Mead Johnson proposes using DHASCO and ARASCO at approximately 0.8 and 1.6 percent, respectively, of total fat in their infant formula. With overages to assure label target levels are met, they may actually include up to about 0.9% DHASCO and 1.9% ARASCO. 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. The data contained in the Mead Johnson food master file (FMF 639) at the Agency further support the use of level and ratios of DHASCO and ARASCO in Mead Johnson's formulas.

The use of the proposed ARASCO and DHASCO levels in the Mead Johnson formula is supported by the following data and information, which are summarized briefly below:

- The Martek Expert Panel found DHASCO and ARASCO to be safe at levels up to 2.5% each of fat in infant formula.
- Mead Johnson's proposed levels of both DHA and ARA delivered by the DHASCO and ARASCO oils are well within the range found in human breast milk (Table 1).
- The DHA and ARA levels proposed for Mead Johnson's commercial infant formula are similar to the levels used in Mead Johnson's clinical safety studies which posed no safety concerns (Table 2).
- This proposed level of ARASCO oil is similar to amounts studied in other infant clinical trials without adverse effects (Table 3).
- The ARA to DHA ratio in the formula is within the 1:1 to 2:1 range specified in Martek's GRAS Notification.
- Martek- and Mead Johnson-sponsored toxicology studies used 2:1 ratios of ARA to DHA, thus providing the same safety factor for ARASCO as DHASCO at the proposed use levels (Table 2).
- A number of organizations have recommended levels above those in the Mead Johnson formula, ranging up to 1.5% of fat as ARA in infant formula (Table 4).

For the reasons stated above, Martek concurs with Mead Johnson that it is safe to use DHASCO and ARASCO in commercial infant formulas at levels that provide 0.8-0.9% of fat from DHASCO and 1.6-1.8% of fat from ARASCO. Indeed, Martek considers ARASCO and DHASCO to be GRAS when used in infant formulas at levels up to 2.5 % each of fat with a DHASCO:ARASCO ratio between 1:1 and 1:2.

Sincerely,

Henry Linsert, Jr.
Chief Executive Officer

EXECUTIVE SUMMARY

The undersigned, an independent panel of recognized experts (hereinafter the Panel), qualified by scientific and/or medical training and relevant international experience to evaluate the safety of food and food ingredients, was requested by Martek Biosciences Corporation to determine whether DHASCO® and ARASCO® oils (macronutrient oils produced by fermentation and containing docosahexaenoic acid (DHA) or arachidonic acid (ARA)), using scientific procedures, are Generally Recognized as Safe (GRAS) for consumption by infants (birth to 1 year) and children (1-12 years) at dose levels up to 2.5% each of total dietary fat. For infants, this dose is equivalent to 1.25% of dietary calories or about 150 mg of the oil/kg body wt/day. A comprehensive search of the scientific literature (published and unpublished) for information on the use and safety of ARASCO and DHASCO through October 15, 1999 was conducted by Martek and was made available to the Panel. The Panel members independently and critically evaluated the materials submitted by Martek Biosciences Corporation and other materials deemed appropriate and conferred by telephone several times before preparation of this report.

Earlier safety concerns regarding growth of infants fed fish oil-supplemented formulas have been addressed. They appear to be associated with the eicosapentaenoic acid (EPA) present in the fish oils. EPA leads to a depression of ARA and associated eicosanoid levels. In contrast, growth inhibition has never been observed when DHASCO, which does not contain EPA, was used as a dietary DHA source for infants. The growth delay seen in some fish oil-based formula studies can be avoided by adding an ARA source along with the DHA to infant formulas. Recent controlled studies indicate that preterm and term infants fed formulas fortified with both ARASCO and DHASCO oils have better growth indices than infants fed unsupplemented formula. Other potential safety concerns associated with fish oil, such as increased bleeding times, have never been linked to the use of DHASCO or ARASCO oils.

The Panel first considered the importance of DHA and ARA for infants and children. After reviewing the literature regarding the efficacy of DHA and ARA (from any source) in infant nutrition, the Panel unanimously agrees that: 1) 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. The link between these two long-chain polyunsaturated fatty acids and developmental outcome has been established by well-controlled clinical studies indicating that addition of DHA and ARA to the formula not only corrects the deficiency, but can also contribute to the elimination of these neurological deficits.

This Panel also considered the opinion of other Expert Panels, associated with prestigious scientific and medical organizations, that convened over the last ten years to consider the question of whether to add DHA and ARA to infant formulas. The general consensus among the different panels was that both of these fatty acids should be added to formulas at levels corresponding to those found in human breast milk. One panel from the Life Science Research Organization (LSRO) of the American Society for Nutritional Sciences, that reviewed the addition of long-chain fatty acids as part of an overall review

of the requirements for term (not preterm) infants formulas did not recommend that DHA and ARA be a required addition to term formulas at this time. However, they did recognize the importance of maintaining the DHA status of infants and suggested that supraphysiological levels of linolenic acid (a precursor to DHA) should be added to help improve the DHA status of the formula-fed infant. This LSRO Panel did not review several of the most recent studies, and recommended a re-evaluation of this issue in the near future as new clinical study data emerge. Another LSRO panel is presently considering the DHA and ARA requirements for preterm infants.

The chemical composition and manufacturing processes for both DHASCO and ARASCO oils were carefully reviewed by the Panel to ensure that oil quality meets standards for food production. DHASCO oil is a triglyceride, produced by the alga *Crypthecodinium cohnii*, which contains 40% by weight of DHA (specifications given in section 6). ARASCO oil, also predominantly triglyceride, is produced by the fungus *Mortierella alpina* and contains 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. Both oils also contain other common saturated and monounsaturated fatty acids. Minor nonsaponifiable fractions of the oils have been characterized and contain primarily cholesterol-related sterols commonly found in other food sources. 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. All oils undergo rigorous analytical and quality assurance testing and meet well-defined product specifications prior to release.

Based on elevation of blood lipid levels following ingestion of either DHASCO or ARASCO, both oils are absorbed in a manner consistent with other dietary triglycerides. 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 eicosanoids, and the omega-6 series of eicosanoids are well recognized as stimulators of immune function. Studies have shown that small amounts of DHA can be retroconverted to 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 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 not inconsistent 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 assays. All studies were modeled after FDA

Redbook guidelines and conducted at GLP-compliant laboratories. The study results were 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 themselves. None of the studies indicated that the oils were toxic, and the No Adverse Effect Levels (NOAELs) were determined to correspond to the highest doses tested. Modestly increased liver weights were noted, but this was not a consistant finding, nor was it accompanied by abnormal histology or serum enzyme levels. Furthermore, the liver weight changes were no longer apparent when assessed relative to other organ weights (*e.g.* brain). These changes were, therefore, deemed not to be toxicological in nature as this is a well-known effect of the Long Chain PUFAs in mammals when administered at high dose levels, well above those intended for infants or children. All other concerns raised by previous reviews have been addressed in this document (Appendix 4). In addition, the crude biomasses containing the DHASCO or ARASCO have also been extensively tested and found to be nontoxigenic.

Studies conducted in twelve different animal species, including nonhuman primates, have provided a large base of experience and extensive toxicological 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 over 1500 infants 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 and mental acuity have also been reported in infant groups supplemented with these oils and no adverse events have been reported. 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 adult volunteers. The preclinical and clinical studies conducted with these oils further support their use as a safe dietary source of DHA and ARA.

In addition, these oils have now been in commercial use in infant formulas in over 60 countries around the world (including the United Kingdom, France and Israel) at levels in accord with WHO/FAO guidelines for as long as three years with no reported adverse findings during that time. 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.

Human breast milk is the "Gold Standard" for infant nutrition. In addition to other important nutrients, human breast-fed infants receive DHA- and ARA-containing triglycerides from their mother's milk. Based on this fact, the growing body of evidence demonstrating the importance of supplying infants with DHA and ARA, and after a critical evaluation and analysis of the safety and clinical information available on DHASCO and ARASCO as DHA- and ARA-containing triglycerides, the undersigned Expert Panel has determined that these oils, derived from the referenced algal and fungal sources, meeting food grade specifications and produced according to current Good Manufacturing Practices (cGMP; 21 CFR 182.1) to be Generally Recognized as Safe (GRAS) for use in supplementing the diets of infants and children up to 15 years of age 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).

[redacted]

08 Nov 1999
Date

Joseph Borzelleca, Ph.D
Professor, Pharmacology and Toxicology
Medical College of Virginia, Richmond, VA

[redacted]

12/10/99
Date

Gary Flamm, Ph.D.
Flamm and Associates
Vero Beach, FL
(former Director of Toxicology, CFSAN, FDA)

[redacted]

Nov 12 1999
Date

Bruce German, Ph.D.
Professor, Nutrition and Food Science
University of California, Davis, CA

[redacted]

Nov 5 1999
Date

Walter Glinsmann, M.D.
Adjunct Professor, Center for Food and Nutrition Policy
Georgetown University, Washington, DC
(former Director of Clinical Nutrition, CFSAN, FDA)

[redacted]

15 December 99
Date

David Kritchevsky, Ph.D.
Professor, Wistar Institute
Philadelphia, PA

[redacted]

Dec 3 1999
Date

David Bechtel, Ph.D., DABT
Senior Toxicologist
CanTox US Inc, Bridgewater, NJ

Table 1: International Breast Milk DHA Level

wt % ARA	wt % DHA	Country	Ref.
0.19	0.29	UK	[1]
0.24	0.14	France	[2]
0.29	0.06	USA	[3]
0.31	0.24	Netherlands	[4]
0.31	0.39	The Gambia	[5]
0.32	0.14	UK	[6]
0.35	0.15	Enshi, China	[7]
0.35	0.31	Finland	[8]
0.35	0.37	UK	[6]
0.36	0.22	Germany	[9]
0.36	0.24	France	[10]
0.36	0.53	Japan	[11]
0.36	0.9	Dominica	[12]
0.37	0.34	Netherlands	[4]
0.37	0.39	Finland	[13]
0.38	0.3	UK	[6]
0.39	0.16	Germany & UK	[14]
0.4	0.1	USA	[15]
0.4	0.21	Australia	[16]
0.4	0.3	Sweden	[17]
0.4	0.3	Canada	[18]
0.4	0.32	Australia	[19]
0.41	0.21	Australia	[20]
0.42	0.15	Nigeria	[21]
0.42	0.68	Sweden	[22]
0.44	0.55	Congo	[23]
0.45	0.23	Germany	[24]
0.45	0.37	France	[25]
0.46	0.2	Belize	[12]
0.46	0.29	Sweden	[26]
0.46	0.29	Netherlands	[27]
0.47	0.26	Netherlands	[28]
0.47	0.43	Sweden	[29]
0.47	0.9	Malaysia	[30]
0.48	0.15	USA	[31]
0.48	0.24	Germany	[24]
0.48	0.26	Netherlands	[32]
0.5	0.1	Hungary	[33]
0.5	0.2	USA	[34]
0.5	0.2	Canada	[35]
0.5	0.31	France	[36]
0.5	0.32	France	[37]
0.5	0.34	Spain	[38]
0.5	0.4	Dominica	[39]
0.5	0.4	Canada	[40]
0.51	0.2	Nigeria	[41]
0.52	0.21	USA	[42]

