source of arachidonic acid (ARA) is generally recognized as safe (GRAS) through scientific procedures when added to infant formulas at a level up to 1.88% of total daily dietary fat under conditions of use where the formula also contains DHASCO® as the source of docosahexaenoic acid (DHA) at levels of up to 1.25% of total daily dietary fat and the DHA:ARA and DHASCO:ARASCO ratios range from 1:1 to 1:2. This use level for ARASCO is equivalent to 0.938% of daily dietary calories or about 113 mg of the oil per kilogram of body weight per day (mg/kg bw/day) for infants.

DHASCO and ARASCO are oils extracted from single cell organisms that are rich in the long chain polyunsaturated fatty acids (LCPUFAs) DHA and ARA, respectively. Both oils are manufactured by Martek Biosciences Corporation (Martek). DHASCO is a triglyceride oil produced by the algae *Crypthecodinium cohnii* and is standardized to contain 40% by weight of DHA by adding high oleic sunflower oil. ARASCO, also a triglyceride oil, is produced by the fungus *Mortierella alpina* and also is standardized with high oleic acid sunflower oil to contain 40% by weight of ARA. Both single cell oils contain DHA and ARA in triglyceride structures that are chemically similar to those delivered to infants from mother's milk. The safe use of these oils in infant formulas has been established in a previous GRAS Notice by Martek Biosciences Corporation (GRAS Notice No. GRN 000041), with no questions raised by the Food and Drug Administration (FDA) in their response letter of May 17, 2001 (Appendix A).

The level of ARASCO addition to infant formula proposed by Mead Johnson Nutritionals is an extension of the 1.25% use level of ARASCO previously requested by Martek in their GRAS Notification for the use of ARASCO and DHASCO as Sources of the LCPUFAs in Infant Formulas of February 18, 2000 (GRAS Notice No. GRN 000041; excerpts included in Appendix B). The response to the Martek GRAS Notice from Alan Rulis and Christine Lewis, of the Center for Food Safety and Applied Nutrition (CFSAN) of FDA, to Henry Linsert, Jr., of Martek, on May 17, 2001 (Appendix A), includes a

table listing the conditions of use for both ARASCO and DHASCO. The conditions of use presented in FDA's May 17, 2001 letter reflect those requested by Martek in their GRAS Notice. The proposed levels of ARASCO, ARA, DHASCO, and DHA for use in Mead Johnson infant formulas are presented in Table 1 of this Notice. In comparison to levels specified in the Martek GRAS Notice and included in FDA's May 17, 2001 letter, only the amounts of ARASCO and ARA have been adjusted (i.e., the levels of DHA and DHASCO and the ratio of DHASCO:ARASCO remain unchanged). Martek is fully in support of this extension for the GRAS level for ARASCO, as expressed in a letter of July 11, 2001 from Henry Linsert, Jr., of Martek to Thomas Ferguson of Mead Johnson Nutritionals (Appendix C).

Table 1. Conditions of Use of ARASCO and DHASCO*

Substance	**Percent of daily fat**	**Percent of calories**	**Mg/kg bw/day**
ARA	Up to 0.75 percent	Up to 0.375 percent	Up to 45 mg/kg bw/day
DHA	Up to 0.50 percent	Up to 0.250 percent	Up to 30 mg/kg bw/day
ARA + DHA	Up to 1.25 percent	Up to 0.625 percent	Up to 75 mg/kg bw/day
ARASCO	Up to 1.88 percent	Up to 0.938 percent	Up to 113 mg/kg bw/day
DHASCO	Up to 1.25 percent	Up to 0.625 percent	Up to 75 mg/kg bw/day
ARASCO + DHASCO	Up to 3.13 percent	Up to 1.563 percent	Up to 188 mg/kg bw/day

*The ratio of DHA to ARA (and of DHASCO to ARASCO) would range from 1:1 to 1:2.

The proposed levels of ARASCO and ARA represent 50% increases (1.5X) compared with the levels included in FDA's letter of May 17, 2001 for ARASCO (up to 1.25% of total fat) and ARA (up to 0.5% of total fat). As noted above, Mead Johnson formulas conform to the established GRAS levels of DHASCO and DHA, and to the established GRAS ratios of DHA:ARA and DHASCO:ARASCO.

Mead Johnson has submitted this notice to cover levels of ARASCO and ARA tested in our preclinical and clinical studies. These levels of addition were initially selected based on a critical review of the literature regarding LCPUFA content of human milk. The goal

was to adopt a level of ARA (and thus ARASCO) supplementation that would be representative of typical ARA levels observed in human milk. The safety of these levels of ARASCO and ARA addition to infant formula is supported by the information reviewed in this Notice, including preclinical studies, infant clinical trials, and the Martek GRAS Expert Panel recommendations regarding inclusion of ARA and ARASCO in infant formula.

Rationale for Level of ARASCO

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I. Rationale for Level of ARASCO Addition in Mead Johnson Infant Formulas

Mead Johnson has maintained constant targets for the levels of DHA and ARA, and thus for DHASCO and ARASCO, over the course of our preclinical and clinical testing of these oils to support inclusion in infant formula. As noted above, the targets for DHA and ARA levels in our formulas were based on our review of LCPUFA levels found in human milk. These levels are typified by the median values reported from 24 studies [reviewed in Koletzko et al. 1992] of European and African women of 0.3% (of total fatty acids) DHA and 0.5 (European) to 0.6% (African) ARA, with the median ratio of DHA to ARA of 1:1.8 (European) to 1:2.2 (African). A review by Innis [1992] reported mean levels of approximately 0.1-1.4% DHA and 0.4-0.7% ARA across 17 population groups from around the world, including the U.S. and Canada, with typical values again of about 0.3-0.4% DHA and 0.5-0.6% ARA. Based on our assessment, Mead Johnson initially selected target levels of 0.3% DHA and 0.6% ARA, with a 1:2 ratio of DHA:ARA, for our preterm and term formulas. The Koletzko et al. 1992 review also served as a basis for the FAO/WHO Joint Expert Consultation [1994] recommendations for DHA and ARA levels for term infant formula, discussed below.

Mead Johnson's initial target levels of 0.3% DHA and 0.6% ARA were standardized to amounts per 100 Cal assuming 50% of calories from fat in human milk, or 5.56 g/100 Cal (assuming 9 Cal/g fat), resulting in target specifications (label claims) of 17 mg DHA and 34 mg ARA per 100 Cal. Specifications for essentially all nutrients in our infant formulas are established in units per 100 Cal, as specified for label claims in accordance with 21 CFR 107.10. Since the level of total fat in our term formulas is slightly below 50% of Cal, at 5.3 g fat/100 Cal, the specifications for the fatty acids in our term formula are equivalent to approximately 0.32% DHA and 0.64% ARA. As is true for most nutrients in infant formulas, an overage for each single cell oil is included to ensure claimed levels of the LCPUFA are maintained across batches and throughout shelf-life.

Given the normal variability inherent in oil composition, manufacturing and analyses, the level for ARA of 0.75% (1.88% ARASCO) of daily fat determined as GRAS by Mead Johnson assures that our formulas will consistently meet our specifications for ARA across multiple batches.

Support for GRAS Level

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II. Support for GRAS Level for ARA and ARASCO

A. Levels of ARA in Human Milk

The appropriateness of the targets Mead Johnson selected as representing typical human milk is further supported by compilations of human milk composition included in the Martek GRAS Panel Evaluation of DHASCO and ARASCO of December 1999 (excerpts in Appendix D), the Martek GRAS Notice of February 18, 2000 (excerpts in Appendix B), and Martek's letter to Mead Johnson of July 11, 2001 (Appendix C). Human milk levels of DHA and ARA from 78 population groups from around the world were reviewed in the GRAS Panel Evaluation (Table 2.1-1, pp. 8-9 in Appendix D) and subsequently in the Martek GRAS Notice (Table 1, pp. 5-7 in Appendix B). The pooled means (not weighted) and standard deviations for levels of DHA and ARA across the 78 population groups are 0.33±0.21% of fatty acids for DHA and 0.53±0.18% for ARA. Thus, the maximum for ARA (0.5% of fat) from the Martek GRAS Notice as reflected in FDA's May 17, 2001 letter (Appendix A) is below the pooled mean for ARA across these studies of human milk composition, while the current GRAS maximum for DHA (also 0.5%) is approximately 50% higher than the pooled mean for DHA from these same studies. The GRAS maximum for DHA is approximately the mean plus one standard deviation for this compilation of human milk DHA values (0.54%). For ARA levels across the same 78 population groups, the mean plus one standard deviation is 0.71%, similar to our proposed GRAS level of up to 0.75%. The median value for ARA across the 78 population groups is 0.5%, with a range of means of 0.19% to 1.56%. In the letter of July 11, 2001 received from Martek (Appendix C), an updated list of studies of human milk fatty acid composition across 93 population groups is included as Table 1 in the letter. In this compilation, the median value for human milk ARA content is 0.52%, slightly greater than the value

provided in the Martek GRAS Notice. The range, pooled mean (not weighted), and standard deviation across the 93 population groups are the same as across the 78 groups in the Martek GRAS Notice.

The recommendations by the FAO/WHO Joint Expert Consultation [1994] for amounts of ARA and DHA to be added to term infant formula also were based on levels found in human milk. This expert panel recommended that term infant formula should provide 40 mg ARA and associated long chain n-6 fatty acids/kg bw/day (a level 33 percent greater than the 30 mg/kg bw/day maximum requested in the Martek GRAS Notice) and 20 mg DHA/kg bw/day (33% less than the 30 mg/kg bw/day maximum requested in the Martek GRAS Notice). The WHO/FAO levels are equivalent to 0.65-0.7% ARA and 0.33-0.35% DHA (in Table 2.1-1, p. 9 and Table 3-1, p. 24, in the Martek GRAS Panel Evaluation [Appendix D] and Table 1, p. 6, and Table 3, p. 23, of the Martek GRAS Notice [Appendix B]), which are very similar to the Mead Johnson targets of 0.64% ARA and 0.32% DHA.

B. Recommendation of the Martek GRAS Expert Panel

The Expert Panel assembled by Martek to review the GRAS status of the single cell oils determined DHASCO and ARASCO to be GRAS for use in supplementing the diets of infants and children (p. 6 in Appendix D) "at levels of 2.5% of dietary fat (1.25% of energy or up to 150 mg DHASCO (or ARASCO) per kg body weight per day)." Thus, the levels determined to be GRAS by this panel are twice as high as those provided in the Martek GRAS Notice of February 18, 2000 (p. 1 of Appendix B) and the final levels as described in FDA's May 17, 2001 letter (up to 1.25% of daily fat, 0.625% of calories, and 75 mg/kg bw/day for each oil; Appendix A). The Martek GRAS Notice does not provide a scientific rationale for deviating from the GRAS Panel recommendation, and Mead Johnson was

not consulted regarding the levels of oils submitted in the GRAS Notice. In the letter of July 11, 2001 to Mead Johnson (Appendix C), Martek states:

> "When Martek submitted its GRAS notification, the company intended to cover all infant formulas with ARASCO and DHASCO to be marketed in the U.S. Martek now realizes, however, that the level selected for ARASCO and the corresponding ARA level did not cover the Mead Johnson formulation. Martek fully supports Mead Johnson's proposed use level, with the expected overages, of ARASCO and DHASCO and believes that the data submitted by Martek, including the findings of the Expert Panel, establish the GRAS status of Martek's oils at the levels and ratios in the Mead Johnson formula."