wt % ARA	wt % DHA	Country	Ref.
0.52	0.22	Xichang, China	[7]
0.52	0.26	Netherlands	[43]
0.52	0.32	Panama	[44]
0.52	0.32	France	[45]
0.52	0.32	Nigeria	[46]
0.53	0.16	USA	[47]
0.54	0.11	USA	[48]
0.54	0.3	Canada	[49]
0.54	0.48	Finland	[50]
0.54	0.59	UK	[51]
0.55	0.24	USA	[52]
0.56	0.16	USA	[53]
0.56	0.34	Nigeria	[11]
0.56	0.41	Surinam	[54]
0.56	0.42	France	[55]
0.57	0.2	Nigeria	[56]
0.57	0.3	Spain	[57]
0.57	0.49	Thailand	[58]
0.57	0.9	Malaysia	[30]
0.58	0.15	USA	[48]
0.58	0.21	USA	[52]
0.58	0.56	St. Lucia	[59]
0.59	0.19	USA	[60]
0.59	0.23	USA	[61]
0.6	0.1	USA	[62]
0.6	0.2	S. Africa	[63]
0.6	0.21	Hungary	[58]
0.6	0.23	USA	[52]
0.6	0.27	Tanzania	[54]
0.6	0.4	Netherlands	[64]
0.6	1.4	Canada	[65]
0.63	0.28	Beijing, China	[7]
0.64	0.71	Malaysia	[30]
0.69	0.29	USA	[66]
0.69	0.38	Spain	[44]
0.7	0.37	USA	[67]
0.7	0.4	Canada	[65]
0.71	0.43	Curacao	[54]
0.72	0.23	UK	[51]
0.77	0.55	Germany	[68]
0.8	0.4	Spain	[69]
0.8	0.5	Canada	[70]
0.82	0.93	Nigeria	[71]
0.99	1.09	Japan	[72]
1	0.1	S. Africa	[63]
1.56	0.15	Poland	[73]

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Table 2: Comparison of ARA and DHA Levels

Dietary Fat	Mead Johnson Proposed Use Levels*	Martek's Expert Panel Opinion	Martek's GRAS Notification	Mead Johnson Clinical Safety Study	Toxicology Studies			
					Martek		Mead Johnson	
					Max Dose	Safety Factor[1]	Max Dose	Safety Factor
ARA (wt % of fat)	0.64%	1.0%	0.5%	0.6%				
DHA (wt % of fat)	0.32%	1.0%	0.5%	0.34%				
ARASCO (wt % of fat)	1.6%	2.5%	1.25%	1.50%				
DHASCO (wt % of fat)	0.8%	2.5%	1.25%	0.85%				
ARASCO (mg oil/kg bw/day)	96	150	75	90	2500	26x	6000	62.5x
DHASCO (mg oil/kg bw/day)	48	150	75	55	1250	26x	3000	62.5x

*Actual values are approximately 10-15% higher as a result of overages included to assure label target levels are met consistently.

[1] Safety factor over Mead Johnson's proposed use level.

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Table 3
ARASCO and DHASCO Contents of Infant Formulas Used in Controlled Clinical Trials

Study	ARA Content (wt %)	DHA Content (wt %)
Preterm Studies		
Carnielli et al. 1994	0.75	0.6
Carnielli et al. 1998	0.84	0.64
Clandinin et al. 1997	0.32 / 0.49 / 1.1	0.24 / 0.35 / 0.76
Foreman-van Drongelen et al. 1996	0.61	0.3
Vanderhoof et al. 1999; 2000	0.50	0.35
Hansen et al. 1997	0.60 / 0	0.33 / 0.34
Term Studies		
Birch et al. 1998; 2000	0 / 0.72	0.35 / 0.36
Carlson 1999	0.6 / 0.6	0.3 / fish oil
Gibson et al. 1997	0.2 / 0.32 / 0.4	0.2 / 0.2 / 0.25
Morris et al. 2000	0.4	0.2

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Table 4: Comparison of Proposed Commercial Use Level and Panel Recommendations

Preterm Formula	MJ Proposed Levels*	ESPGAN (1991)	BNF (1992)	ISSFAL (1994)	FAO/WHO (1994)	ISSFAL (1999) (Adequate Intakes)	Child Health Foundation (2001)
ARA (wt% of fat)	0.64%	1%	0.4%	0.9-1.5%	0.9%	0.5%	at least 0.4%
DHA (wt% of fat)	0.32%	0.5%	0.4%	0.5-1.1%	0.6%	0.35%	at least 0.35%
Term Formula							
ARA (wt% of fat)	0.64 %		0.4%		0.7%	0.5%	at least 0.35%
DHA (wt% of fat)	0.32 %		0.4%		0.35%	0.35%	at least 0.2%

*Actual values are approximately 10-15% higher as a result of overages included to assure label target levels are met consistently.

Appendix D

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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®
December, 1999

Martek Biosciences Corporation

OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ARA AND DHA SINGLE CELL OILS FOR INFANTS AND CHILDREN

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EXECUTIVE SUMMARY

The undersigned, an independent panel of recognized experts (hereinafter the Panel), qualified by scientific and/or medical training and relevant international experience to evaluate the safety of food and food ingredients, was requested by Martek Biosciences Corporation to determine whether DHASCO® and ARASCO® oils (macronutrient oils produced by fermentation and containing docosahexaenoic acid (DHA) or arachidonic acid (ARA)), using scientific procedures, are Generally Recognized as Safe (GRAS) for consumption by infants (birth to 1 year) and children (1-12 years) at dose levels up to 2.5% each of total dietary fat. For infants, this dose is equivalent to 1.25% of dietary calories or about 150 mg of the oil/kg body wt/day. A comprehensive search of the scientific literature (published and unpublished) for information on the use and safety of ARASCO and DHASCO through October 15, 1999 was conducted by Martek and was made available to the Panel. The Panel members independently and critically evaluated the materials submitted by Martek Biosciences Corporation and other materials deemed appropriate and conferred by telephone several times before preparation of this report.

Earlier safety concerns regarding growth of infants fed fish oil-supplemented formulas have been addressed. They appear to be associated with the eicosapentaenoic acid (EPA) present in the fish oils. EPA leads to a depression of ARA and associated eicosanoid levels. In contrast, growth inhibition has never been observed when DHASCO, which does not contain EPA, was used as a dietary DHA source for infants. The growth delay seen in some fish oil-based formula studies can be avoided by adding an ARA source along with the DHA to infant formulas. Recent controlled studies indicate that preterm and term infants fed formulas fortified with both ARASCO and DHASCO oils have better growth indices than infants fed unsupplemented formula. Other potential safety concerns associated with fish oil, such as increased bleeding times, have never been linked to the use of DHASCO or ARASCO oils.

The Panel first considered the importance of DHA and ARA for infants and children. After reviewing the literature regarding the efficacy of DHA and ARA (from any source) in infant nutrition, the Panel unanimously agrees that: 1) 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. The link between these two long-chain polyunsaturated fatty acids and developmental outcome has been established by well-controlled clinical studies indicating that addition of DHA and ARA to the formula not only corrects the deficiency, but can also contribute to the elimination of these neurological deficits.

This Panel also considered the opinion of other Expert Panels, associated with prestigious scientific and medical organizations, that convened over the last ten years to consider the question of whether to add DHA and ARA to infant formulas. The general consensus among the different panels was that both of these fatty acids should be added to formulas at levels corresponding to those found in human breast milk. One panel from the Life Science Research Organization (LSRO) of the American Society for Nutritional Sciences, that reviewed the addition of long-chain fatty acids as part of an overall review

of the requirements for term (not preterm) infants formulas did not recommend that DHA and ARA be a required addition to term formulas at this time. However, they did recognize the importance of maintaining the DHA status of infants and suggested that supraphysiological levels of linolenic acid (a precursor to DHA) should be added to help improve the DHA status of the formula-fed infant. This LSRO Panel did not review several of the most recent studies, and recommended a re-evaluation of this issue in the near future as new clinical study data emerge. Another LSRO panel is presently considering the DHA and ARA requirements for preterm infants.

The chemical composition and manufacturing processes for both DHASCO and ARASCO oils were carefully reviewed by the Panel to ensure that oil quality meets standards for food production. DHASCO oil is a triglyceride, produced by the alga *Crypthecodinium cohnii*, which contains 40% by weight of DHA (specifications given in section 6). ARASCO oil, also predominantly triglyceride, is produced by the fungus *Mortierella alpina* and contains 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. Both oils also contain other common saturated and monounsaturated fatty acids. Minor nonsaponifiable fractions of the oils have been characterized and contain primarily cholesterol-related sterols commonly found in other food sources. 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. All oils undergo rigorous analytical and quality assurance testing and meet well-defined product specifications prior to release.

Based on elevation of blood lipid levels following ingestion of either DHASCO or ARASCO, both oils are absorbed in a manner consistent with other dietary triglycerides. 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 eicosanoids, and the omega-6 series of eicosanoids are well recognized as stimulators of immune function. Studies have shown that small amounts of DHA can be retroconverted to 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 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 not inconsistent 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 assays. All studies were modeled after FDA

Redbook guidelines and conducted at GLP-compliant laboratories. The study results were 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 themselves. None of the studies indicated that the oils were toxic, and the No Adverse Effect Levels (NOAELs) were determined to correspond to the highest doses tested. Modestly increased liver weights were noted, but this was not a consistant finding, nor was it accompanied by abnormal histology or serum enzyme levels. Furthermore, the liver weight changes were no longer apparent when assessed relative to other organ weights (*e.g.* brain). These changes were, therefore, deemed not to be toxicological in nature as this is a well-known effect of the Long Chain PUFAs in mammals when administered at high dose levels, well above those intended for infants or children. All other concerns raised by previous reviews have been addressed in this document (Appendix 4). In addition, the crude biomasses containing the DHASCO or ARASCO have also been extensively tested and found to be nontoxigenic.

Studies conducted in twelve different animal species, including nonhuman primates, have provided a large base of experience and extensive toxicological 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 over 1500 infants 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 and mental acuity have also been reported in infant groups supplemented with these oils and no adverse events have been reported. 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 adult volunteers. The preclinical and clinical studies conducted with these oils further support their use as a safe dietary source of DHA and ARA.

In addition, these oils have now been in commercial use in infant formulas in over 60 countries around the world (including the United Kingdom, France and Israel) at levels in accord with WHO/FAO guidelines for as long as three years with no reported adverse findings during that time. 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.

Human breast milk is the "Gold Standard" for infant nutrition. In addition to other important nutrients, human breast-fed infants receive DHA- and ARA-containing triglycerides from their mother's milk. Based on this fact, the growing body of evidence demonstrating the importance of supplying infants with DHA and ARA, and after a critical evaluation and analysis of the safety and clinical information available on DHASCO and ARASCO as DHA- and ARA-containing triglycerides, the undersigned Expert Panel has determined that these oils, derived from the referenced algal and fungal sources, meeting food grade specifications and produced according to current Good Manufacturing Practices (cGMP; 21 CFR 182.1) to be Generally Recognized as Safe (GRAS) for use in supplementing the diets of infants and children up to 15 years of age 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).

[redacted]

08 Nov 1999
Date

Joseph Borzelleca, Ph.D
Professor, Pharmacology and Toxicology
Medical College of Virginia, Richmond, VA

[redacted]

12/10/99
Date

Gary Flamm, Ph.D.
Flamm and Associates
Vero Beach, FL
(former, Director of Toxicology, CFSAN, FDA)

[redacted]

Nov 12 1999
Date

Bruce German, Ph.D.
Professor, Nutrition and Food Science
University of California, Davis, CA

[redacted]

Nov 5 1999
Date

Walter Glinsmann, M.D.
Adjunct Professor, Center for Food and Nutrition Policy
Georgetown University, Washington, DC
(former Director of Clinical Nutrition, CFSAN, FDA)

[redacted]

15 December 99
Date

David Kritchevsky, Ph.D.
Professor, Wistar Institute
Philadelphia, PA

[redacted]

Dec 3 1999
Date

David Bechtel, Ph.D., DABT
Senior Toxicologist
CanTox US Inc, Bridgewater, NJ

1. INTRODUCTION

An independent panel of recognized experts (hereinafter the Panel), qualified by scientific and/or medical training and relevant international experience to evaluate the safety of food and food ingredients, was requested to determine whether DHASCO® and ARASCO® oils (macronutrient oils produced by microbial fermentation and containing docosahexaenoic acid (DHA) or arachidonic acid (ARA)), using scientific procedures, are Generally Recognized as Safe (GRAS) for consumption by infants (birth to 1 year) and children (1-12 years) at dose levels up to 2.5% of total dietary fat. In an infant, this dose is equivalent to 1.25% of dietary calories or about 150 mg of the oil/kg body wt/day. A comprehensive search of the scientific literature (published and unpublished) for information on the use and safety of ARASCO and DHASCO through October 15, 1999 was conducted by Martek Biosciences Corporation (Martek) and was made available to the Panel. Reprints, abstracts, laboratory data and other materials were also provided. The Panel members independently and critically evaluated the materials submitted by Martek and other materials deemed appropriate. The Panel conferred by telephone several times and also conferred with members of Martek Biosciences Corporation for issues requiring clarification before the preparation of this report, which includes a review of the pertinent literature and recommendations.

2. THE IMPORTANCE OF DHA AND ARA IN INFANT NUTRITION

Prior to assessing the safety of the reference materials DHASCO and ARASCO oils, the Panel considered the importance of dietary DHA and ARA from any source for infants and children. The Panel unanimously agrees that there is a biochemical deficiency in infants receiving formulas with no supplemental DHA and ARA relative to infants being fed their own mother's milk -- the gold standard for infant nutrition. This biochemical deficiency is reflected in abnormally low levels of circulating DHA and ARA in the blood of unsupplemented formula-fed babies relative to breast-fed babies, and there is clinical evidence to suggest that there may be both short term and long term detrimental consequences as a result of this deficiency.

2.1 DHASCO AND ARASCO ARE SUBSTANTIALLY EQUIVALENT TO DHA AND ARA TRIGLYCERIDES IN HUMAN 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 also used as 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 2.1-1. 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 (165). 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 2.1-1).

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 (32), and the DHA content of breast milk would therefore 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 2.1-1 Breast milk DHA and ARA levels from women around the world.

Author/year	*Reference*	*wt % DHA*	*wt % ARA*	*Country*
Finley, et al. (1985)	(89)	0.06	0.29	USA
Harris, et al. (1984)	(114)	0.1	0.4	USA
Putnam, et al. (1982)	(198)	0.1	0.6	USA
van der Westhuizen, et al. (1988)	(235)	0.1	1	S. Africa
Sas, et al. (1986)	(208)	0.1	0.5	Hungary
Spear, et al. (1992)	(221)	0.11	0.54	USA
Sanders and Reddy (1992)	(207)	0.14	0.32	UK (vegan)
Maurage, et al. (1998)	(171)	0.14	0.24	France
Auestad, et al. (1997)	(9)	0.15	0.48	USA
Spear, et al. (1992)	(221)	0.15	0.58	USA
Okolska, et al. (1983)	(189)	0.15	1.56	Poland
Dotson, et al. (1992)	(80)	0.16	0.53	USA
Jackson, et al. (1994)	(134)	0.16	0.56	USA
Harzer, et al. (1983)	(115)	0.16	0.39	Germany & UK
Carlson, et al. (1986)	(44)	0.19	0.59	USA
Francois, et al. (1998)	(92)	0.2	0.5	USA
van der Westhuizen, et al. (1988)	(235)	0.2	0.6	S. Africa
Innis, et al. (1994)	(132)	0.2	0.5	Canada
Bitman, et al. (1983)	(23)	0.21	0.58	USA
Henderson, et al. (1998)	(118)	0.21	0.52	USA
Drury and Crawford (1990)	(81)	0.21	0.6	Hungary
Makrides, et al. (1995)	(163)	0.21	0.4	Australia
Makrides, et al. (1996)	(165)	0.21	0.41	Australia
Koletzko, et al. (1988)	(147)	0.22	0.36	Germany
Bitman, et al. (1983)	(23)	0.23	0.6	USA
Sanders, et al. (1978)	(206)	0.23	0.72	UK (vegan)
Genzel-Boroviczeny, et al. (1997)	(94)	0.23	0.45	Germany
Bitman, et al. (1983)	(23)	0.24	0.55	USA
Beijers and Schaafsma (1996)	(16)	0.24	0.31	Netherlands
Genzel-Boroviczeny, et al. (1997)	(94)	0.24	0.48	Germany
Martin, et al. (1993)	(170)	0.24	0.36	France
Carnielli, et al. (1998)	(51)	0.26	0.48	Netherlands
Foreman-van Drongelen, et al. (1996)	(91)	0.26	0.52	Netherlands
van Beusekom, et al. (1993)	(233)	0.26	0.47	Netherlands
Muskiet, et al. (1987)	(181)	0.27	0.6	Tanzania
Specker, et al. (1987)	(222)	0.29	0.69	USA
Hall (1979)	(111)	0.29	0.19	UK
Yu, et al. (1998)	(257)	0.29	0.46	Sweden

Author/year	*Reference*	*wt % DHA*	*wt % ARA*	*Country*
Sanders and Reddy (1992)	(207)	0.3	0.38	UK
Jansson, et al. (1981)	(137)	0.3	0.4	Sweden
Villacampa, et al. (1982)	(240)	0.3	0.57	Spain
Cherian and Sim (1996)	(56)	0.3	0.4	Canada
Clandinin, et al. (1997)	(61)	0.3	0.54	Canada
Babin et al. (1999)	(10)	0.31	0.5	France
Kaila, et al. (1999)	(142)	0.31	0.35	Finland
Rueda, et al. (1998)	(202)	0.32	0.52	Panama
Billeaud, et al. (1997)	(19)	0.32	0.52	France
Chardigny, et al. (1995)	(55)	0.32	0.5	France
Gibson, et al. (1981)	(95)	0.32	0.4	Australia
FAO/WHO RECOMMENDATIONS FOR FULL TERM INFANTS	(84)	**0.33**[1]	**0.65**	
de la Presa-Owens, et al. (1998)	(71)	0.34	0.5	Spain
Ogunleye, et al. (1991)	(188)	0.34	0.56	Nigeria
Beijers and Schaafsma (1996)	(16)	0.34	0.37	Netherlands
ISSFAL 1999 RECOMMENDATIONS FOR FULL TERM INFANTS	(133)	**0.35**	**0.50**	
Sanders and Reddy (1992)	(207)	0.37	0.35	UK
Guesnet, et al. (1993)	(110)	0.37	0.45	France
Rueda, et al. (1998)	(202)	0.38	0.69	Spain
Prentice, et al. (1989)	(197)	0.39	0.31	The Gambia
Luukkainen, et al. (1994)	(162)	0.39	0.37	Finland
de Lucchi, et al. (1988)	(72)	0.4	0.8	Spain
Jacobs, et al. (1996)	(135)	0.4	0.6	Netherlands
van Beusekom, et al. (1993)	(233)	0.4	0.5	Dominica
Clandinin, et al. (1981)	(58)	0.4	0.5	Canada
Innis, et al. (1988)	(126)	0.4	0.7	Canada
Muskiet, et al. (1987)	(181)	0.41	0.56	Surinam
Horby Jorgensen, et al. (1996)	(124)	0.43	0.47	Sweden
Muskiet, et al. (1987)	(181)	0.43	0.71	Curacao
Luukkainen, et al. (1995)	(162)	0.48	0.54	Finland
Drury and Crawford (1990)	(81)	0.49	0.57	Thailand
Innis, et al. (1990)	(130)	0.5	0.8	Canada
Ogunleye, et al. (1991)	(188)	0.53	0.36	Japan
Fidler, et al. (1998)	(87)	0.55	0.77	Germany
Rocquelin, et al. (1998)	(200)	0.55	0.44	Congo
Boersma, et al. (1991)	(25)	0.56	0.58	St. Lucia
Sanders, et al. (1978)	(206)	0.59	0.54	UK
Kneebone, et al. (1985)	(144)	0.71	0.64	Malaysia
Kneebone, et al. (1985)	(25)	0.9	0.47	Malaysia
Kneebone, et al. (1985)	(25)	0.9	0.57	Malaysia
Koletzko, et al. (1991)	(149)	0.93	0.82	Nigeria
Innis, et al. (1988)	(126)	1.4	0.6	Canada

[1] 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.

clinical studies indicate that supplementing formulas with DHASCO and ARASCO reduces or eliminates the detrimental consequences of the DHA and ARA deficiency. Consequently, this Panel does not find any reason to disagree with other Expert Panels' opinions on the importance of DHA and ARA in infant nutrition, and further believes that there is now sufficient evidence that it is beneficial to supplement infants with DHA and ARA as a means to eliminate the biochemical deficiency in these infants and to more closely approximate the condition of a breast-fed infant. The Panel further believes that such an addition is in line with the general tenet of providing infants with nutritive components that most closely match the nutritive components of human milk.

3. CURRENT AND PROPOSED USES FOR DHASCO AND ARASCO

As described in the previous section, several other Expert Panels have addressed the issue of use levels of DHA and ARA in infant formulas (31, 83, 84, 133). The levels recommended by those Expert Panels are given in Table 3-1 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. Although the recent LSRO Panel in the U.S. (159) concluded that there is not enough information at this time to mandate the addition of DHA and ARA to infant formulas (see previous discussion), they did conclude that there is ongoing research in this area, and that the issue should be reopened when those data are available. A much larger group of world-wide experts in lipid nutrition more recently (April, 1999) reviewed the latest data (not reviewed or discussed by the LSRO Panel), and 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 (217). They also went on to identify 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 3-1. 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)[5]	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

[5] Assmptions 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 2.2-1). 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 0.1% to 1.1% of total fat in preterm formulas, and from 0.20% to 0.72% for term formulas. Of particular relevance to this Panel were the 14 clinical studies in which DHASCO and ARASCO were used as the DHA and ARA sources for supplementation (see Appendix 2). In those trials, the DHA and ARA dose levels ranged from 0.24 % to 0.76 % of total formula fat for DHA and 0.24 % to 1.1 % of total formula fat for ARA in preterm formulas. 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 2.1-1) and the Expert recommendations (Table 3-1).