C. Safety of ARASCO and DHASCO in Preclinical Studies

The GRAS Expert Panel determination was based to a large extent on their evaluation of the very extensive body of preclinical and clinical studies reviewed in both the GRAS Panel Evaluation of December 1999 and in the Martek GRAS Notice of February 2000. With regard to preclinical testing, both the GRAS Panel Evaluation (p. 68 in Appendix D) and the Martek GRAS Notice (p. 60 in Appendix B) state, "These studies, in total, represent a very large experience base of dietary treatment of many different mammalian species with DHASCO and ARASCO, and there have been no suggestions from these reports of any toxicological or safety issues with these oils."

Key safety studies reviewed include the toxicity studies summarized in Tables 7.5.1 through 7.5.5, pp. 54-56 of the GRAS Panel Evaluation (in Appendix D) and in Tables 9-12, pp. 45-47 of the Martek GRAS Notice

(in Appendix B). As summarized in those tables, all of the toxicity studies that included blends of ARASCO with DHASCO tested the two oils at dietary ratios of 1:1.5 to 1:2 (DHASCO:ARASCO). Similarly, in toxicity studies that evaluated the oils separately, the highest levels of ARASCO tested were typically two times the highest levels of DHASCO tested. In sub-chronic (28- and 90-day) [Boswell et al. 1996; Koskelo et al. 1997] and developmental [Arterburn et al. 2000] toxicity studies of the individual single cell oils, a maximum level of 2.5 g/kg bw/day ARASCO was tested and found to be the No Observed Adverse Effect Level (NOAEL). When administered as a mixture of DHASCO and ARASCO (1:2) in the diet, the maximum level of about 9 g/kg bw/day (about 3 g/kg bw/day DHASCO and 6 g/kg bw/day ARASCO) was found to be the NOAEL in both 28-day [Wibert et al. 1997] and a 90-day [Burns et al. 1999] feeding studies in rats. This NOAEL of 3 g/kg bw/day DHASCO and 6 g/kg bw/day ARASCO represents a 40-fold excess over the existing GRAS maximum established for DHASCO (75 mg/kg bw/day) and a 53-fold excess over the upper bound for ARASCO use in infant formula established by Mead Johnson (113 mg/kg bw/day).

Based on a critical review of the toxicology studies, the Expert Panel concluded (p. 5 in Appendix D) that, "None of the studies indicated that the oils were toxic, and the No Adverse Effects Levels (NOAELs) were determined to correspond to the highest doses tested." The Martek GRAS Notice (p. 3 in Appendix B) states, "None of the thirty-two toxicological studies undertaken indicated any toxigenicity related to these oils . . . The No Adverse Effect Levels (NOAELs) in these studies corresponded to the highest doses tested." Mead Johnson's determination of 1.88% of daily fat as ARASCO in infant formula as GRAS is consistent with the preclinical safety data and is 25% below the GRAS recommendation of the Martek Expert Panel of 2.5% daily fat as ARASCO.

D. Additional Preclinical (Neonatal Piglet) Studies

Mead Johnson sponsored two additional preclinical studies, conducted by Dr. J. Thomas Brenna at Cornell University and by Dr. Jack Odle at North Carolina State University, utilizing the neonatal piglet model. This widely used animal model allows direct feeding of the materials of interest (ARASCO and DHASCO) in a neonatal animal with many physiologic similarities to the human infant.

Dr. Brenna's study [Huang et al. 2000, in press; Sarkadi Nagy et al. 2000] was a dose response of DHASCO plus ARASCO (1:2 ratio) fed in milk-based formulas to provide DHA and ARA at 0/0, 17/34, 34/78, and 85/170 mg/100 Cal (6 piglets per group), i.e., 0, 1, 2 and 5 times the Mead Johnson infant formula specifications. As noted in the manuscript by Huang et al. [in press], piglets fed the 5X dose received on average 585 mg ARA and 290 mg DHA/kg bw/day during week 1 of the study, falling to 420 mg ARA and 210 mg DHA/kg bw/day at 25 days of age. These intakes correspond to approximately 1460 and 1050 mg ARASCO/kg bw/day, which are 9.3 (day 25) to 13 (week 1) times the maximum levels proposed by Mead Johnson for ARASCO (up to 113 mg/kg bw/day). For DHASCO, these intakes correspond to 7 (day 25) to 9.7 (week 1) times the GRAS level of up to 75 mg/kg bw/day in FDA's May 17, 2001 letter (Appendix A).

Piglets were fed the formulas from day 2 of life through sacrifice at day 30. There were no significant differences between groups in body weights or weight gain, organ weights (liver, brain, heart, spleen, kidney, and lung; absolute or relative to body weight), or serum chemistry values (alkaline phosphatase, ALT, AST, BUN, creatinine, albumin, glucose, cholesterol, triglycerides, plasma protein, or hematocrit), either by two-way analysis of variance or by linear regression to identify any dose-response trends.

Also, no significant histopathological changes were noted in liver sections from any piglets, and liver protein content did not differ between groups. The results of this study of direct feeding to neonatal piglets supports the safety of ARASCO, provided with DHASCO at a 1:2 (DHA:ARA) ratio, at levels up to approximately 10-fold higher than the level proposed by Mead Johnson for infant formula.

Dr. Odle's neonatal piglet study [Mathews et al. 2001a, 2001b] compared formulas with two different sources of DHA and ARA, Martek's single cell triglyceride oils or an egg phospholipid source. Two groups of piglets (10 piglets per group) were fed milk-based formulas containing DHASCO and ARASCO to provide approximately 0.3% DHA and 0.6% ARA. The formulas provided 53.8% of calories from fat, with DHA providing about 0.16% of calories and ARA about 0.32%. Based on intakes and weights of the piglets in this study, it was calculated that piglets received about 40 mg DHA and 80 mg ARA/kg bw/day at the mid-point of the study. This level of ARA is approximately 1.8 times the upper level proposed by Mead Johnson.

Piglets were fed experimental formulas from day 1 of life until sacrifice at day 16. No statistically significant differences were observed between piglets fed control (no LCPUFA) formula and either of the two DHASCO+ARASCO-supplemented formula groups with respect to body weight, weight gain or feed efficiency; plasma concentrations of cholesterol, glucose, ALT, AST, urea nitrogen, or alkaline phosphatase; intestinal villi height or crypt depth or lactase activity in the jejunum or ileum; ileal or rectal apparent dry matter digestibility; liver or spleen weight (absolute or relative to body weight); liver crude protein or fat composition; or liver histopathology. The results of this safety study in piglets support the level of ARASCO proposed by Mead Johnson, with an NOAEL 1.8 times the proposed upper level for addition to infant formula.

E. Safety of ARASCO and DHASCO in Infant Clinical Trials

The infant clinical trials of formulas containing DHASCO and/or ARASCO are summarized in the GRAS Panel Evaluation of December 1999 (excerpts in Appendix D), the Martek GRAS Notice of February 2000 (excerpts in Appendix B), and the Martek letter to Mead Johnson of July 11, 2001 (Table 3 in Appendix C). Of the formulas that included both DHASCO and ARASCO, the ratio of the two oils ranged from 1:1 to 1:2 DHASCO:ARASCO. As summarized by the Expert Panel (p. 25 in the GRAS Panel Evaluation in Appendix D, and p. 23 of the Martek GRAS Notice in Appendix B), "In trials where supplemental ARA has been used, the concentration ranged from 0.1% to 1.1% of total fat in preterm formulas, and from 0.2% to 0.72% for term formulas. . . These values clearly fall well within the normal range of mother's milk and the Expert recommendations." The Expert Panel did not identify any safety issues or concerns related to the results of any of the infant clinical trials that used DHASCO and/or ARASCO as sources of LCPUFA supplementation.

Mead Johnson has conducted two large, prospective, randomized, double-blind growth studies, in preterm [Hansen et al. 1997; Diersen-Schade et al. 1998] and term [Carlson et al. 1999] infants. These studies were included in the Martek GRAS Panel Evaluation (p. 2 of Appendix 2, included in Appendix D) and the GRAS Notice (p. 86 in Appendix B). As noted above, the specifications for LCPUFA levels in all of our clinical studies were 17 mg DHA and 34 mg ARA per 100 Cal. In the clinical trial with term infants, target levels as a percent of total fatty acids were approximately 0.32% DHA and 0.64% ARA. Targets were slightly higher for the preterm formula, at 0.33% DHA and 0.67% ARA, because this formula contains somewhat lower total fat (5.1 g/100 Cal) than term formula.

The term infant study [Carlson et al. 1999] included three groups of formula-fed infants: 122 infants enrolled at about 14 days of age to receive control (marketed) milk-based formula without added DHA or ARA; 129 enrolled to receive formula with added DHASCO and ARASCO; and 126 enrolled to receive formula with added fish oil (as the source of DHA) and ARASCO. Formulas were fed to a year of age, with 98, 100 and 87 infants completing the study within each of the formula groups, respectively. In the preterm infant study [Hansen et al. 1997; Diersen-Schade et al. 1998], very low birthweight infants were fed one of three study formulas while in the hospital – control (marketed) premature formula without LCPUFA (n=62 enrolled), formula with added DHASCO (n=66), or formula with added DHASCO and ARASCO (n=66). Study formulas were fed for at least 28 days, from, on average, 32 weeks post-menstrual age (PMA) to 36 weeks PMA. At hospital discharge, all preterm infants were switched to marketed term formula (without added LCPUFA), and followed for about an additional 5 months, to 4 months adjusted age (57 weeks PMA). A group of 90 breastfed term infants also was enrolled and followed to 4 months of age. The number of infants completing the study to 57 weeks PMA included 47 fed the control preterm formula, 49 fed the preterm formula with DHASCO, 55 fed the preterm formula with DHASCO and ARASCO, and 76 term breastfed infants.

Both of these studies demonstrated that addition of DHASCO and ARASCO to infant formula to provide the targeted levels and ratio (1:2) of DHA and ARA did not negatively impact infant growth, using one-sided tests designed to maximize the power to detect a decrease in weight gain (the measure traditionally used by FDA and infant formula manufacturers to assess safety of new or reformulated infant formulas). For both studies, post-hoc analysis using two-sided tests of equality found significantly

higher early weight gain when DHASCO and ARASCO were both included in the formulas. No significant differences were observed in measures of acceptance and tolerance or in incidence of serious adverse events or other negative effects in comparing the LCPUFA-supplemented preterm and term formulas with the control (marketed) formulas.

Mead Johnson has also provided the same term infant formulas used in our term infant growth study to Dr. Eileen Birch and colleagues at the Retina Foundation of the Southwest/Anderson Vision Research Center in Dallas, Texas, for a series of clinical trials evaluating the effects of LCPUFA on infant visual and mental development. Peer-reviewed publications from these studies report improved visual acuity [Birch et al. 1998; Hoffman et al. 2000] to a year of age and improved mental development at 18 months [Birch et al. 2000] when infants were fed formula containing DHASCO and ARASCO for the first four months of life, compared with those fed marketed formula. Analysis of one batch of LCPUFA-supplemented formula in their laboratory found levels of 0.36 % DHA and 0.72 % ARA, as reported in their published papers [Birch et al. 1998, 2000; Hoffman et al 2000]. More recently, this group has reported data from infants at one year of age that were weaned from human milk to either control (marketed) or LCPUFA-supplemented formula, with the study formulas fed to a year of age [Hoffman et al. 2001]. Although these studies were not designed to evaluate safety-related parameters such as growth or incidence of adverse events, infants were closely followed and the authors did not report any negative effects from feeding the LCPUFA formulas compared with control products. From the first study discussed above [Birch et. al. 1998, p. 207], the authors note:

> "With the caveats that the current study was not designed to fully assess safety issues (rare events could not be detected with these sample sizes) and had sufficient

power to assess a 0.9 SD difference in growth (approximately 9% weight, 3% length, and 2.5% head circumference), infants in all diet groups had similar rates of growth and tolerated all diets well."