In the past five years, commercial infant formulas containing supplemental DHA and ARA have been introduced around the world. Of particular interest to this Panel were the formulas containing DHASCO and ARASCO as the forms of supplementation (Table 3-2). The Panel also noted that the LBW formula produced by Wyeth/Ayerst is provided to low birth weight infants under strict doctor's supervision. The manufacturer estimates that over 100,000 infants have been given these formulas under doctor's guidance. In fact, DHASCO/ARASCO-supplemented formulas are used more extensively around the world than formulas supplemented with any other source of DHA and ARA. The DHA levels in current commercial use for those formulas are 0.30% to 0.40% of formula fat for preterm formulas and from 0.25% to 0.40% of formula fat for term formulas. The ARA levels in current commercial use for those formulas are 0.45% to 0.60% of formula fat for preterm formulas and from 0.45% to 0.50% of formula fat for term formulas.

Table 3-2. Commercial infant formulas containing DHASCO and ARASCO

Country	*Company*	*Product Name*	*Term*	*Preterm*
Argentina	Wyeth	SMA LBW		X
Australia	Wyeth	SMA LBW		X
		SMA Gold	X	
	Numico	Nenatal		X
		KariCare First[6]	X	
Bahrain	Wyeth	SMA LBW		X
Bolivia	Wyeth	SMA LBW		X
Belgium	Numico	Nenatal		X
		Premilon		X
Chile	Wyeth	SMA LBW		X
China (P.R.C.)	Wyeth	SMA LBW		X
Colombia	Wyeth	SMA LBW		X
Cyprus	Wyeth	SMA LBW		X
Dominica	Wyeth	SMA LBW		X
Ecuador	Wyeth	SMA LBW		X
Egypt	Wyeth	SMA LBW		X

[6] KariCare First contains fish oil and ARASCO oil.

Since 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 (151). 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* genotoxicity assays and a variety of animals studies, such as acute, subchronic, developmental and multigenerational reproductive studies. Since 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 4. Only the significant findings will be presented here. These data have also been reported in a number of publications and summarized by Kyle and Arterburn (154).

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 problems (26). 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, is the elevation of serum cholesterol in a human who changes their diet from one containing 25% of dietary calories as fat to one with 45% of dietary calories as fat 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. Several studies have shown that at acute oral doses of 20 grams of DHASCO or ARASCO/kg body wt in rats, there were no deaths (Table 7.5.1). 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. Since 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.

Table 7.5.1. Acute toxicity studies with DHASCO and ARASCO 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	(27)
DHASCO	Martek	20	0/5	0/5	>20 g/kg bw	(100)
C. cohnii algal biomass	Martek	7	0/5	0/5	>7 g/kg bw	(103)
C. cohnii biomass	Martek	7	0/5	0/5	>7 g/kg bw	(104)
Delipidated C. cohnii biomass	Martek	10	0/5	0/5	>10 g/kg bw	(101)
Delipidated algal biomass	Martek	6	0/5	0/5	>6 g/kg bw	(105)
ARASCO	Martek	20	0/5	0/5	>20 g/kg bw	(27)
ARASCO	Martek	20	0/5	0/5	>20 g/kg bw	(102)
ARASCO	Gist-brocades	18.2	0/5	0/5	>18.2 g/kg bw	(117)
Fungal biomass	Martek	5	0/5	0/5	>5 g/kg bw	(99)
Delipidated fungal biomass	Martek	4	0/5	0/5	>4 g/kg bw	(106)
Microencapsulated ARASCO/DHASCO blend	Martek	5	0/5	0/5	>5 g/kg bw	(107)

7.5.2 SUB-CHRONIC (28-63 DAY) TOXICITY STUDIES. In several independent studies, DHASCO or ARASCO was administered by gavage, or as a dietary admixture, daily for 28 or 63 days using doses of 0.025 to 9.4 grams of test material /kg body wt/day (Table 7.5.2). The No Adverse Effect Levels (NOAEL) were 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 bw/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 were not accompanied by changes in histology or clinical chemistry. Rather, they were considered normal physiological responses to high doses of polyunsaturated fatty acids and to the use of artificial diets in some cases.

Table 7.5.2. 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	63 days in the diet; 10 rats/sex/dose	Not toxic	(88)
DHASCO	Martek	1.25	28 days by gavage; 5-10 rats/sex/dose	No toxic	(27)
ARASCO	Martek	2.5	28 days by gavage; 5-10 rats/sex/dose	Not toxic	(27)
ARASCO	Gist-brocades	3.0	28 days by gavage; 10 rats/sex/dose	Not toxic	(117)
ARASCO/DHASCO 2:1 blend	Martek	3.75	28 days by gavage; 5-10 rats/sex/dose	Not toxic	(27)
ARASCO/DHASCO 2:1 blend	Mead Johnson	9.1/9.4	28 days in the diet; 10 rats/sex/dose	Not toxic	(247)
Algal biomass	Martek	5	28 days in diet, 5-10 rats/sex/dose	Not toxic	(249)

7.5.3 SUB-CHRONIC (90-DAY) STUDIES. DHASCO and ARASCO were administered by gavage or as a dietary admixture for 90 days at doses up to 8.9 grams of test material/kg body wt/day with 20 rats per sex per dosing group. The No Adverse Effect Levels 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 per kg bw per day and up to 8.9 g/kg body wt/day for combined oils) except for the Gist-brocades study (see Table 7.5.3) (8, 36, 150). A NOAEL of 8.9 gm/kg/day (about 6 grams ARASCO and 3 grams of DHASCO) represents at least a 60-fold excess of this macronutrient from the intended use level of ARASCO, and a 50-fold excess from the intended use level of DHASCO. At the highest dose levels, there were some findings (see section 7.5.9) that were not considered to be adverse test material because they were not accompanied by changes in histology or clinical chemistry. Rather, they were considered normal physiological responses to high doses of polyunsaturated fatty acids and to the use of artificial diets in some cases. Some of these studies also involved detailed assessments of neurotoxicity (8, 143) as outlined by the FDA Redbook II Guidelines, and other studies also involved an *in utero* supplementation phase (36).

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.

Table 7.5.3. 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	(8)
DHASCO/ARASCO blend	Numico	2.0	in diet	Not toxic	(113)
ARASCO	Martek	2.5	gavage, full neurotox assessment	Not toxic	(150)
DHASCO/ARASCO 1.5:1 blend	Wyeth Ayerst	2.5	in diet	Not toxic	(88)
ARASCO	Gist-brocades	4.9	in diet, *in utero* phase, full neurotox assessment	Not toxic	(157)
DHASCO/ARASCO 2:1 blend	Mead Johnson	8.9	in diet, *in utero* phase, full neurotox assessment	Not toxic	(36)
Algal biomass	Martek	5.8	in diet, full neurotox assessment	Not toxic	(225)

7.5.5 REPRODUCTIVE/DEVELOPMENTAL TOXICOLOGY STUDIES. Developmental toxicology studies were conducted with DHASCO and ARASCO administered by gavage (Table 7.5.5). There were no adverse developmental effects at the doses tested as determined by the independent study site toxicologists.

Table 7.5.5. 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	(7)
ARASCO	Developmental	Martek	2.5	gavage 25/sex/dose	Not toxic	(7)
Algal biomass	Developmental	Martek	4.3	in diet 20/sex/dose	Not toxic	(242)
Algal biomass	Reproductive	Martek	8	in diet 20/sex/dose	Not toxic	(241)

7.5.6 GENOTOXICITY STUDIES - *IN VITRO*. The test materials were determined to be neither mutagenic, clastogenic, nor genotoxic through a number of standard *in vitro* mutagenicity tests (Table 7.5.6) when tested with and without *in vitro* metabolic activation using rat liver microsomal S9 fractions.

The above biochemical evidence explains why eliminating DHA and ARA from the diet of infants, particularly preterm infants, may put them at higher risk for oxidation dependent pathologies such as NEC, BPD and ROP. This has been recently confirmed in an animal model for NEC by Caplan *et al.* (38). Those authors demonstrated that the incidence of NEC was significantly lower in rats fed formulas containing DHA and ARA (using the DHASCO and ARASCO as sources) than in rats fed formulas containing no DHA or ARA. This has also been confirmed in human infants by Carlson and colleagues (42) using egg yolk as a source of DHA and ARA. In her controlled clinical study, infants who received standard preterm infant formula had an incidence of NEC of 18%, whereas the incidence was reduced to only 3% if the babies were given a formula that was supplemented with DHA and ARA. About 4,000 babies die each year in the United States as a result of NEC (35). Extrapolating Carlson's data to all U.S. babies, the inclusion of DHA and ARA into the formulas could substantially reduce the morbidity and mortality associated with this disease.

We conclude, therefore, that if DHASCO and ARASCO are provided to infants at the GRAS levels (*i.e.*, DHA and ARA up to 1.0% of total milk fat lipids), no oxidative injury would be expected. In fact, based on laboratory and clinical evidence, it is quite likely that the addition of DHA and ARA to formulas at these levels (similar to breast milk) may well reduce the incidence of oxidative damage in predisposed infants because of the stimulation of intracellular antioxidants such as SOD.

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.

APPENDIX 2

CLINICAL STUDIES WITH INFANTS USING DHASCO AND/OR ARASCO [22] (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.
Damil, 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.

[22] 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.
Monsolls 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 Nutridonals)	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.

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

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1634

Lower the Neonatal Weight, Higher the Plasma Coenzyme Q10 (CoQ10) Levels: Which Is the Meaning?

Isabella Bosi, Gina Ancora, Alberto M Bargossi, Gabriele Grossi, Fabrizio Sandri, Gian P Salvioli, Institute of Neonatology, University of Bologna, Italy, Central Laboratory, Sant'Orsola Hospital, Bologna, Italy (Spon by: Giuseppe Buonocore)

Introduction: CoQ10 is an important antioxidant molecule. Scanty data are available about neonatal levels and needs. We have measured maternal and neonatal plasma CoQ10 values at the time of birth and studied their possible correlation with different perinatal variables.

Patients and methods: plasma CoQ10 levels were determined in 60 cord blood samples from healthy newborns (Apgar score 1'-5' > 6, no malformations) of gestational ages ranging from 32 to 42 weeks, 12 born by cesarean section and 48 by vaginal delivery. Paired maternal peripheral blood samples were drawn at the same time. An HPLC-ED method was used to determine total CoQ10 concentration. Statistical package SPSS v. 4.2 was used for statistical analysis.