Summary & Conclusions

III. Summary and Conclusions

Mead Johnson has determined that ARASCO is generally recognized as safe (GRAS) as a source of arachidonic acid (ARA) when added to infant formulas at a level up to 1.88% of total daily dietary fat. This level of addition is an extension of the ARASCO level of 1.25% (of total daily dietary fat) specified in the Martek GRAS Notice (GRAS Notice No. GRN 000041) and reflected in FDA's response letter of May 17, 2001 (Appendix A). The purpose of this change is to encompass the levels of ARASCO and ARA that have been maintained as targets for Mead Johnson infant formulas throughout all of our preclinical and clinical testing of these oils. This value provides a level of ARA that is well within the range found in human milk. Martek is fully in support of this extension, as stated in the letter of July 11, 2001 to Mead Johnson (Appendix C). Mead Johnson's proposed maximum level of use for ARASCO is consistent with the recommendation of Martek's GRAS Expert Panel and is in fact, 25% below the Panel's finding of safe use for ARASCO up to 150 mg/kg bw/day in infant formula (Appendix D).

Additional support for the safe use of ARASCO in infant formula comes from two recent Mead Johnson sponsored studies in piglets. These studies, conducted in a well-accepted model for human infants, found no adverse effects on growth, organ weights, serum chemistries or other parameters associated with feeding ARASCO at levels ranging from approximately two-fold to greater than 10-fold higher than the level proposed by Mead Johnson.

The safety of ARASCO in infant formula at 1.88% of total daily dietary fat is further supported by two large clinical trials conducted by Mead Johnson in preterm and term infants, and a series of term infant studies conducted by Dr. Birch and colleagues. All of these studies utilized the Mead Johnson target level for ARASCO. Results from all of these studies indicated no negative impact on infant growth or any association of ARASCO and DHASCO supplementation with increased incidence of adverse events.

Based upon the large body of preclinical and clinical data reviewed by the GRAS Expert Panel, as well as results from Mead Johnson-sponsored piglet studies and infant clinical trials, Mead Johnson has established that ARASCO is GRAS for use in infant formula at levels up to 1.88% of total daily dietary fat or 113 mg per kg body weight per day. This corresponds to an ARA level of 0.75% of total daily dietary fat, 0.375% of energy, or up to 45 mg of ARA per kg body weight per day.

List of Appendices

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Appendix A. Rulis AM, Lewis CJ. Letter to Henry Linsert, Jr., Martek Biosciences Corporation. May 17, 2001.

Appendix B. Excerpts from: Martek Biosciences Corporation GRAS Notification for the use of DHASCO and ARASCO (single cell sources of DHA and ARA) as Sources of the LCPUFAs in Infant Formulas. February 18, 2000.

Appendix C. Linsert H. Letter to Thomas Ferguson, Mead Johnson Nutritionals. July 11, 2001.

Appendix D. Excerpts from: Opinion of an Expert Panel on the Generally Recognized as Safe (GRAS) Status of ARA and DHA Single Cell Oils for Infants and Children. GRAS Panel Evaluation of DHASCO® and ARASCO®. Martek Biosciences Corporation. December 1999.

List of References

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Appendix A

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U. S. Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety*

Agency Response Letter
GRAS Notice No. GRN 000041

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
Washington, DC 20204

May 17, 2001

Henry Linsert, Jr.
Martek Biosciences Corporation
6480 Dobbin Road
Columbia, Maryland 21045

Re: GRAS Notice No. GRN 000041

Dear Mr. Linsert:

The Food and Drug Administration (FDA) is responding to the notice, dated February 29, 2000, that Hogan and Hartson submitted on behalf of Martek Biosciences Corporation (Martek) in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)). FDA received the notice on March 1, 2000 and designated it as GRAS Notice No. GRN 000041.

The subject of the notice is DHASCO (docosahexaenoic acid-rich single-cell oil) and ARASCO (arachidonic acid-rich single-cell oil). DHASCO is derived from the microalgal species *Crypthecodinium cohnii*; ARASCO is derived from the soil fungus *Mortierella alpina*. In its notice, Martek informs FDA of its view that DHASCO and ARASCO as sources of docosahexaenoic acid (DHA) and arachidonic acid (ARA) are GRAS, through scientific procedures, when added to term infant formulas as described in Table 1.

Table 1
Conditions of Use of ARASCO and DHASCO Proposed by Martek*

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Substance	Percent of daily fat	Percent of calories	Mg/kg bw/day
ARA	Up to 0.5 percent	Up to 0.25 percent	Up to 30 mg/kg bw/day
DHA	Up to 0.5 percent	Up to 0.25 percent	Up to 30 mg/kg bw/day
ARA + DHA	Up to 1.0 percent	Up to 0.5 percent	Up to 60 mg/kg bw/day
ARASCO	Up to 1.25 percent	Up to 0.625 percent	Up to 75 mg/kg bw/day
DHASCO	Up to 1.25 percent	Up to 0.625 percent	Up to 75 mg/kg bw/day
ARASCO + DHASCO	Up to 2.5 percent	Up to 1.25 percent	Up to 150 mg/kg bw/day
*The ratio of DHA to ARA (and of DHASCO to ARASCO) would range from 1:1 to 1:2.			

Data and information that Martek presents to support its determination that ARASCO and DHASCO are GRAS for use in infant formula

As part of its notice, Martek includes the report of a panel of individuals (Martek's GRAS Panel) who evaluated the data and information that are the basis for Martek's GRAS determination. Martek considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food.

Martek describes published information about the levels of the fatty acids ARA and DHA in human milk and considers that the presence of ARA and DHA in human milk establishes that these fatty acids are GRAS. Martek cites published information to support its view that ARA and DHA have a role as nutrients. Martek describes the recommendations of several international bodies that these fatty acids be added to preterm infant formulas, term infant formulas, or both, at levels similar to the levels proposed by Martek.

Martek describes published information about the digestion of triglycerides by infants. Martek describes published information relevant to the biodisposition of ARASCO and DHASCO, focusing on a comparison of the biodisposition of ARASCO and DHASCO to that of ARA- and DHA-containing triglycerides in human milk. Martek concludes that the digestion and absorption of DHA from DHASCO, and ARA from ARASCO, would be the same as that of DHA and ARA from triglycerides present in human milk.

Martek describes published information about the identity and characteristic properties of ARASCO and DHASCO, including published information about the source microorganisms for ARASCO and DHASCO and the method of manufacture of ARASCO and DHASCO. Martek provides food grade specifications for ARASCO and DHASCO. Martek describes published information about the composition of sterols in the nonsaponifiable fractions of DHASCO and ARASCO. Martek states that the principal components of the sterol fraction in DHASCO (i.e., 4-methyl sterols) are found in the normal metabolic pathway of cholesterol biosynthesis and have been identified in several common food sources including fish and shellfish. Martek further states that the principal component of the sterol fraction in ARASCO (i.e, desmosterol) also is found in the normal metabolic pathway of cholesterol biosynthesis and is commonly found in several common food sources including animal fat, vegetable oils, and human milk.

Martek describes published and unpublished toxicity studies conducted in rats treated with ARASCO and DHASCO. These studies include acute studies, 28-day studies, a 63-day study, 90-day studies, and developmental and reproductive toxicity studies. Martek concludes that the relatively large number of toxicity studies provides a degree of redundancy in the standard toxicological assessments.

In Martek's view, ARASCO and DHASCO pose unique testing problems because it can be difficult to distinguish whether an observed effect related to the test material is a normal physiological response to

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the high dietary load of that particular macronutrient, a dietary deficiency that is related to presence of a large amount of the test material in the diet, or a toxicological effect.(1) Martek acknowledges that some of the available animal toxicity studies have reported statistically significant effects at the highest doses tested, including an increase in relative liver weights, an increase in relative spleen weights, and a change in some blood chemistries. For each of these reported effects, Martek explains the reasons for its conclusion that the reported effects are not adverse toxicological findings that are related to ARASCO and DHASCO as sources of ARA and DHA. Martek concludes that the No Observed Effect Level (NOEL) in each study should be the highest dose tested.

Martek also acknowledges that some studies have reported changes attributed to possible effects on the kidney. Martek presents an analysis of these studies and concludes that there are no consistent treatment related effects of DHASCO and ARASCO, either alone or in combination, with respect to kidney weight, kidney function or kidney histopathology. Martek discusses a published subchronic rat study with an *in utero* phase and a published developmental toxicology study and concludes that there is no evidence of any treatment-related adverse reproductive effects in rats treated with DHASCO and ARASCO.

Martek cites 14 published clinical intervention trials involving 1,500 term or preterm infants. In all, more than 700 of the term or preterm infants enrolled in these studies consumed formulas supplemented with DHASCO or ARASCO oils. Martek reports that none of the studies conducted with ARASCO and DHASCO as sources of ARA and DHA demonstrated any adverse effect on infant growth.

Martek notes that some studies reported that infants who consumed term and preterm infant formula supplemented with fish oil as a source of DHA exhibited reduced growth and a decline in measures of ARA status. To address these reports, Martek notes that fish oil contains eicosapentaenoic acid (EPA), which is not present in DHASCO and which is known to be an antagonist of ARA metabolism. In Martek's view, the reduced growth seen in these studies could be related to (1) the presence of EPA, which is present in fish oils; (2) the lack of a source of ARA in the formula supplemented with fish oil; or (3) a combination of the presence of EPA and the lack of ARA. Martek contrasts the reports of negative effects on growth in infants who consumed formulas supplemented with fish oil to the reports of studies conducted with DHASCO as a source of DHA, which have not reported negative effects on growth.

Martek presents information about DHASCO- and ARASCO-supplemented infant formulas that have been introduced to the international marketplace during the past 5 years. According to Martek, more than 100,000 infants have consumed a formula that is marketed for low birth weight infants and is administered under a physician's strict supervision, with no reported adverse effects associated with the consumption of the formula.

Martek discusses a published clinical study that reported more deaths attributed to Sudden Infant Death Syndrome (SIDS) in infants who consumed a formula supplemented with fish oil compared to infants in the control group. According to Martek, an independent safety committee, which included specialists with expertise in SIDS, carefully reviewed each case and concluded that none of the SIDS deaths was related to dietary treatment. In support of the opinion expressed by the independent safety committee, Martek notes that more than 30 other clinical trials have been conducted using formulas supplemented with DHA and ARA from various sources, and that none of these other trials reported any similar observation. Martek also notes that there have been no reports of an increase in SIDS-related deaths in countries where formulas supplemented with a source of DHA and ARA have been used for several years. Martek also notes that there have been no concerns reported by physicians worldwide who have monitored the administration of formulas supplemented with DHASCO and ARASCO to low birth-weight infants.