Initially, correlation between cord blood and maternal CoQ10 levels was tested. Regression analysis was then undertaken using birth weight, gestational age, sex and type of delivery as independent variables against cord blood CoQ10 (CBCoQ10) levels.

Results: there was a significant positive correlation between maternal and cord blood levels of CoQ10 ($r=0.46$, $p<0.05$). Multiple regression analysis showed a significant negative correlation between birth weight and CBCoQ10 levels. The other independent variables resulted not correlated with CBCoQ10.

Discussion: present data suggest a transplacental crossing of CoQ10. Moreover, CBCoQ10 levels show a tend toward higher levels in lower birth weight newborns. We hypothesize that higher levels of CBCoQ10 in lower birtweight newborns could reflect lower tissutal utilization as also reported in hypothyroxinemic adults. Indeed, preterm neonates atre hypothyroxinemic compared to term babies. At present, in order to test this hypothesis we are conducting a study on tissutal CoQ10 levels with the aim to evaluate their possible correlation with plasma levels.

1635 *Poster Session I, Saturday, 5/1 (poster 122)*

Effects of Hypoxia on Tyrosine Phosphorylation Pyruvate Kinase and Aconitate on Synaptosomes of Newborn Guinea Pigs

Guiseppe Buonocore, Sabrina Liberotori, Luca Bini, Om P Mishra, Rodolfo Bracci, Vitaliano Pallini, Department of Molecular Biology, Inst. of Prev. Ped. and Neonatology, University of Siena, ITA, Department of Pediatrics, MCP and Hahnemann University and St. Christopher's Hospital for Children, Philadelphia, PA (Spon by: Maria Delivoria-Papadopoulos)

Previous studies have shown that cerebral glycolytic enzymes are up-regulated following hypoxia. The present study tests the hypothesis that tissue hypoxia results in increased phosphorylation of pyruvate kinase and aconitate hydratase, key enzymes of the tricarboxylic acid pathway in cerebral cortex of newborn guinea pigs. Studies were conducted on six newborn guinea pigs divided into normoxic (n=3) and hypoxic (n=3) groups. Hypoxia was induced by decreasing the inspired oxygen from 21% to 7% for 60 min. Brain tissue hypoxia was documented biochemically by determining tissue levels of ATP and phosphocreatine. P2 membrane fractions were prepared and global protein expression was analyzed by two-dimensional electrophoresis using the immobiline-polyacrylamide system (2-D-PAGE). The isoelectric focusing (first dimension) was carried out on non-linear wide-range immobilized pH gradients (pH 3-10). The second dimension was carried out on 9-16% polyacrylamide linear gradient gels at 40 mA/gel constant current and 10°C until the dye front reached the bottom of the gel. Gels were stained with ammoniacal silver nitrate. After 2-D-PAGE, gels were electroblotted onto nitrocellulose membrane and stained in 0.2% w/v Ponceau S in 3% w/v trichloroacetic acid for 3 min. Immunoblot analysis was performed using mouse monoclonal antibody to phosphotyrosine and horseradish peroxidase-conjugated goat antimouse IgGs with chemiluminescence detection. Protein identification was achieved by computer-aided matching with a 2-D reference map of human brain proteins, using the Melanie II software, on the basis of the known high degree of homology between human and rodent brain proteins. In hypoxic synaptosomes, 49 tyrosine-phosphorylated spots (out of total 130) were detected as compared to only 20 in normoxic synaptosomes. Pyruvate kinase was phosphorylated only in the hypoxic synaptosomes. The tyrosine-phosphorylation of pyruvate kinase was absent in normoxia. Aconitate hydratase was more phosphorylated in the hypoxic synaptosomes as compared to normoxic synaptosomes. We conclude that during hypoxia an increased phosphorylation of pyruvate kinase and aconitate hydratase is a potential mechanism of regulation of these enzyme activities. We speculate that the increased activities of these key enzymes of the tricarboxylic acid pathway during hypoxia will result in increased turnover of the tricarboxylic acid pathway leading to increased intracellular accumulation of reducing equivalents.

(Funded by NIH 20337)

1636 *Human Milk Poster Symposium, Sunday, 5/2*

Enteral Absorption of Recombinant Human Granulocyte Colony-Stimulating Factor (rhG-CSF) in G-CSF-Receptor Deficient (-/-) and Wild-Type (WT) Mice

Darlene A Calhoun, Mathilde Lunoe, Yan Du, Robert D Christensen, Pediatrics/Neonatology, University of Florida College of Medicine, Gainesville, FL

G-CSF is a lineage specific hematopoietic cytokine known to stimulate neutrophil proliferation and differentiation. It is present in human milk and that its receptor (G-CSF-R) is present in human fetal intestine. However, it is not clear if enterally administered rhG-CSF is absorbed and if so, whether the G-CSF-R is integral to this process. We hypothesized that rhG-CSF would be absorbed when administered enterally to normal (WT) mice and that in G-CSF-R(-/-) mice absorption would be significantly reduced or absent. WT and G-CSF-R(-/-) pups (total n=17) received 1000 pg rhG-CSF via a feeding tube. Pups were returned to their mothers to feed and pups from each liter were sacrificed at 30 min, 1hr, 1.5 hrs, 2 hrs, 4 hrs, and 5 hrs after the dosing. Serum samples were collected and G-CSF concentrations were determined by ELISA. Immunostaining for G-CSF-R protein in the intestines of pups (n=5) and their mothers (n=3) was performed using the chromagen diaminobenzidine tetrahydrochloride (DAB). The primary antibody consisted of a rabbit polyclonal antibody specific for G-CSF-R of mouse origin. Serum concentrations of G-CSF in WT animals which received 1000 pg of rhG-CSF ranged from 11-238pg/mL at 30 min and 11-193pg/mL at 1 hr. G-CSF was not detected in the serum of any G-CSF-R(-/-) pups at any time point examined for either dose of rhG-CSF. G-CSF-R protein was localized to the villus enterocytes of WT mice. No G-CSF-R protein was detectable in the intestine of G-CSF-R(-/-) mice. We conclude that 1) rhG-CSF is enterally absorbed in WT mice, 2) maximal absorption occurs between 30 min and 1 hr after administration, 3) no detectable absorption occurs in G-CSF-R(-/-) pups, and 4) G-CSF-R protein is present in enterocytes of WT animals and absent in G-CSF-R(-/-) mice. We speculate that the presence of the G-CSF-R is integral for the enteral absorption of rhG-CSF.

1637

Lactate Dehydrogenase and Isoenzymes Activity in Colostrum, Transitional Milk and Serum from Mothers Delivering Term Infants Appropriate or Small for Gestational Age

Valdenise M L T Calil, Orlando C O Barretto, Kimiyo Nonovama, Clea R Leone, Department of Pediatrics, Department of Hematology, University of Sao Paulo, Department of Hematology, Adolfo Lutz Institute, Sao Paulo, SP, Brazil (Spon by: Waldemar A. Carlo)

Introduction: Investigations concerning the composition of milk produced by mothers delivering newborn infants small for gestational age have demonstrated its capacity of adaptation to their elevated energy and growth requirements.The few reported studies about lactate dehydrogenase (LDH) and its isoenzymes (LD-1 to LD-5) activity in human milk have assessed a small number of patients, yielded controversial results and have not included mothers from newborn infants small for gestational age. **Objectives:** Evaluating the enzymatic profile of human milk and maternal serum with respect to variations in the ativity of LDH and its isoenzymes throughout lactation, the possibility of enzyme secretion by the mammary gland and its adequacy to the newborn metabolic requirements, as well as the effects of the elevated requirements of small for gestational age infants on the milk enzyme content. **Methods:** Forty-three lactating mothers were selected according to preestablished criteria and divided into 2 groups: **Group 1**, 22 lactating mothers delivering term infants appropriate for gestational age. **Group 2**, 21 lactating mothers delivering term infants small for gestational age. The author personally collected samples of colostrum between 24 and 72 hours post-partum, of transitional milk at the 7th day and of maternal blood soon after milk extraction. This material was used for determination of the activities of total LDH and of its isoenzymes. Statistical analysis was by chi-square, Student's t and paired t tests, two-way analysis of variance and Pearson's correlation method. Significance was set at 0.05, with an allowed beta error of 0.2. **Results:** The results showed a marked increase in the activity of LDH in colostrum, when compared with transitional milk ($p<0.01$); the concentrations of total LDH and its isoenzymes in milk were higher than those detected in maternal serum at the 2nd / 3rd and 7th days post-partum. The milk isoenzyme profile showed predominance of LD-5 in colostrum, of LD-1 in transitional milk and of LD-2 and LD-3 in the mother's serum.There was no correlation between the activities of LDH and its isoenzymes in milk and serum.The differences between LDH and isoenzymes activities from groups 1 and 2 were not significant. **Conclusion:** The results suggest that LDH and its isoenzymes are secreted by the mammary gland and may be adjusted to the increased metabolic requirements during perinatal adaptation, with an initial predominance of an anaerobiosis pattern and progressive evolution to an aerobiosis pattern. These enzymatic activities in milk, however, do not seem to be influenced by the elevated requirements of small for gestational age infants.

1638 *Neonatal Nutrition and Metabolism II Poster Symposium, Monday, 5/3*

Higher Protein Intake Improves Growth of VLBW Infants Fed Fortified Breast Milk

Susan J Carlson, Karen J Johnson, Gretchen A Cress, Natalie W Connolly, Ekhard E Ziegler, Dept. of Pediatrics, University of Iowa, Iowa City, IA

The protein intakes of VLBW infants are typically lower than requirements, particularly when breast milk with nutrient fortifier is fed. We hypothesized that increasing the protein content of feedings (fortified breast milk, premature formula) for VLBW infants improves growth. In this double-blind study, 66 infants with birth weight < 1250g were randomly assigned to receive protein (Elementra) or lactose in the amount of 0.8 g/dl as supplements to their regular feedings. Nutritional management was otherwise by parents' choice (breast vs formula) and under the jurisdiction of the medical team. Study supplements were started when enteral feeds reached 50 ml/kg/d and ended when the infant was discharged or reached a weight of 2000 g. Blood for urea nitrogen, albumin and blood gases was obtained weekly and later biweekly. Forty-six infants completed the study. Growth was evaluated from the day feeds reached 90 kcal/kg/d to the end of the study. Results were as follows (means (SD)):

	Fortif. breast milk		Formula	
	Lactose (n=13)	Protein (n=13)	Lactose (n=9)	Protein (n=11)
Cal. int.(kcal/kg/d)	120.7 (6.9)	113.7 (7.6)*	117.0 (5.3)	114.4 (4.6)
Weight gain (g/d)	22.7 (5.9)	26.7 (2.9)*	28.5 (5.3)	29.4 (5.7)
(g/kg/d)	14.9 (3.2)	17.3 (1.9)*	18.4 (1.9)	19.1 (2.2)
(g/100 kcal)	13.0 (3.3)	16.0 (1.9)*	16.4 (2.3)	16.9 (1.8)

* significantly different from lactose group.

It is evident that among infants fed fortified breast milk, protein supplementation led to significantly greater weight gain despite significantly lower energy intake. There was a trend toward shorter hospital stay by ~ 4 days. Among formula-fed infants protein supplementation had no significant effect on growth. In neither group did protein have adverse effects. Serum urea nitrogen was higher in infants receiving the protein supplement, but albumin concentrations and acid-base status were not different. We conclude that breast milk fortifiers provide too little protein.

1639 *Poster Session III, Monday, 5/3 (poster 67)*

Growth and Development of Term Infants Fed Formulas with Docosahexaenoic Acid (DHA) from Algal Oil or Fish Oil and Arachidonic Acid (ARA) from Fungal Oil

Susan E Carlson, Sunil Mehra, William J Kagey, Kimberly L Merkel, Deborah A Diersen-Schade, Cheryl L Harris, James W Hansen, Schools of Nursing and Medicine, Univ. of Missouri, Kansas City, MO, Oshawa Clinic, Oshawa, Ontario, CAN, Lewis Gale Clinic, Salem, VA, Mead Johnson Research Center, Evansville, IN

Background. In several published, randomized trials of preterm and term infants, infants fed formula with the n-3 fatty acid DHA had higher neural development than infants fed formula containing the DHA precursor, α-linolenic acid, but no preformed DHA. However, preterm infants fed DHA also experienced lower growth in several trials, the possible result of not including the n-6 fatty acid ARA, a necessary factor for growth. This study evaluated whether ARA with DHA prevented the lower growth seen with DHA alone. Methods. This multicenter, randomized, double-masked trial compared first-year growth of term (39-41 wk PMA), AGA infants fed formula without DHA and ARA (Control, C) with that of infants fed experimental formulas with DHA (0.3% of total fatty acids) and ARA (0.6% of total fatty acids). One experimental formula contained fish oil DHA and fungal oil ARA (S-FF). The other contained algal oil DHA and fungal oil ARA (S-AF). Effects of DHA and ARA on visual acuity (Teller Acuity Card procedure, TAC) and the Bayley Scales of Mental (MDI) and Psychomotor Development(PDI)were assessed as secondary aims. At baseline (0.5 mo), 377 infants were divided among the three groups with 335 of these infants (112 C, 113 S-FF, 110 S-AF) returning to be studied at 2 mo, and 322 infants (107 C, 109 S-FF, 106 S-AF) returning to be studied at 4 mo. Results. S-FF and S-AF had higher weight gain between 0.5 mo and 2 mo (34.4 g/d, $p=0.041$ and 35.2 g/d, $p=0.007$, respectively) than C (32.1 g/d). Similarly, S-FF (28.4 g/d, $p=0.031$)and S-AF (29.1 g/d, $p=0.002$) gained more rapidly than C (26.7 g/d) between 0.5 and 4 mo. Body weights of S-FF and S-AF were somewhat higher (not significantly) than C at 6, 9 and 12 mo; the means for length and head circumference were virtually identical in all three diet groups at all study ages. Visual acuity did not differ at any of the assessment ages (4, 6 or 12 mo). Mean 12-mo MDI and PDI scores were S-AF > S-FF > C, but did not reach significance for an effect of diet with 93 C, 86 S-FF and 97 S-AF studied at 12 mo ($p=0.31$ and $p=0.15$, respectively). Summary. Infants fed the experimental formulas with DHA and ARA gained weight more rapidly than the control group from 0.5 to 4 mo of age, and weighed somewhat more than the control group through 12 mo of age. MDI and PDI scores were higher in the experimental groups than the control, but did not reach statistical significance as the study was designed to detect a 5-pt or larger effect of diet. While visual acuity was not higher at 4, 6 or 12 mo, several other studies have found higher acuity at 2 mo but not at these ages in infants fed DHA. Conclusion. Supplementation with DHA and ARA increased early weight gain and may have improved first year neural development of term infants. Feeding fish or algal and fungal oil sources of DHA and ARA for the first year had no adverse effects on growth and development.

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1639 *Poster Session III, Monday, 5/3 (poster 67)*

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Susan E Carlson, Sunil Mehra, William J Kagey, Kimberly L Merkel, Deborah A Diersen-Schade, Cheryl L Harris, James W Hansen, Schools of Nursing and Medicine, Univ. of Missouri, Kansas City, MO, Oshawa Clinic, Oshawa, Ontario, CAN, Lewis Gale Clinic, Salem, VA, Mead Johnson Research Center, Evansville, IN

Background. In several published, randomized trials of preterm and term infants, infants fed formula with the n-3 fatty acid DHA had higher neural development than infants fed formula containing the DHA precursor, α-linolenic acid, but no preformed DHA. However, preterm infants fed DHA also experienced lower growth in several trials, the possible result of not including the n-6 fatty acid ARA, a necessary factor for growth. This study evaluated whether ARA with DHA prevented the lower growth seen with DHA alone. Methods. This multicenter, randomized, double-masked trial compared first-year growth of term (39-41 wk PMA), AGA infants fed formula without DHA and ARA (Control, C) with that of infants fed experimental formulas with DHA (0.3% of total fatty acids) and ARA (0.6% of total fatty acids). One experimental formula contained fish oil DHA and fungal oil ARA (S-FF). The other contained algal oil DHA and fungal oil ARA (S-AF). Effects of DHA and ARA on visual acuity (Teller Acuity Card procedure, TAC) and the Bayley Scales of Mental (MDI) and Psychomotor Development(PDI)were assessed as secondary aims. At baseline (0.5 mo), 377 infants were divided among the three groups with 335 of these infants (112 C, 113 S-FF, 110 S-AF) returning to be studied at 2 mo, and 322 infants (107 C, 109 S-FF, 106 S-AF) returning to be studied at 4 mo. Results. S-FF and S-AF had higher weight gain between 0.5 mo and 2 mo (34.4 g/d, p=0.041 and 35.2 g/d, p=0.007, respectively) than C (32.1 g/d). Similarly, S-FF (28.4 g/d, p=0.031)and S-AF (29.1 g/d, p=0.002) gained more rapidly than C (26.7 g/d) between 0.5 and 4 mo. Body weights of S-FF and S-AF were somewhat higher (not significantly) than C at 6, 9 and 12 mo; the means for length and head circumference were virtually identical in all three diet groups at all study ages. Visual acuity did not differ at any of the assessment ages (4, 6 or 12 mo). Mean 12-mo MDI and PDI scores were S-AF > S-FF > C, but did not reach significance for an effect of diet with 93 C, 86 S-FF and 97 S-AF studied at 12 mo (p=0.31 and p=0.15, respectively). Summary. Infants fed the experimental formulas with DHA and ARA gained weight more rapidly than the control group from 0.5 to 4 mo of age, and weighed somewhat more than the control group through 12 mo of age. MDI and PDI scores were higher in the experimental groups than the control, but did not reach statistical significance as the study was designed to detect a 5-pt or larger effect of diet. While visual acuity was not higher at 4, 6 or 12 mo, several other studies have found higher acuity at 2 mo but not at these ages in infants fed DHA Conclusion. Supplementation with DHA and ARA increased early weight gain and may have improved first year neural development of term infants. Feeding fish or algal and fungal oil sources of DHA and ARA for the first year had no adverse effects on growth and development.

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IOVS
March 15, 2001, Vol. 42, No. 4

Monday, April 30
PGM#'s 502—687

Room 222
Monday 5:15—7:00 PM

Visual Psychophysics & Physiological Optics
Development

MODERATORS: Davida Y Teller
Ruxandra Sireteanu

PGM#	TIME	AUTHORS
653	5:15	Sireteanu, Wagner, Bucher
654	5:30	Kiorpes, Bassin, Movshon
655	5:45	Shams, Shimojo
656	6:00	Hoffman, Birch, Castaneda, Fawcett, Birch, Uauy
657	6:15	Chien, Teller, Palmer
658	6:30	Pitchford, Mullen
659	6:45	Lawton

NOTE: Potential conflicts of interest of presenters and authors are noted at the end of the abstract either by "None" or with codes (see code definitions under the section "Abstract Content/Commercial Relationships" Disclosure Codes" in the front matter).

653—05:15

What features do little creatures look at? R. Sireteanu, M. Wagner, I. Bucher. Neurophysiology, Max Planck-Inst Brain Research, Frankfurt, Germany.

Purpose: To investigate perceptual mechanisms in infants and children for different visual search tasks. Methods: Infants between 2 and 12 months and children of 1-4 years of age were tested with different visual search tasks using a preferential looking technique (7 age groups; 12 infants per group). The stimuli were displayed on cardboard cards containing a target in a surround of 15 distractors. The target consisted of: a) a patch of a low-frequency, high-contrast vertical grating, among high-frequency distractors (and vice-versa), b) a closed quadrangle among open-quadrangle distractors (and vice-versa), and c) horizontal or vertical single line segments among quadrangles (and vice-versa). Results: Children aged 2-4 years showed high preferences for the discrepant item in all tasks. Infants younger than 6 months of age showed a reverse preference for some tasks (e.g. closed quadrangle among open quadrangles), by orienting reliably towards the field without target. Preference for some targets (e.g. open quadrangle among closed ones; single lines among quadrangles, quadrangle among single lines) started at chance level and increased progressively to reach a reliably positive preference around the end of the first year of life. All tasks involving grating patches of different spatial frequencies showed a positive preference starting from 4 months of age. Conclusions: Our findings confirm and extend previous observations (Sireteanu and Encke, IOVS 1999;40:4:343) and suggest that the common mechanisms responsible for orientation towards targets of different salience are different in their development during the course of the first years of life.
CR: None Support: DFG (Si344/11-2)

654—05:30

Development of contour integration. L. Kiorpes[1], S.A. Bassin[1], J.A. Movshon[1,2]. Center for Neural Science, New York University, New York, NY[1], Howard Hughes Medical Institute[2].

Purpose: Basic measures of spatial vision such as acuity and contrast sensitivity develop to adult levels within the first postnatal year in primates. Recent data from human and non-human primates suggest that more global tasks such as integration of motion signals and detection of features in noise develop over a much slower time scale. These global perceptual tasks require integration of information over wide areas of space and extended time and may depend on higher level neural mechanisms. Methods: To compare the relative development of basic spatial vision and more global perception of form, we studied the development of contour integration in monkeys (*Macaca nemestrina*) ranging in age from 3 months to 2 years. The animals detected a ring of collinear Gabor elements in a field of randomly arrayed Gabor patches. We measured detection thresholds as a function of noise density and Gabor element orientation jitter (up to +/-60 deg). Patch spatial frequency was 3 c/deg, contour element spacing was 1.6 deg, and the noise field subtended 22 deg at 1m. Results: We found that infants younger than 3 months were unable to detect the contour without the aid of a dummy eye, and performance improved slowly to adult levels over at least the first two years. Orientation jitter did not degrade performance differently in infants and older monkeys. Conclusions: On the basis of physiological data on the development of orientation selectivity and receptive field organization, we speculate that the protracted development of contour integration reflects the development of integrative mechanisms beyond the early spatial filters in V1.
CR: None Support: NIH grants EY02864 and 02017, and HHMI.