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FDA's evaluation of the data and information in Martek's notice

On January 29, 2001, at Martek's request, Martek met with representatives of the Center for Food Safety and Applied Nutrition (CFSAN). In a letter dated January 31, 2001, you reiterated a concern that you expressed on January 29 about a delay in the agency's response to your GRAS notice. In your January

31 letter, you outlined your understanding of a commitment made by CFSAN at the January 29 meeting to define, as soon as possible, any specific unresolved scientific questions related to this notice.

In a letter dated March 1, 2001, CFSAN explained that the delay in its response to your GRAS notice was related to broader issues than those related to your notice and described specific scientific concerns related to the use of infant formulas containing long chain polyunsaturated fatty acids (LCPUFAs) such as the fatty acids ARA and DHA. As we explained in our March 1 letter, some publications and panel reports, which considered multiple sources of ARA and DHA (e.g, sources such as fish oil and egg phospholipid), have questioned the scientific adequacy of data to support the use of LCPUFAs in infant formula. In addition, some studies have reported unexpected deaths among infants who consumed formula supplemented with LCPUFAs. These unexpected deaths were attributed to Sudden Infant Death Syndrome (SIDS), sepsis or necrotizing enterocolitis. Also, some studies have reported adverse events and other morbidities including diarrhea, flatulence, jaundice, and apnea in infants fed LCPUFAs. In addition, CFSAN noted that your notice had not accounted for the fact that the bioactive fatty acids ARA and DHA when consumed in mature human milk are part of a complex matrix that includes, for example, linoleic acid, alpha-linolenic acid, and other polyunsaturated fatty acids and that important physiologic considerations relative to the matrix are not accounted for by the simple addition of LCPUFAs to infant formula.

In our letter dated March 1, 2001, we informed you that CFSAN intended to convene a group of experts to address the broader scientific issues raised by your specific determination that the ingredients DHASCO and ARASCO are GRAS sources of DHA and ARA for use in infant formula.

In a letter dated March 23, 2001, Martek expressed its view that the reports and publications that questioned the scientific adequacy of data to support the use of LCPUFAs in infant formula recommended additional studies, which are now available. Martek also noted that studies conducted in term and preterm infants with balanced addition of ARASCO and DHASCO as sources of both bioactive fatty acids showed no difference in deaths between treatment and control groups. In Martek's view, the results of clinical studies conducted with infant formulas manufactured by three different firms are the most direct and conclusive evidence that there are no concerns related to physiologic considerations associated with the overall physiological matrix of infant formula compared to human milk.

In its March 23 letter, Martek expressed its view that some of the concerns described in CFSAN's March 1 letter are hypothetical and that convening a group of scientific experts to answer such hypothetical concerns would not be productive.

Conclusions

Based on all of the information provided by Martek, as well as other information available to FDA, the agency has no questions at this time regarding Martek's conclusion that ARASCO and DHASCO are GRAS sources of ARA and DHA under the intended conditions of use - i.e., when added to infant formulas intended for consumption by healthy term infants at a level of up to 1.25 percent each of total dietary fat and at a ratio of DHA to ARA of 1:1 to 1:2. The agency has not, however, made its own determination regarding the GRAS status of the subject use of ARASCO and DHASCO. As always, it is the continuing responsibility of Martek to ensure that food ingredients that the firm markets are safe. As discussed in detail below, it is the responsibility of an end user of ingredients that Martek markets for use in infant formula to ensure that an infant formula that contains such ingredients is otherwise in compliance with all applicable legal and regulatory requirements.

As noted above, in its March 23 letter, Martek expressed its view that convening a panel of experts to discuss the broad scientific issues raised by its notice would not be productive. We disagree with that view. Rather, we have initiated a discussion with an authoritative body of scientific experts to address those broader issues, such as, for example, the addition of bioactive ingredients to an infant formula matrix, which is different from the physiological matrix of mature human milk.

As you are aware, under section 412 of the Federal Food, Drug, and Cosmetic Act (the FFDCA), a

manufacturer of a new infant formula must make a submission to FDA, providing required assurances about the formula, at least 90 days before the formula is marketed. We realize that Martek is a supplier of an ingredient that would be used in infant formula, rather than a manufacturer of infant formula. Thus, we would not expect Martek to be making the submission required by section 412 about an intent to market a new infant formula that contains ARASCO and DHASCO. However, you should be aware that FDA's response to your GRAS notice does not alleviate the responsibility of any infant formula manufacturer who intends to market an infant formula that contains ARASCO and DHASCO to make the submission required by section 412.

As noted in our March 1 letter, the specific use of the ingredients DHASCO and ARASCO in infant formula raises broad scientific issues about the use of bioactive ingredients such as LCPUFAs in infant formula. In addition, it is FDA's view that any evaluation that a use of a food ingredient is safe is a time-dependent judgment that is based on general scientific knowledge as well as specific data and information about the ingredient. For these reasons, FDA would expect any infant formula manufacturer who lawfully markets infant formula containing ARASCO and DHASCO to monitor, through scientific studies and rigorous post-market surveillance, infants who consume such a formula. We also would expect regular reports of such studies and post-market surveillance. Because the use of ARASCO and DHASCO in infant formula would be based on the GRAS provision of the FFDCA, we also would expect that these reports would not be considered to be confidential so that the broader scientific community can contribute to this continuing evaluation. In light of these expectations, we strongly encourage any manufacturer of infant formula who is considering using Martek's ARASCO and DHASCO in infant formula to consult with FDA's Office of Nutritional Products, Labeling, and Dietary Supplements (ONPLDS) before making the required premarket submission. Moreover, we strongly encourage any such manufacturer to consult with ONPLDS before initiating premarket clinical studies, and FDA would plan to engage manufacturers concerning any needed postmarket clinical studies or postmarket surveillance.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the Office of Premarket Approval's homepage on the Internet (at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,

/s/
Alan M. Rulis, Ph.D.
Director
Office of Premarket Approval
Center for Food Safety and Applied Nutrition

/s/
Christine J. Lewis, Ph.D., R.D.
Director
Office of Nutritional Products, Labeling, and Dietary Supplements
Center for Food Safety and Applied Nutrition

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(1)As an analogy, Martek presents a scenario of elevated serum cholesterol in a human who changes a diet from one with 25 percent of calories from fat to one with 45 percent of calories from fat. In this analogy, Martek notes that the elevation in serum cholesterol ordinarily is viewed as a normal physiological response to increased dietary fat rather than as a toxicological effect of the particular fat used.

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* The Office of Premarket Approval became the Office of Food Additive Safety on June 18, 2001.

Content last updated by pmg/lsk on 2001-MAY-29
Hypertext last updated by pmg/lsk on 2001-MAY-29

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Appendix B

Martek Biosciences Corporation
GRAS Notification for the use of
DHASCO and ARASCO
(single cell sources of DHA and ARA)
as Sources of the LCPUFAs in Infant Formulas
February 18, 2000

EXECUTIVE SUMMARY

This notification establishes that DHASCO® and ARASCO® are generally recognized as safe (GRAS) sources of docosahexaenoic acid (DHA) and arachidonic acid (ARA), when added to infant formulas at a level of up to 1.25% each of total dietary fat at DHA:ARA ratio of 1:1 to 1:2. For infants, this use level is equivalent to 0.625% of dietary calories, about 75 mg of the oil/kg body wt/day, or about 30 mg DHA or ARA/kg body wt/day. These values are well within the normal range of DHA and ARA levels found in human milk.

DHA and ARA are long-chain polyunsaturated fatty acids (LCPUFA) that are found naturally in breast milk. DHA and ARA are considered important for the developing infant because these long chain polyunsaturated fatty acids (LCPUFAs) are the primary building blocks for the brain and retina. DHASCO and ARASCO are oils extracted from single cell organisms that are rich in DHA and ARA. DHASCO oil is a triglyceride, produced by the algae *Crypthecodinium cohnii* and is standardized with high oleic sunflower oil to contain 40% by weight of DHA. ARASCO oil, also a triglyceride, is produced by the fungus *Mortierella alpina* and also is standardized with high oleic acid sunflower oil to contain 40% by weight of ARA. In both cases, these oils contain DHA and ARA in triglyceride structures that are chemically equivalent to those delivered to infants from mother's milk.

Martek convened a panel of esteemed experts to review the available data on DHASCO and ARASCO (hereinafter referred to as the "Expert Panel") 1/ A copy of the Expert Panel's report has been submitted to FDA and can be found in the food master file number 636. After reviewing the available data and information regarding the importance of DHA and ARA in the infant diet, the Expert Panel unanimously agreed:

1) that there is a deficiency in the DHA and ARA status in infants fed formula not fortified with DHA and ARA;

2) that this is reflected in a decreased blood and other tissue (*e.g.*, brain) levels of DHA and ARA; and

3) that this deficiency contributes to the visual and neurological deficits observed in formula-fed compared with breast-fed infants.

1/ The following individuals comprise the Expert Panel: Joseph Borzelleca, Ph.D; Gary Flamm, Ph.D.; Bruce German, Ph.D.; Walter Glinsman, M.D.; David Kritchevsky, Ph.D.; David Bechtal, Ph.D.

In making these conclusions regarding the importance of DHA and ARA in the infant diet, the Expert Panel noted that numerous independent scientific bodies from around the world have recommended the addition of DHA and ARA to infant formulas. For example, the Food and Agriculture Organization (FAO) of the World Health Organization (WHO) reviewed the available data and recommended in 1994 that infant formulas be fortified with "40 mg of ARA and 20 mg of DHA per kg per day." (84). The Expert Panel also considered the findings in the 1998 Report prepared by the Life Science Research Organization (LSRO) of the American Society for Nutritional Sciences. The LSRO Panel is one of the few expert panels that failed to recommend fortification with DHA and ARA after reviewing the available data. The LSRO Panel did acknowledge, however, the importance of the LCPUFAs in the infant diet and recommended that this issue be reevaluated in the near future as new studies emerge. The Expert Panel convened by Martek reviewed studies not considered by the LSRO Panel and concluded that the weight of the evidence supported the addition of DHA and ARA to infant formulas to address the deficiency found in the infant formulas commercially available in the United States.

In addition to recognizing the importance of DHA and ARA in the infant diet, the Expert Panel also carefully reviewed the published and unpublished data from studies involving the use of DHASCO and ARASCO. The presence of DHA and ARA in breast milk establishes that these LCPUFAs are GRAS. The focus of this notification, and of the Expert Panel's review, therefore, is not on whether DHA or ARA are GRAS, but on whether the data establish that DHASCO and ARASCO are GRAS sources of DHA and ARA. After reviewing the available data, the Expert Panel concluded that DHASCO and ARASCO are GRAS as sources of DHA and ARA in infant formula when provided at a maximum level of 2.5% each of total dietary fat. The level found to be GRAS by the Expert Panel is twice as high as that proposed for use in this notification.

The Expert Panel considered numerous factors in its assessment of the GRAS status of DHASCO and ARASCO, including the chemical composition and manufacturing processes for these oils. Both oils are manufactured by a controlled fermentation process, followed by oil extraction and purification using methods common to the vegetable oil industry. All ingredients used in the processing of the oils are either food grade, or of higher quality, and the entire process meets current Good Manufacturing Practices for foods. The oils undergo rigorous analytical and quality assurance testing and meet well-defined product specifications prior to release. DHASCO and ARASCO do contain other common saturated and monounsaturated fatty acids found in human milk. Minor nonsaponifiable fractions of the oils have been characterized and contain primarily cholesterol-related sterols, which can be found in other food sources. The studies conducted on the oil and the biomass, from which the oil is extracted, establish the safety of these oils as sources of DHA and ARA.

Data also establish that DHASCO and ARASCO are absorbed in a manner consistent with other dietary triglycerides and that their supplementation in the infant formula will normalize blood DHA and ARA levels to those found in breast fed babies. The DHA and ARA are distributed throughout the body and are found at the highest levels in brain, retina, testes, and heart. DHA and ARA can be catabolized completely to CO_2 and H_2O, but the catabolic rate is slower than with other dietary fatty acids. This is necessary in order to maintain DHA and ARA levels in the rapidly expanding neurological tissues of infants and children.

In addition, ARA, but not DHA, serves as a precursor molecule to the omega-6 class of eicosanoids, which are well recognized as stimulators of immune function. Studies have shown that small amounts of DHA can be retroconverted to eicosapentaenoic acid (EPA) in humans, although accumulation of EPA is negligible at doses of DHASCO used for infant supplementation. Although oxidation of these highly unsaturated fatty acids in blood or tissues has been raised as a potential concern, studies have shown that DHA, in particular, activates antioxidant defense systems in the body and may actually protect against oxidation of polyunsaturated fatty acids. Animal and human studies have confirmed that supplementation with DHA and ARA protects, rather than accentuates oxidative damage. This is consistent with the finding that infants who receive breast milk (containing DHA and ARA) are more protected from Necrotizing Enterocolitis (NEC) than infants fed formulas without DHA and ARA.

A large number of safety studies have been conducted using DHASCO and ARASCO oils, including acute, subchronic, developmental and reproductive toxicology studies in rats and *in vitro* mutagenicity and clastogenicity assays with bacterial and mammalian cells. All studies were modeled after FDA Redbook guidelines and conducted at GLP-compliant laboratories. The study results must be evaluated relative to well known effects of supplementation with high doses of polyunsaturated fatty acids (PUFAs) in order to distinguish between PUFA-related effects and effects due to the sources of DHA and ARA themselves. None of the thirty-two toxicological studies undertaken indicated any toxigenicity related to these oils (summarized in Appendix 5). The No Adverse Effect Levels (NOAELs) in these studies corresponded to the highest doses tested.

Studies conducted in twelve different animal species, including nonhuman primates, have provided a large base of experience and extensive safety data with DHASCO and ARASCO oils (summarized in Appendix 1). None of the reports (published or unpublished) have suggested any toxicological or safety issues associated with the use of these oils. At least fourteen well-controlled clinical studies involving about 1,500 infants (700 infants receiving the supplemented formula) have confirmed that DHASCO and ARASCO increase circulating levels of DHA and ARA in preterm and term infants (summarized in Appendix 2). Significant improvements in growth, visual acuity and mental acuity have also been reported in infant groups supplemented with these oils and no adverse events have been reported.

Some, although not all, of the animal toxicology studies found modest increases in liver and spleen weights with very high doses of the oils that were considered statistically significant. Increased liver and spleen weights were not found consistently across the studies and the increased weights were within the normal range for liver and spleen. The modest increase in liver and spleen weights was not accompanied by abnormal histology or serum enzyme levels, which would have been expected if the increase was attributable to a toxicological effect. Moreover, when the liver and spleen weights were assessed relative to other organ weights (such as the brain), the weight changes in almost all instances were no longer apparent.

Modest increases in liver and spleen weights is a well-known effect in mammals given large doses of LCPUFAs of any source (*e.g.*, fish oils). The modest increases in liver and spleen weights are, therefore, considered to be an LCPUFA effect, and not an effect unique to the DHASCO and ARASCO. To further support the safety of DHASCO and ARASCO, studies have been conducted on the biomass in an attempt to assess whether there could be any component in

the biomass other than the purified oil (the subject of this Notification), that may be of toxicological concern. These studies consistently found the ARASCO and DHASCO biomasses to be nontoxigenic.

In addition, twenty-nine separate, well-controlled clinical intervention studies using DHASCO and/or ARASCO have been conducted on adults or children with no reported adverse effects of the treatment (summarized in Appendix 3). Two such studies with particular emphasis on safety and bioavailability were conducted by the U.S. Department of Agriculture using high doses of either ARASCO (3 g/day) or DHASCO (15 g/day) with healthy adult volunteers. The preclinical and clinical studies conducted with these oils further support their use as a safe dietary source of DHA and ARA.

The safety of DHASCO and ARASCO as sources of DHA and ARA is further supported by the highly controlled conditions under which they would be used. DHASCO and ARASCO would be added to infant formulas at levels and ratios comparable to that found in breast milk. The proposed fortification levels and ratios are consistent with the WHO/FAO recommendations and with the recommendations of other independent scientific panels. Because infant formulas are manufactured under strict controls, the levels and ratios of DHASCO and ARASCO proposed in this notification will not be exceeded.

The use of DHASCO and ARASCO in commercial infant formulas around the world is yet further evidence of the safety of these oils as sources of DHA and ARA. These oils have now been in commercial use in infant formulas in over 60 countries including the United Kingdom, France and Israel, at levels in accord with WHO/FAO guidelines. These infant formulas have been marketed for as long as three years with no reported adverse findings attributable to the DHASCO and ARASCO. One of these formulas in extensive use around the world is a Low Birth Weight (LBW) formula that is given to infants under strict supervision by doctors. This LBW formula has been introduced in 56 countries and has been provided to an estimated 100,000 LBW babies of most races, cultures and both sexes with no adverse events reported by the physicians. In addition, over 40 million capsules have been sold as dietary supplements to an estimated 250,000 individuals, primarily in the United States, with no significant adverse events reported to the Company. The large numbers of individuals (infants through adults) who have consumed the DHASCO or ARASCO oils as commercial products or in clinical trials with no adverse effects provides additional support for the establishment of GRAS status for these products at use levels that can commonly be obtained in the diet.

Based upon the historical presence of DHA- and ARA-containing triglycerides in human milk, the substantial equivalence of DHASCO and ARASCO triglycerides to those triglycerides found in human milk, and a critical evaluation and analysis of the preclinical and clinical information available on DHASCO and ARASCO, the data establish that these oils are GRAS on the basis of scientific procedures for use in supplementing the diets of infants at levels of 1.25% of dietary fat (0.625% of energy or up to 75 mg DHASCO (or ARASCO) per kg body weight per day). This corresponds to a DHA or ARA level of 0.50% of dietary fat, 0.25% of energy, or up to 30 mg DHA or ARA per kg body weight per day.

1 DHA AND ARA ARE GRAS INGREDIENTS AND DHASCO AND ARASCO ARE GRAS SOURCES OF THESE LCPUFAS

1.1 DHA and ARA are GRAS Because They are Components in Breast Milk

From an evolutionary point of view, breast milk represents the optimal source of nutrition for the human infant and it is often referred to as the "gold standard." Infant formulas are the sole source of nutrition for a human infant and should, therefore, be as nutritionally balanced as human milk. DHA and ARA are found in human milk in low, but significant quantities. The DHA and ARA content of human milks from 65 published reports around the world are given in Table 1. It is clear that the DHA content of human milk is quite variable ranging from 0.06% to 1.4% of total fat and has been shown to be dependent on the dietary DHA intake of the mother (1). Mothers with diets low in fish and other sources of DHA, but otherwise high in fat (*e.g.*, a typical Western diet), have breast milk DHA levels on the low end of the range. Women from the United States, for example, have among the lowest levels of DHA in their breast milk compared to worldwide averages (Figure 1).

To determine the optimal level of DHA in breast milk we must consider the diets to which our species evolved. Such Paleolithic diets were thought to contain much more DHA and much less total fat than the typical Western diet (2). The DHA content of breast milk, therefore, would have been much higher than it is today. Thus, an estimated optimal level of DHA in the breast milk would likewise be much higher than it is in the United States today.

Table 1. Breast milk DHA and ARA levels from women around the world.

Author/year	*Reference*	*wt % DHA*	*wt % ARA*	*Country*
Finley, et al. (1985)	(3)	0.06	0.29	USA
Harris, et al. (1984)	(4)	0.1	0.4	USA
Putnam, et al. (1982)	(5)	0.1	0.6	USA
van der Westhuizen, et al. (1988)	(6)	0.1	1	S. Africa
Sas, et al. (1986)	(7)	0.1	0.5	Hungary
Spear, et al. (1992)	(8)	0.11	0.54	USA
Sanders and Reddy (1992)	(9)	0.14	0.32	UK (vegan)
Maurage, et al. (1998)	(10)	0.14	0.24	France
Auestad, et al. (1997)	(11)	0.15	0.48	USA
Spear, et al. (1992)	(8)	0.15	0.58	USA
Okolska, et al. (1983)	(12)	0.15	1.56	Poland
Dotson, et al. (1992)	(13)	0.16	0.53	USA
Jackson, et al. (1994)	(14)	0.16	0.56	USA
Harzer, et al. (1983)	(15)	0.16	0.39	Germany & UK
Carlson, et al. (1986)	(16)	0.19	0.59	USA
Francois, et al. (1998)	(17)	0.2	0.5	USA
van der Westhuizen, et al. (1988)	(6)	0.2	0.6	S. Africa
Innis, et al. (1994)	(18)	0.2	0.5	Canada
Bitman, et al. (1983)	(19)	0.21	0.58	USA
Henderson, et al. (1998)	(20)	0.21	0.52	USA
Drury and Crawford (1990)	(21)	0.21	0.6	Hungary
Makrides, et al. (1995)	(22)	0.21	0.4	Australia