655—05:45

An enhanced Preferential Looking method for infant studies. L.H. Shams, S. Shimojo. Division of Biology, California Institute of Technology, Pasadena, CA

Purpose: Preferential Looking (PL) has remained one of the most important psychophysical methods of infant research since its inception in 1974. This method suffers, however, from a serious practical shortcoming. It relies on a 2-alternative forced-choice (2AFC) scheme for individual trials. As a result, a large number of trials are required for achieving statistical significance. We have explored a variation of PL which operates upon 4-AFC trials. The forced chance level in each trial (25% vs. 50%) drastically reduces the number of trials needed to achieve that significance. We used a grating acuity test to verify the feasibility of carrying out 4-AFC gaze judgements in infants. Methods: The grating acuity of 13 infants (6-20 weeks of age) was tested using both the conventional 2-AFC PL method, and our 4-AFC variation. The stimuli in the 4-AFC condition differed from those in the conventional PL test in that they contained two additional distractors. The four disks were presented at equal distance from the fixation point, and the target appeared at random in one of the four possible disks. In both conditions a staircase method was used, trials started from low spatial frequency gratings, and ended when the gratings were not detected reliably any longer. 8/10 and 5/6 correct judgements were used as the criteria in the 2-AFC and 4-AFC conditions, respectively, both corresponding to p=0.050. Results: In 12 out of 13 infants, we obtained the same estimates of acuity in both conditions. All of these estimates were within the expected range of acuity (in terms of participants age). In other words, the 4-AFC method results in the same acuity estimates as those obtained by the conventional method but with 60% fewer trials. Conclusions: The results clearly indicate that the 4-AFC judgments of infants gaze directions can be made reliably. By substituting the traditional 2-AFC paradigm with a 4-AFC in PL experiments, a substantial reduction in the number of required trials is achieved. This is due to the substantial increase in the amount of information obtained in individual trials. This substantial increase in efficiency can prove crucial in making a number of infant experiments possible which would be practically very difficult to complete otherwise.
CR: None Support: NIH grant HD08509

656—06:00

Dietary Docosahexaenoic Acid (DHA) and Visual Maturation in the Post-Weaning Term Infant. D.R. Hoffman[1,2], E.E. Birch[1,2], T.S. Castaneda[1], S.L. Fawcett[1], D.G. Birch[1,2], R. Uauy[1]. Retina Foundation of the Southwest, Dallas, TX[1], Dept.of Pediatrics, UT Southwestern[2], Dept of Ophthalmology, UT Southwestern, Dallas, TX[2]

Purpose: Breast-fed infants receive the long-chain polyunsaturated fatty acids, DHA and arachidonic acid (AA) until they are weaned. Since commercial formulas in the U.S. do not contain these important components of neural membranes, we evaluated the benefits of post-weaning dietary supplementation of DHA+AA on visual maturation in a randomized clinical trial. Methods: Healthy term infants (n=61) were breast-fed for 4 to 6 months then randomized to commercial formula (CF: Enfamil® with no DHA or AA) or Enfamil® formula enriched with DHA (0.36%) and AA (0.72%). Measurements of red blood cell (RBC) fatty acids, growth, visual acuity (assessed by swept VEP) and stereoacuity (Infant Random Dot Stereocards) were taken during the pre- and post-weaning period to 1 year of age. Results: Throughout the trial, body weight, length, head circumference and skin-fold measurements were not significantly different between study groups. Prior to weaning, there were no differences in RBC-DHA or -AA content of study infants. However at 1 year of age, RBC-DHA in the CF group was reduced by 32% from the weaning level (4.6% to 2.3% of total fatty acids) while the DHA+AA enriched formula group had a 31% increase in RBC-DHA (4.9% vs. 6.9%). RBC levels of AA also dropped 8% in the CF group in the post-weaning period whereas the DHA+AA group maintained RBC-AA levels at 15.9%. VEP acuity was significantly more mature in the DHA+AA group compared to the CF group (p<0.0005; 0.152 vs. 0.255 logMAR; equivalent to one line on the eye chart). Stereoacuity was equivalent in the two groups at 1 year. There was a significant correlation at 1 year of age such that elevated RBC-DHA levels are associated with more mature VEP acuity ($r = -0.42$; $p = 0.0007$). Conclusions: The need for DHA+AA in infant formula is supported by diet-induced differences in RBC fatty acid levels, mean visual acuities and a correlation between these values at one year of age. These data extend the critical period through the first year of life in which a dietary supply of DHA+AA can optimize visual development in term infants.
CR: 5 Support: NIH grant HD 26980

657—06:15

Simultaneous lightness contrast in 4-month-olds: Do infants use Wallach's ratio rule? S.H.L. Chien, D.Y. Teller, J. Palmer. Department of Psychology, University of Washington, Seattle, WA 98195-1525.

Purpose. Wallach's ratio rule (1948) suggests that the perceived lightness of a disk embedded in a higher luminance surround is determined by the surround/disk (S/D) luminance ratio. Our goal was to test whether infants' lightness perception follows Wallach's ratio rule.

Methods. We developed a new hybrid technique combining familiarization-novelty paradigm (Fagan, 1974) and forced-choice preferential looking procedures (Teller, 1979) to measure infantor novelty responses to a change in perceived lightness. In the familiarization phase of each trial, infants were exposed to two identical 9° disks embedded in a large surround (30°× 48°), with a S/D ratio of 6:1. In the test phase, the surround luminance was increased or decreased by a factor of 3. The infants were presented one disk that maintained the same luminance as the familiarization disks (but with a different S/D ratio) and another disk that maintained the original S/D ratio (but with a different luminance). It is expected that infants will prefer the disk that looks novel to them. The intrinsic preference for each test disk was measured in additional control conditions.

Results. Infants stared consistently more at the luminance-matched disk than at the ratio-matched disk. This behavior indicates that the ratio-matched disk was seen as familiar, whereas the luminance-matched disk was seen as novel in the new surround.

Conclusions. We demonstrated simultaneous lightness contrast in 4-month-olds. Moreover, at least to a first approximation, infants' lightness perception follows Wallach's ratio rule. Experiments with a more finely graded series of test disks are in progress.
CR: None Support: UWRRF, NIH grant 04670-18

000173

Dietary Docosahexaenoic Acid (DHA) and Visual Maturation in the Post-Weaning Term Infant.

D.R. Hoffman[1,2], E.E. Birch[1,3], Y.S. Castaneda[1], S.L. Fawcett[1], D.G. Birch[1,3], R. Uauy[1]
Retina Foundation of the Southwest, Dallas, TX[1]; Dept. of Pediatrics[2] & Dept. of Ophthalmology.[3], UT Southwestern, Dallas, TX

Purpose. Breast-fed infants receive the long-chain polyunsaturated fatty acids, DHA and arachidonic acid (AA) until they are weaned. Since commercial formulas in the U.S. do not contain these important components of neural membranes, we evaluated the benefits of post-weaning dietary supplementation of DHA+AA on visual maturation in a randomized clinical trial.

Methods. Healthy term infants (n=61) were breast-fed for 4 to 6 months then randomized to commercial formula (CF; Enfamil® with no DHA or AA) or Enfamil® formula enriched with DHA (0.36%) and AA (0.72%). Measurements of red blood cell (RBC) fatty acids, growth, visual acuity (assessed by swept VEP) and stereoacuity (Infant Random Dot Stereocards) were taken during the pre- and post-weaning period to 1 year of age.

Results. Throughout the trial, body weight, length, head circumference and skin-fold measurements were not significantly different between study groups. Prior to weaning, there were no differences in RBC-DHA or -AA content of study infants. However at 1 year of age, RBC-DHA in the CF group was reduced by 52% from the weaning level (4.6% to 2.3% of total fatty acids) while the DHA+AA enriched formula group had a 31% increase in RBC-DHA (4.5% vs. 5.9%). RBC levels of AA also dropped 8% in the CF group in the post-weaning period whereas the DHA+AA group maintained RBC-AA levels at 15.5%. VEP acuity was significantly more mature in the DHA+AA group compared to the CF group ($p<0.0005$; 0.152 vs. 0.255 logMAR; equivalent to one line on the eye chart). Stereoacuity was equivalent in the two groups at 1 year. There was a significant correlation at 1 year of age such that elevated RBC-DHA levels are associated with more mature VEP acuity ($r = -0.42$; $p = 0.0007$).

Conclusions. The need for DHA+AA in infant formula is supported by diet-induced differences in RBC fatty acid levels, mean visual acuities and a correlation between these values at one year of age. These data extend the critical period through the first year of life in which a dietary supply of DHA+AA can optimize visual development in term infants.

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Support: NIH grant HD 22380

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Invest. Ophthalmol. Vis. Sci. 42: S122 (2001)

DIETARY SINGLE CELL LONG CHAIN POLYUNSATURATED OILS AT PHYSIOLOGICAL LEVELS DO NOT ALTER SERUM CLINICAL INDICATORS OR ORGAN WEIGHTS IN PIGLETS FED FROM BIRTH TO ONE MONTH.

M.-C. Huang, A. Chao, R. Kirwan, C. Tschanz, J. M. Peralta[1], D. A. Diersen-Schade[2], S. Cha, J. T. Brenna.
Division of Nutritional Sciences and [1]College of Veterinary Medicine, Cornell University, Ithaca, NY, and [2]Mead-Johnson Nutritionals, 2400 West Lloyd Expressway, Evansville, Indiana, USA

Single cell oils are currently included in human infant formula as sources of the long chain polyunsaturates (LCP) docosahexaenoic acid (DHA) and arachidonic acid (AA) in many countries, but have not yet been approved for use in the USA. We prepared four bovine-milk-based formulas with of AA/DHA = 0, 34/17, 68/34, and 170/85 (mg per 100 kcal formula) provided by two commercial single cell oils to test in newborn piglets. These levels correspond approximately to 0, 1, 2, and 5 times the amounts used in infant formulas. All other dietary fat (47% of calories) was provided by a vegetable oil blend used in commercial human infant formulas. Domestic piglets were allowed to nurse with the sow for 24 h after parturition, then removed to individual cages and maintained on one of the four diets. At 30 d of age the piglets were sacrificed, and serum collected and organs weighed. With litters treated as a blocked variable, no significant differences among groups were found by analysis of variance for the following serum assays: alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, albumin, glucose, cholesterol, triglycerides, and total protein. No significant differences were found for hematocrit or body weight. No significant differences were found among groups for weights of any measured organ, analyzed as absolute weight and as a fraction of body weight, for liver, brain, heart, lung, spleen, kidneys, or lung. Hematoxylin/eosin liver sections examined by light microscopy showed no abnormalities. We conclude that single cell oils consumed at levels 5-fold greater than proposed for human infants in the USA do not produce gross toxic effects in neonatal pigs.