Author/year	Reference	wt % DHA	wt % ARA	Country
Makrides, et al. (1996)	(1)	0.21	0.41	Australia
Koletzko, et al. (1988)	(23)	0.22	0.36	Germany
Bitman, et al. (1983)	(19)	0.23	0.6	USA
Sanders, et al. (1978)	(24)	0.23	0.72	UK (vegan)
Genzel-Boroviczeny, et al. (1997)	(25)	0.23	0.45	Germany
Bitman, et al. (1983)	(19)	0.24	0.55	USA
Beijers and Schaafsma (1996)	(26)	0.24	0.31	Netherlands
Genzel-Boroviczeny, et al. (1997)	(25)	0.24	0.48	Germany
Martin, et al. (1993)	(27)	0.24	0.36	France
Carnielli, et al. (1998)	(28)	0.26	0.48	Netherlands
Foreman-van Drongelen, et al. (1996)	(29)	0.26	0.52	Netherlands
van Beusekom, et al. (1993)	(30)	0.26	0.47	Netherlands
Muskiet, et al. (1987)	(31)	0.27	0.6	Tanzania
Specker, et al. (1987)	(32)	0.29	0.69	USA
Hall (1979)	(33)	0.29	0.19	UK
Yu, et al. (1998)	(34)	0.29	0.46	Sweden
Sanders and Reddy (1992)	(9)	0.3	0.38	UK
Jansson, et al. (1981)	(35)	0.3	0.4	Sweden
Villacampa, et al. (1982)	(36)	0.3	0.57	Spain
Cherian and Sim (1996)	(37)	0.3	0.4	Canada
Clandinin, et al. (1997)	(38)	0.3	0.54	Canada
Babin et al. (1999)	(39)	0.31	0.5	France
Kaila, et al. (1999)	(40)	0.31	0.35	Finland
Rueda, et al. (1998)	(41)	0.32	0.52	Panama
Billeaud, et al. (1997)	(42)	0.32	0.52	France
Chardigny, et al. (1995)	(43)	0.32	0.5	France
Gibson, et al. (1981)	(44)	0.32	0.4	Australia
FAO/WHO RECOMMENDATIONS FOR FULL TERM INFANTS*	(45)	**0.33**	**0.65**	
de la Presa-Owens, et al. (1998)	(46)	0.34	0.5	Spain
Ogunleye, et al. (1991)	(47)	0.34	0.56	Nigeria
Beijers and Schaafsma (1996)	(26)	0.34	0.37	Netherlands
ISSFAL 1999 RECOMMENDATIONS FOR FULL TERM INFANTS	(48)	**0.35**	**0.50**	
Sanders and Reddy (1992)	(9)	0.37	0.35	UK
Guesnet, et al. (1993)	(49)	0.37	0.45	France
Rueda, et al. (1998)	(41)	0.38	0.69	Spain
Prentice, et al. (1989)	(50)	0.39	0.31	The Gambia
Luukkainen, et al. (1994)	(51)	0.39	0.37	Finland
de Lucchi, et al. (1988)	(52)	0.4	0.8	Spain
Jacobs, et al. (1996)	(53)	0.4	0.6	Netherlands
van Beusekom, et al. (1993)	(30)	0.4	0.5	Dominica
Clandinin, et al. (1981)	(54)	0.4	0.5	Canada
Innis, et al. (1988)	(55)	0.4	0.7	Canada
Muskiet, et al. (1987)	(31)	0.41	0.56	Surinam
Horby Jorgensen, et al. (1996)	(56)	0.43	0.47	Sweden
Muskiet, et al. (1987)	(31)	0.43	0.71	Curacao
Luukkainen, et al. (1995)	(51)	0.48	0.54	Finland
Drury and Crawford (1990)	(21)	0.49	0.57	Thailand
Innis, et al. (1990)	(57)	0.5	0.8	Canada
Ogunleye, et al. (1991)	(47)	0.53	0.36	Japan

Author/year	Reference	wt % DHA	wt % ARA	Country
Fidler, et al. (1998)	(58)	0.55	0.77	Germany
Rocquelin, et al. (1998)	(59)	0.55	0.44	Congo
Boersma, et al. (1991)	(60)	0.56	0.58	St. Lucia
Sanders, et al. (1978)	(24)	0.59	0.54	UK
Kneebone, et al. (1985)	(61)	0.71	0.64	Malaysia
Kneebone, et al. (1985)	(60)	0.9	0.47	Malaysia
Kneebone, et al. (1985)	(60)	0.9	0.57	Malaysia
Koletzko, et al. (1991)	(62)	0.93	0.82	Nigeria
Innis, et al. (1988)	(55)	1.4	0.6	Canada

* Fatty acid recommendations given in mg/kg body weight and were converted to weight % fat assuming that term infants consume 110 kcal/kg body wt/day and that 50% of calories in formula are from fat.

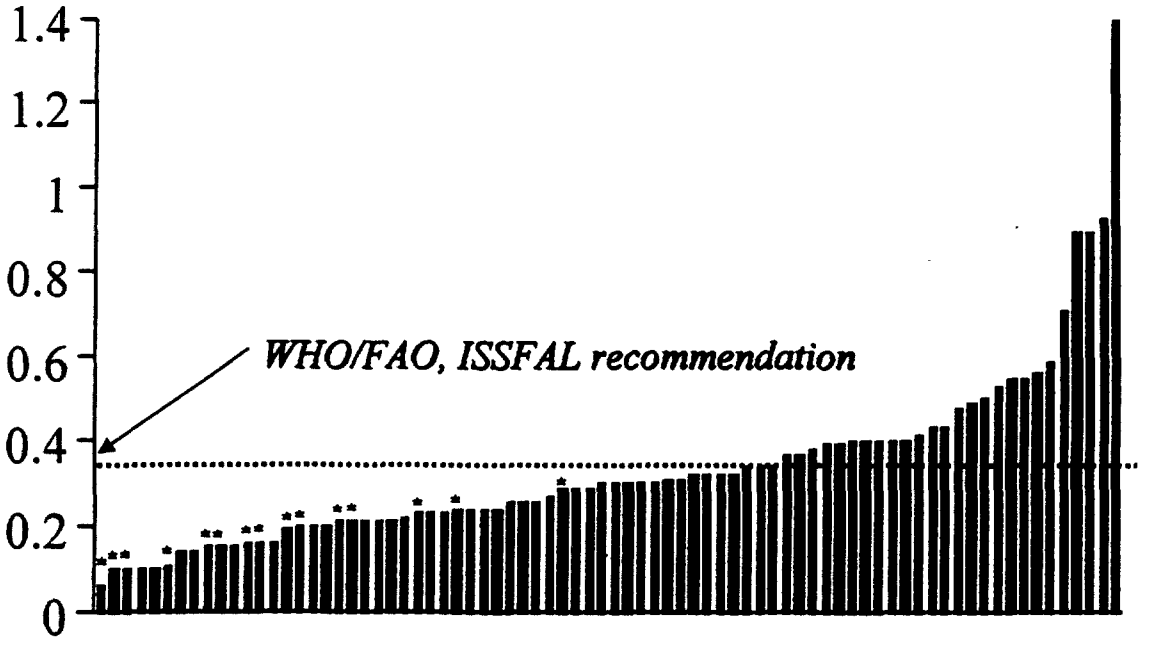

Figure 1. DHA content (% of total milk fat) from the 65 separate references in Table 1 arranged in ascending order. * indicates values from the United States.

1.2 The DHA and ARA in DHASCO and ARASCO are Structurally Similar to the DHA and ARA in Breast Milk.

DHA and ARA are found in both triglycerides and phospholipids in human milk. However, breast milk fat is primarily triglyceride (*ca.* 98%), with only about 1% phospholipid, and 1% nonsaponifiable fats such as cholesterol and phytosterols (63). Although the DHA level in the phospholipid fraction of breast milk is generally higher than in the triglyceride fraction (1.4% *vs.* 0.2% respectively) (63), there is substantially less of the phospholipid fraction in the breast milk. The vast majority of DHA in breast milk, therefore, is in the triglyceride, rather than the phospholipid, form (*i.e.*, it is estimated that 6.6% of the DHA in breastmilk is a phospholipid while 93.3% is triglyceride).

providing infants with nutritive components that most closely match the formula that nature developed for nourishing infants (*i.e.*, breast milk).

3 PROPOSED USES FOR DHASCO AND ARASCO

Several Scientific Panels have addressed the issue of use levels of DHA and ARA in infant formulas (45, 48, 125, 126). The levels recommended by those Panels are given in Table 3 for pre-term and term infant formulas and range from 0.4% to 1.5% of total formula fat for ARA, and 0.35% to 1.1% of total formula fat for DHA. These values closely approximate levels of DHA and ARA found in human milks from around the world.

Perhaps most relevant, are the recommendations coming from the April 1999 expert panel assembled by NIH, The Center for Genetics, Nutrition and Health, and ISSFAL (and co-sponsored by several industry groups). This group of world-wide experts in lipid nutrition reviewed the latest data, including many studies not reviewed or discussed by the LSRO Panel. This Expert Panel concluded that an infant formula would need to contain at least 0.35% of lipid as DHA and 0.50% of lipid as ARA for an infant to receive an adequate intake (AI) of these components (109). The experts also identified the potential problems with EPA and recommended that there should be an upper limit (not to exceed) for EPA of not more than one quarter the level of DHA.

Table 3. Recommendations of five expert panels for the inclusion of ARA and DHA in infant formulas.

	ESPGAN	*BNF*	*ISSFAL*	*FAO/WHO*	*ISSFAL*
Year	1991	1992	1994	1994	1999
Preterm Formula					
*ARA (% of fat)**	1%	0.40 %	0.90 -1.5 %	0.90 %	0.50 %
DHA (% of fat)	0.5%	0.40 %	0.5-1.1 %	0.60 %	0.35 %
Term Formula					
ARA (% of fat)		0.40 %		0.70 %	0.50 %
DHA (% of fat)		0.40 %		0.35 %	0.35 %
DHA/EPA ratio			> 5 to 1	10 to 1	> 4 to 1

*Assumptions for the calculated values are 1) preterm infants consume 120 kcal/kg/d and term infants consume 110 kcal/kg/d, 2) fat comprises 50% of energy of the formula, and 3) 1 g fat has 9 kcal.

In the last 15 years, at least 32 well-controlled clinical trials have been completed using DHA/ARA supplemented formulas (see Table 2). All trials have used supplemental DHA at levels in preterm formulas from 0.15% to 0.78%, and for term formulas from 0.1% to 0.36% of total formula fat. Supplemental ARA has been used in most, but not all trials. In trials where supplemental ARA has been used, the concentration ranged from 0.10% to 1.1% of total fat in preterm formulas, and from 0.20% to 0.72% for term formulas. Of particular relevance to this submission are the 14 clinical studies in which DHASCO and ARASCO were used as the DHA and ARA sources for supplementation (see Appendix 2). In full term infant formula trials, dose levels ranged from 0.20 % to 0.36 % of total formula fat for DHA, and from 0.20 % to 0.72 % of total formula fat for ARA. These values clearly fall well within the normal range of mother's milk (Figure 1) and the Expert recommendations (Table 3).

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Some of these sterols, such as desmosterol, are already known to be in an infant's diet via its mother's milk (63). 4-Methyl sterols are normal intermediates in cholesterol biosynthesis, and humans have the enzymatic capability to convert 4-methyl sterols into 4-desmethyl sterols like cholesterol (135, 136). Microalgae such as *C. cohnii* are commonly found in the food web of filter feeders like mollusks (*e.g.*, oysters and clams), crustaceans (*e.g.*, shrimp and lobster), and certain fish, and therefore, these sterols can also be found in such animals. As a result, these sterols have been in the human food chain, both directly and indirectly, ever since man was eating marine foods.

Because there have been no literature reports on the effects of consumption of such 4-methyl sterols directly, a feeding study was undertaken using a high concentration of the nonsaponifiable fraction of *C. cohnii* in rats. This study compared the effects of consuming a high concentration of the nonsaponifiable fraction from DHASCO with the nonsaponifiable fraction of soybean oil, which contains predominantly sitosterol (191). Although sitosterol is known to inhibit the re-uptake of cholesterol from the gut, the nonsaponifiable fraction of DHASCO did not show a similar effect. Furthermore, when a very large portion (0.5% by weight) of the diet of rats was comprised of this nonsaponifiable fraction, there was no adverse effect on growth, general physiology, or the gross organ morphology in the recipient animals. The study concluded that there were "*no toxicological effects on the rats of consuming up to 0.5% of their diet in the DHASCO nonsaponifiables*". This dose would be equivalent to giving the rat a diet containing about 50% by weight pure DHASCO.

7.5 Toxicological Studies (Non-Human)

A large number of safety tests have been conducted on ARASCO and DHASCO oils by several different organizations. As a result, there is a large degree of redundancy in the standard toxicological assessments. This redundancy allows for an unprecedented and extremely valuable assessment of these oils. The studies include *in vitro* mutagenicity and genotoxicity assays, and a variety of controlled animal studies, such as acute, subchronic, developmental and multigenerational reproductive studies. Because these components represent macronutrients, all studies included appropriate positive as well as negative controls. All studies reported herein were conducted in Good Laboratory Practice (GLP)-compliant laboratories following guidelines outlined by the FDA in *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Foods*, commonly referred to as Redbook I, or from their draft Redbook II. The audited summaries from each toxicology study appear in Appendix 5. Only the significant findings will be presented here. These data have also been reported in a number of publications and summarized recently by Kyle and Arterburn (192).

At the outset, it is important to recognize that DHASCO and ARASCO are macronutrients, not micronutrients, vitamins, or drugs. Safety testing of macronutrients poses several unique problems (193). For example, it is often difficult to distinguish whether an observed response is related to a toxicological effect of the test material, or due to a dietary deficiency of some other component caused by the use of such large amounts of the test macronutrient in the diet. Clearly, one cannot achieve safety margins of greater than 50-fold for a macronutrient that comprises more than 2% of the diet. Finally, when a macronutrient test material is added to the diet at a very high dose, one must carefully distinguish a truly toxicological response due to the test material from a normal physiological response resulting from the high dietary load of that particular

macronutrient. For example, serum cholesterol is elevated in a human who changes their diet from one with 25% of dietary calories as fat to one with 45% of dietary calories as fat. Is this a toxicological response to the particular fat used, or a normal physiological response to increasing total dietary fat? With this caveat, the toxicological studies completed on DHASCO and ARASCO are summarized below.

7.5.1 Acute Toxicity Studies

Five independent studies have shown that very high acute oral doses (up to 20 grams of DHASCO or ARASCO/kg body wt) did not have any major toxicological consequences in rats (Table 9). Soft stools were typically noted in the first day, but all animals gained weight during the two-week post-dosing period. Soft stools are a normal and expected consequence of a large, single dose of any fatty substance, and it was not considered to be an adverse event. Because no animals died, acute oral LD50 values could not be determined, but based on the lack of toxicity in the tests, they were greater than 20 g/kg body wt/day for each oil. In addition to the DHASCO/ARASCO studies, six acute oral dose studies using either whole biomass, delipidated biomass (residual material after removal of the oils), or a microencapsulated oil powder at doses from 4-10 g/kg also failed to show any significant toxicological finding (Table 9).

Table 9. Acute toxicity studies with DHASCO and ARASCO and various biomass forms in Rats.

Test Material	*Sponsor/ Study Date*	*Dose (g/kg bw)*	*Mortality (male female)*		*LD50*	*Reference*
DHASCO	Martek	20	0/5	0/5	>20 g/kg bw	(173)
DHASCO	Martek	20	0/5	0/5	>20 g/kg bw	(194)
C. cohnii algal biomass	Martek	7	0/5	0/5	>7 g/kg bw	(195)
C. cohnii biomass	Martek	7	0/5	0/5	>7 g/kg bw	(196)
Delipidated C. cohnii biomass	Martek	10	0/5	0/5	>10 g/kg bw	(197)
Delipidated algal biomass	Martek	6	0/5	0/5	>6 g/kg bw	(198)
ARASCO	Martek	20	0/5	0/5	>20 g/kg bw	(173)
ARASCO	Martek	20	0/5	0/5	>20 g/kg bw	(199)
ARASCO	Gist-brocades	18.2	0/5	0/5	>18.2 g/kg bw	(200)
Fungal biomass	Martek	5	0/5	0/5	>5 g/kg bw	(201)
Delipidated fungal biomass	Martek	4	0/5	0/5	>4 g/kg bw	(202)
Microencapsulated ARASCO/DHASCO blend	Martek	5	0/5	0/5	>5 g/kg bw	(203)

7.5.2 Sub-Chronic (28-63 Day) Toxicity Studies

In six independent studies, DHASCO and/or ARASCO was administered by gavage, or as a dietary admixture, daily for 28 or 63 days using doses ranging from 0.025 to 9.4 grams of test material /kg body wt/day (Table 10). The No Adverse Effect Levels (NOAEL) were determined to be the highest doses tested for each individual oil during the specific study (up to 2.5 g oil/kg body wt/day for ARASCO and 1.25 g/kg body wt/day for DHASCO) and for the combined oils (up to 9 g/kg body wt/day). At the highest dose levels there were some recurrent findings (see section 7.5.9), but these were not considered to be adverse effects of the test material because they

did not occur consistently and were not accompanied by changes in histology or clinical chemistry. The differences in organ weights were considered to be normal physiological responses to high doses of polyunsaturated fatty acids and, in some cases, due to the use of artificial diets. An additional subchronic 28-day study was undertaken with the whole *C. cohnii* biomass at a dose level of 5 g/kg body wt/d and, once again, there were no test material related toxicological findings. For the biomass, the NOAEL was therefore found to be 5 g biomass/kg body wt/d in rats.

Table 10. Sub-chronic (28 to 63 day) oral safety studies with DHASCO and ARASCO in Rats.

Test Material	*Sponsor*	*Highest Dose (g/kg bw/day)*	*Details*	*Conclusions*	*Reference*
ARASCO/DHASCO 1.5:1 blend	Wyeth Ayerst	1.2 .48/.72	63 days in the diet; 10 rats/sex/dose	Not toxic	(204)
DHASCO	Martek	1.25	28 days by gavage; 5-10 rats/sex/dose	No toxic	(173)
ARASCO	Martek	2.5	28 days by gavage; 5-10 rats/sex/dose	Not toxic	(173)
ARASCO	Gist-brocades	3.0	28 days by gavage; 10 rats/sex/dose	Not toxic	(200)
ARASCO/DHASCO 2:1 blend	Martek	3.75 1.25/2.5	28 days by gavage; 5-10 rats/sex/dose	Not toxic	(173)
ARASCO/DHASCO 2:1 blend	Mead Johnson	9.1/9.4 3.3/6.1-6.3	28 days in the diet; 10 rats/sex/dose	Not toxic	(205)
Algal biomass	Martek	5	28 days in diet, 5-10 rats/sex/dose	Not toxic	(206)

7.5.3 Sub-Chronic (90-Day) Studies

In six independent subchronic 90-day studies, DHASCO and/or ARASCO were administered by gavage or as a dietary admixture at doses up to 8.9 grams of test material/kg body wt/day to 20 rats per sex per dosing group. The NOAELs as determined following a critical evaluation of the data by the study site toxicologists, were the highest doses tested for each material in all studies (up to 2.5 grams of ARASCO and 1.25 grams DHASCO/kg body wt/day and up to 8.9 g/kg body wt/day for combined oils) except for the Gist-brocades study (see Table 11) (174, 188, 207). A NOAEL of 8.9 gm/kg body wt/day (about 6 grams ARASCO and 3 grams of DHASCO) represents at least a 80-fold excess of this macronutrient from the maximum intended use level of ARASCO, and a 40-fold excess from the maximum intended use level of DHASCO as recognized by this GRAS Notification (*i.e.*, 75 mg/kg body wt/day). At the highest dose levels, there were some recurrent findings (see section 7.5.9) that were not considered to be adverse because they were not consistent or accompanied by changes in histology or clinical chemistry. These "findings" were considered normal physiological responses to high doses of polyunsaturated fatty acids and/or to be due to the use of artificial diets in the feeding studies. Some of these studies also involved detailed assessments of neurotoxicity (188, 208) as outlined by the FDA Redbook II Guidelines, and other studies also involved an *in utero* supplementation phase (207). One additional 90-sub-chronic study with a full neurotoxicological assessment was also completed on the intact *C. cohnii* biomass. As in the other studies, there were no test material related

toxicological findings at the highest dose used and the NOAEL for was determined to be 5.8 g biomass/kg body wt/day (Table 11)

Table 11. Sub-chronic 90-day toxicity studies with DHASCO and ARASCO in Rats.

Test Material	*Sponsor*	*Highest Dose (g/kg bw/d)*	*Details*	*Findings*	*Reference*
DHASCO	Martek	1.25	gavage, full neurotox assessment	Not toxic	(188)
DHASCO/ARASCO blend	Numico	2.0	in diet	Not toxic	(209)
ARASCO	Martek	2.5	gavage, full neurotox assessment	Not toxic	(174)
DHASCO/ARASCO 1.5:1 blend	Wyeth Ayerst	2.5 .48/.72	in diet	Not toxic	(204)
ARASCO	Gist-brocades	4.9	in diet, *in utero* phase, full neurotox assessment	Not toxic	(210)
DHASCO/ARASCO 2:1 blend	Mead Johnson	8.9 3/5.9	in diet, *in utero* phase, full neurotox assessment	Not toxic	(207)
Algal biomass	Martek	5.8	in diet, full neurotox assessment	Not toxic	(211)

7.5.4 Chronic Toxicity/Carcinogenicity Studies

Based on *in vitro* toxicological information (see Section 7.5.6) and knowledge of the macronutrient nature of the test material, it was determined that chronic toxicity/carcinogenicity studies were not necessary for this nutrient.

7.5.5 Reproductive/Developmental Toxicology Studies

Two developmental toxicology studies were conducted with DHASCO and ARASCO administered by gavage at dose levels of 1.25 and 2.5 g/kg body wt/day respectively (Table 12). There were no adverse developmental effects at the doses tested as determined by the independent study site toxicologists setting the NOAEL at those highest doses. Developmental and reproductive (multigenerational) studies were also completed using the biomass at dose levels of 4.3 and 8.0 g/kg body wt/day (5% by weight of diet) (Table 12). In both cases there were no test material related toxicologically significant findings at the highest doses used and the NOAELs were determined to be 4.3 and 8.0 g biomass/kg body wt/day respectively.

Table 12. Developmental and reproductive toxicity studies with DHASCO and ARASCO in Rats.

Test Material	*Study Type*	*Sponsor*	*Highest Dose (g/kg bw/d)*	*Details*	*Findings*	*Reference*
DHASCO	Developmental	Martek	1.25	gavage 25/sex/dose	Not toxic	(212)
ARASCO	Developmental	Martek	2.5	gavage 25/sex/dose	Not toxic	(212)
Algal biomass	Developmental	Martek	4.3	in diet 20/sex/dose	Not toxic	(213)
Algal biomass	Reproductive	Martek	8	in diet 20/sex/dose	Not toxic	(214)

8 PRECLINICAL STUDIES.

There have been a number of reports involving the supplementation of the diets of various mammalian species including mice, rats, pigs, cats, dogs, monkeys and baboons with DHASCO and/or ARASCO. We are also aware of ongoing or unpublished studies with hamsters, cows, horses, and chickens. Some of these studies are tabulated and summarized in Appendix 1. Dosages used ranged from levels normally expected to be used as a dietary supplement, to doses wherein the total fat intake of the animal was comprised solely of DHASCO or ARASCO. None of these studies were designed as toxicology studies such as those described in Section 7, but rather, each of the investigators studied the effects of enriching tissue levels of DHA and/or ARA on a particular physiological response in a selected animal model. DHASCO and/or ARASCO are now commonly used as the dietary source of DHA and/or ARA by researchers because they are the most concentrated and purest sources available for use in such studies. These studies, in total, represent a very large experience base of dietary treatment of many different mammalian species with DHASCO and ARASCO, and there have been no suggestions from these reports of any toxicological or safety issues with these oils.

9 CLINICAL STUDIES

9.1 INFANT STUDIES

ARASCO and/or DHASCO oils have been the subject of at least 14 well-controlled clinical intervention trials involving approximately 1,500 term or preterm infants, with over 700 of the infants receiving DHASCO and/or ARASCO oils (29, 38, 69, 77, 78, 89, 90, 244). These trials are summarized in Appendix 2. All of the trials analyzed blood lipids (either erythrocyte or plasma phospholipids) and growth as primary endpoints. In all cases, the supplementation of DHASCO and ARASCO resulted in an improvement of the circulating DHA and ARA status of the infant equivalent to that of a breast-fed infant and no study had any adverse effect on infant growth.

One trial (38) was of particular interest in this respect because it involved the comparison of various doses of DHASCO and ARASCO to optimize the blood lipids of the infants. Other functional endpoints were also studied in a few cases. In contrast to preliminary reports of a reduced growth rate when using a fish oil (*i.e.*, EPA-containing) formula (100, 245), none of these studies reported reduced growth. In fact, two studies using DHASCO/ARASCO supplemented formulas report significantly increased growth in the supplemented formula-fed infants (90, 112). As discussed in section 2.7, reduced growth rate in the fish oil trials may have been due to EPA, which is always present in fish oils, and/or the lack of ARA in the formulas.

Significant improvements in visual and mental acuity have also been reported when infants were fed DHASCO/ARASCO supplemented formulas. Visual acuity improvements were reported to be equivalent to one line in an eye chart at one year of age (78). Mental acuity improvements in the same study were seven IQ points at 18 months of age, as determined by a Bayley MDI assessment (106). In one preterm study (89) where visual acuity was assessed but no significant improvements were observed, the authors explain that the lack of a statistically significant response was likely due to the short duration of the supplementation (*ca.* only 4 weeks during the

APPENDIX 2

CLINICAL STUDIES WITH INFANTS USING DHASCO AND/OR ARASCO 6 (14 trials/1,478 babies)

Author/Principal Investigator: Location	Ref	Dose	Duration	Subjects (n)	Outcome
Pre-Term Infants					
Carnielli, et al. (1994) (abs.), Carnielli, et al. (1996) (abs.); Europe (Numico)	1 2	1) CF, 2) SF with 0.75 ARA from ARASCO + 0.6% DHA from DHASCO, 3) human milk (HM)	(not given)	n=60	Plasma PL of SF group similar to HM group; no SD between groups in growth; visual acuity and neurodevelopment outcomes pending.
Carnielli, et al. (1998); Sophia Children's Hospital, Rotterdam, The Netherlands (Numico)	3	1) CF, 2) SF with 0.24% DHA + 0.35 ARA from PL, 3) SF with 0.84% ARA from ARASCO + 0.64% DHA from DHASCO, 4) HM	up to 5 weeks of age	n=46	triglyceride form of DHA and ARA (ARASCO+DHASCO) absorbed as efficiently as DHA and ARA in HM.
Clandinin et al. (1997); Children's Health Center, Edmonton, Alberta, Canada (Wyeth)	4	1) CF, 2) Low SF with 0.32% ARA from ARASCO + 0.24% DHA from DHASCO, 3) Medium SF with 0.49% ARA from ARASCO + 0.35% DHA from DHASCO, 4) High SF with 1.1% ARA from ARASCO + 0.76 DHA from DHASCO, 5) HM	4-6 weeks	n=91	Dose ranging trial established that plasma PL of Low SF and Medium SF groups similar to HM group, while plasma PL of High SF group higher than HM group; no difference in growth or clinical parameters between formula groups.
Damli, et al. (1996) (abs.); Europe (Humana)	5	1) CF, 2) SF with 0.3% ARA from ARASCO + 0.2% DHA from DHASCO, 3) HM	3 months	n=57	No difference in visual acuity; significantly higher Bayley Mental Development in SF group.
Foreman-van Drongelen et al. (1996); The Netherlands (Numico)	6	1) CF, 2) SF with 0.61% ARA from ARASCO + 0.3% DHA from DHASCO, 3) HM	approx. 22 weeks	n=43	Plasma PL and RBC of SF group higher than CF group; no difference between groups in growth or clinical events.
Gross et al. (1997) (abs.), Vanderhoof et al. (1997) (abs.); Vanderhoof et al (1999) Multi-center trial-USA & Canada (Wyeth)	7 8 9	1) CF, 2) SF with 0.6% ARA from ARASCO + 0.4% DHA from DHASCO, 3) HM	from full GI feeds to 40 weeks post conceptual age	n=191	No difference in growth, serum chemistries or GI symptoms between formula groups; Plasma PL of SF group similar to HM group.

6 Abbreviations used in this table: CF, control formula; HM, human milk; MDI, mental development index; PL – phospholipid; RBC, red blood cell; SF, supplemented formula; VEP –visual evoked potential.

Author/Principal Investigator; Location	*Ref*	*Dose*	*Duration*	*Subjects (n)*	*Outcome*
Hansen, et al. (1997) (abs.), Diersen-Schade, et al. (1998); Multi-center trial-North America (Mead Johnson)	10 11	1) CF, 2) DHA SF with 0.34% DHA from DHASCO, 3) DHA/ARA SF with 0.6% ARA from ARASCO + 0.33% DHA from DHASCO, 4) HM	approx. 28 d	n=284	ARA and/or DHA SF is safe as measured by growth and absence of adverse events; growth in the DHA/ARA SF group was better than the CF group; no difference in visual acuity between groups.
Koletzko (ongoing); Multi-center trial-Europe (Humana)		1) CF, 2) SF with fish oil + borage oil, 3) SF with fish oil + egg phospholipid, 4) SF with DHASCO/ ARASCO 1:1, 5) SF with DHASCO/ ARASCO 1:2, 6) HM	28 d	n=75	Ongoing. Evaluating endogenous synthesis of LCPUFAs and cholesterol and physiological responses to different LCPUFA doses and sources.
Monsolis et al, 1997 (abs.); Spain (Novartis)	12	1) control formula (CF); 2) supplemented formula (SF) with ARASCO:DHASCO 2:1	8 weeks	n=45	No differences between groups in growth or tolerance of formula, plasma RBC of SF group maintained while CF group declined.
Full Term Infants					
Birch, et al. (1998) Retina Foundation of the Southwest, Dallas, TX (Mead Johnson)	13	1) CF; 2) SF with 0.35% DHA; 3) SF with 0.35% DHA + 0.72% ARA; 4) HM	4 months	n=108	All groups had similar growth rates and tolerated all diets well. DHA or DHA + ARA SF groups had better sweep VEP acuity at 6, 17, and 52 weeks of age. DHA SF groups had better visual acuity, equivalent to "one line on an eye chart". Blood lipids and visual acuity of SF groups similar to those of HM group. Blood lipids and visual acuity of CF group significantly lower than all groups.. Recent analysis (unpublished) indicates a 6 IQ point advantage in DHA/ARA SF group by Bayley MDI at 18 months of age.
Carlson, et al. (1999; abstract) Multi-center trial-USA & Canada (Mead Johnson)	14	1) CF, 2) SF with 0.3% DHA from fish oil + 0.6% ARA from ARASCO; 3) SF with 0.3% DHA from DHASCO + 0.6% ARA from ARASCO	(not stated)	n=322	ARA and DHA SF had no adverse effects on growth or development. Infants fed DHA/ARA SF gained weight more rapidly and weighed more than the CF group through 12 months of age. Bayley Scales of Mental and Psychomotor Development scores were highest in the DHASCO/ARASCO SF group at 12 months, but did not reach statistical significance. No differences in visual acuity.

Author/Principal Investigator; Location	Ref	Dose	Duration	Subjects (n)	Outcome
Gibson, et al. (1997a; abstract) Flinders Medical Center, Australia (Wyeth Nutritionals)	15	1) CF; 2) SF1 (0.2% ARA/0.2% DHA); 3) SF2 (0.32% ARA/0.2% DHA); 4) SF3 (0.4% ARA/0.25% DHA); 5) HM	6 weeks	n=113	The formulas containing ARASCO and DHASCO (SF2 & 3) resulted in plasma ARA and DHA levels similar to those in the HM group, while supporting normal growth during the first 6 weeks of life.
Gibson, et al. (1997b; abstract) Flinders Medical Center, Australia	16	1) CF; 2) SF with 0.3% DHA; 3) SF with 0.3% DHA + 0.3% ARA	16 weeks	n=67	RBC PL DHA levels in the SF groups exceeded the published range in breast-fed infants. When ARA was absent from the formula, RBC ARA levels were below values seen in CF infants. The VEP acuity scores were not significantly different between dietary groups, and all were within the normal expected values. There were no growth differences between any of the groups
Weizman (unpublished) Saroka Medical Center, Israel (Maabarot Products)		1) SF (0.45% DHA+ 0.45% ARA of 3.6% fat); 2) CF (Materna Plus Meshuperet)	3 months	n=20	A presentation at the Annual Meeting of the Israeli Pediatric Society concluded that locally produced SF is safe and clinically efficient. No differences were observed in developmental assessments at 3 months. No effects on growth.

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Appendix 2 - References for Term and Preterm Infant Studies with DHASCO and/or ARASCO

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Appendix C

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Martek®
Martek Biosciences Corporation

July 11, 2001

Mr. Thomas Ferguson
Director, Regulatory Affairs,
The Americas
Mead Johnson Nutritionals
2400 West Lloyd Expressway
Evansville, IN 47721-0001

RE: Use of DHASCO® and ARASCO® in Infant Formulas at a Level of 2.5 Percent Each of Fat

Dear Mr. Ferguson:

This letter is being sent to you in conjunction with Mead Johnson's submission of a GRAS notification to the Food and Drug Administration (the "Agency") relating to Mead Johnson's infant formula product. Martek understands and agrees that this letter will be included as a part of such submission.

In the Agency's letter to Martek of May 17, 2001, the Agency responded to Martek's GRAS notification dated February 18, 2000 (GRN 000041) by stating that the Agency has no questions at this time regarding Martek's conclusion that ARASCO® and DHASCO® are GRAS sources of ARA and DHA under the intended conditions of use. That notification identified the maximum use level for DHASCO and ARASCO at 1.25 percent each of fat with a DHASCO:ARASCO ratio between 1:1 and 1:2. For the reasons discussed below, Martek believes that, in fact, a blend of DHASCO and ARASCO, when used at levels up to 2.5 percent each of fat with DHASCO:ARASCO ratios between 1:1 and 1:2, is GRAS. Martek therefore fully supports Mead Johnson's proposed use of a higher level of ARASCO in an infant formula product than was provided for in the GRAS notification considered by the Agency, so long as the level of either oil does not exceed 2.5 percent of fat and the DHASCO:ARASCO ratio is within the 1:1 to 1:2 range.

The GRAS notification submitted by Martek for its DHASCO and ARASCO summarized many published studies supporting the safety of these oils. The GRAS notification also referenced a report prepared by an Expert Panel convened by Martek that concluded DHASCO and ARASCO are safe when used at levels up to 2.5% each of fat in infant formulas (see attached Executive Summary of Martek GRAS Opinion, first paragraph). Martek agrees with and endorses the finding of the GRAS Expert Panel regarding the safe levels of ARASCO and DHASCO in infant formula. The Mead Johnson proposed use levels of both DHASCO and ARASCO are within the levels and ratios found to be GRAS by the Martek Expert Panel.

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