CORNELL
UNIVERSITY
DIVISION OF NUTRITIONAL SCIENCES

J. THOMAS BRENNA, PHD
Associate Professor
Member, Graduate Fields Of Nutrition, Of Chemistry & Chemical Biology, And Of Geological Sciences

May 1, 2000

Dr. Deborah A. Diersen-Schade
Principal Research Scientist
Mead-Johnson Nutritionals
2400 West Lloyd Expressway
Evansville, IN 47721-0001

Dear Dr. Diersen-Schade:

I am writing to confirm that our abstract "Dietary single cell long chain polyunsaturated oils at physiological levels do not alter serum clinical indicators or organ weights in piglets fed from birth to one month.", by M.-C. Huang, C. Chao, R. Kirwan, C. Tschanz, J. M. Peralta, D. A. Diersen-Schade, S. Cha, and J. T. Brenna, has been accepted for presentation at the 4th International Conference of the International Society for the Study of Fatty Acids and Lipids (ISSFAL). The conference is to be held in Japan, June 1-5, 2000.

As you know, the abstract is the first report of our recent study of single cell oils as sources of long chain polyunsaturates in neonates. The tested oils were DHASCO™ and ARASCO™, manufactured by Martek Biosciences, Inc.

Sincerely,

J. Thomas Brenna

000176

SAVAGE HALL • ITHACA, NEW YORK, USA • 14853
PHONE: (607)255-9182 • FAX: (607)255-1033 • E-MAIL: JTB4@CORNELL.EDU

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FASEB J 2001;15:A643 (abstract)

505.13

Comparison of dietary sources of long-chain polyunsaturated fatty acids (LCPUFA) in the neonatal piglet: Assessment of clinical parameters.

Susan A Mathews[1], Robert J Harrell[1], William T Oliver[1], Jack Odle[1], Deborah A Diersen-Schade[2]: [1]North Carolina State University, Box 7621, Raleigh, NC 27695-7621, [2]Mead-Johnson Nutritionals, 2400 West Lloyd Expressway, Evansville, IN 47721

Supplementation of infant formulas with arachidonic acid (AA) and docosahexaenoic acid (DHA) promotes visual and neural development. The objective of this study was to determine if the dietary source of LCPUFA would affect growth and various plasma parameters. Piglets (n=10/group) had ad libitum access from day 1 to 16 of age to a skim milk-based formula with different fat sources added to provide 50% of the energy. Formulas included LCPUFA as follows: single cell oil triglyceride (TG), egg phospholipid (EPL), TG with phospholipid and cholesterol added to match EPL (TG+PL), and a control with no added LCPUFA (CTL). Formulas with LCPUFA provided 0.6% of fatty acids as AA and 0.3% as DHA. All piglets grew at a similar rate (326±17 g/d) ($P>0.2$). Total plasma cholesterol concentrations were similar in all piglets. No differences in alanine aminotransferase were detected among treatments, but aspartate aminotransferase, a marker of putative cellular damage, was elevated ($P<0.03$) in EPL fed piglets compared to TG+PL fed piglets. Total plasma AA concentration (expressed as weight % of total lipid fatty acids) was greater in the TG fed piglets compared to the CTL piglets ($P<0.05$), but there were no differences among the TG, TG+PL or EPL fed piglets ($P>0.2$). Total plasma DHA concentrations were greater in the TG fed piglets compared to the EPL piglets ($P<0.06$) or the CTL piglets ($P<0.02$), but no differences were detected between the EPL and CTL piglets ($P>0.4$). These data demonstrate that the single cell TG source of AA and DHA may be a more efficacious supplement for infant formulas based on the plasma fatty acid profiles.

505.11

DIETARY POLYUNSATURATED FATTY ACIDS DO NOT AFFECT FETAL STEROL SYNTHESIS RATES.

Kara E Schmid, Laura A Woollett: University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267

The rate of growth during gestation is rapid and unmatched by any other stage of life. For this rate to be possible, the fetus needs a large quantity of cholesterol. Cholesterol is necessary for membrane formation, hormone synthesis and development of the central nervous system. Consequently, defects in cholesterol metabolism lead to severe developmental abnormalities. Preliminary studies in male hamsters demonstrated that dietary polyunsaturated fatty acids cause a significant decrease in hepatic cholesterol synthesis rate. If fetal tissues respond to dietary fatty acids comparable to the adult, then fatty acids that decrease cholesterol synthesis rates could impair growth and differentiation in the fetus. To study the impact of maternal dietary fatty acids on fetal sterol metabolism, female hamsters were fed diets enriched in either medium chain triglycerides (MCT oil), oleic acid (olive oil), linoleic acid (corn oil), or saturated fatty acid (hydrogenated coconut oil (HCO)) for 21 days, and then mated. On gestational day 11, *in vivo* sterol synthesis rates were measured in fetal tissues. Contrary to the adult, sterol synthesis rates in the fetus and fetal tissues were not affected in dams fed corn oil as compared to MCT-fed dams. However, sterol synthesis rates did decrease by 20% ($p<0.05$) in fetuses of dams fed HCO. In the yolk sac, sterol synthesis rates were 40% and 20% lower ($p<0.05$) in dams fed HCO or olive oil, respectively. Despite a decrease in sterol synthesis rates, cholesterol concentrations in the fetus and fetal tissues were similar, independent of maternal diet. These data show that fetal sterol synthesis does not respond to dietary fatty acids like the adult. These differences may be due to differential expression of regulatory proteins that control cholesterol synthesis in the fetus versus the adult.

505.12

SN-2 PALMITIC ACID IS ONLY A PARTIAL EXPLANATION FOR FAT ABSORPTION LEVELS IN RATS FED INFANT FORMULA FAT BLENDS

Eric L. Lien, Rebecca J. Yuhas, Suzanne M. Mason, Frances G. Boyle: Wyeth Nutritionals International, P O. Box 42528, Philadelphia, PA 19101

Absorption of total fat and saturated fatty acids (SFA) is dependent on the triglyceride (TG) SFA positional distribution. Absorption of TGs containing high levels of palmitic acid (PA) may be more efficient if PA is present in the sn-2 position, due to intestinal production of 2-monopalmitin. Betapol and butterfat have relatively high sn-2 PA levels and would be expected to have relatively efficient absorption levels.

Methods: Male rats were fed a fat-free diet prior to the study. Rats received test diets (control-C, Betapol blend-B or butterfat blend-BF; all fat blends contained equivalent amounts of SFA) for three days followed by fat-free diet for three days. The C fat blend was a typical infant formula blend containing soy, high oleic safflower, coconut and palm oils. Food consumption was measured and feces were collected during the six day study period. Total fat and SFA absorption were measured. Fatty acid positional distribution and fatty acid configuration of TGs were determined.

Results: The sn-2 PA levels in the fat blends were similar in the B and BF groups (48.0%, 47.5% respectively) and much higher than the C group (15.2%). Surprisingly, PA excretion was similar for C (12.8 ± 1.2%, mean ± SEM) and BF (10.4 ± 0.9%) groups and significantly higher than the B group (3.5 ± 0.4%). Total fat excretion followed a similar pattern. TG structure analysis of the fat blends indicated that the BF diet had a higher proportion of TGs with tri-SFAs than the B group.

Conclusions: Both positional distribution and triglyceride structure are important determinants in fat absorption in infant formula fat blends.

505.13

Comparison of dietary sources of long-chain polyunsaturated fatty acids (LCPUFA) in the neonatal piglet: Assessment of clinical parameters.

Susan A Mathews[1], Robert J Harrell[1], William T Oliver[1], Jack Odle[1], Deborah A Diersen-Schade[2]: [1]North Carolina State University, Box 7621, Raleigh, NC 27695-7621, [2]Mead-Johnson Nutritionals, 2400 West Lloyd Expressway, Evansville, IN 47721

Supplementation of infant formulas with arachidonic acid (AA) and docosahexaenoic acid (DHA) promotes visual and neural development. The objective of this study was to determine if the dietary source of LCPUFA would affect growth and various plasma parameters. Piglets (n=10/group) had ad libitum access from day 1 to 16 of age to a skim milk-based formula with different fat sources added to provide 50% of the energy. Formulas included LCPUFA as follows: single cell oil triglyceride (TG), egg phospholipid (EPL), TG with phospholipid and cholesterol added to match EPL (TG+PL), and a control with no added LCPUFA (CTL). Formulas with LCPUFA provided 0.6% of fatty acids as AA and 0.3% as DHA. All piglets grew at a similar rate (326±17 g/d) ($P>0.2$). Total plasma cholesterol concentrations were similar in all piglets. No differences in alanine aminotransferase were detected among treatments, but aspartate aminotransferase, a marker of putative cellular damage, was elevated ($P<0.03$) in EPL fed piglets compared to TG+PL fed piglets. Total plasma AA concentration (expressed as weight % of total lipid fatty acids) was greater in the TG fed piglets compared to the CTL piglets ($P<0.05$), but there were no differences among the TG, TG+PL or EPL fed piglets ($P>0.2$). Total plasma DHA concentrations were greater in the TG fed piglets compared to the EPL piglets ($P<0.06$) or the CTL piglets ($P<0.02$), but no differences were detected between the EPL and CTL piglets ($P>0.4$). These data demonstrate that the single cell TG source of AA and DHA may be a more efficacious supplement for infant formulas based on the plasma fatty acid profiles.

505.14

Nutrient intake, Complementary Feeding Practices, and Mothers' Beliefs about Young Children in central Mexico.

Guadalupe Rodriguez[1], Raul Borbolla[1], Lourdes Flores[2], Cecilia Cortes[2], Nicté Castañeda[2], Homero Martinez[2], Marian Villanueva[2], Helena Pachon[4], Elena Hurtado[4], Juan Rivera[3]: [1]Cornell University, Savage, B18, Ithaca, NY 14850, [2]Instituto Nacional de Salud Publica, Mexico, Av. Universidad 655, Cuernavaca, Mor 62508 Mexico, [3]Instituto Mexicano del Seguro Social, Av. Cuahutemoc, DF, Mexico, [4]Emory University, 1518 Clifton Road, Atlanta, GA 30322

Two of the main problems associated with complementary feeding in poor, rural, Mexican villages are the use of foods with low energy and micronutrient density and the presence of taboos related to foods. Inadequate micronutrient intakes are associated with stunting, higher morbidity risk, and impaired intellectual performance. To determine complementary feeding intake, knowledge, and beliefs, 159 mother-children pairs in two rural communities in central Mexico were studied. Formative research using rapid assessment methods was conducted to examine feeding practices and beliefs regarding complementary feeding among children 0-24 mo. Quantitative dietary assessment methods included 24-recall, recipe trials, and market surveys. Qualitative methods included free-listing, pile-sort and attributes surveys, semi-structured interviews, focus groups, and behavioral observations. The energy density of the diet, the frequency of feeding and the intake of animal products were low relative to recommendations. Forty seven percent of the children received solid foods before 4 mo of age and 20% after 7 mo of age. Gastro-intestinal illness and the presence of teeth in young children were the negative attributes most related to the non-consumption of iron- and zinc-rich foods. Lack of knowledge about preparation of some legumes and vegetables were observed. Many mothers were found to be passive feeders of their infants. Mothers' perceptions and values about foods, feeding dynamics, and food preparation are limiting the appropriate introduction and intake of highly nutritious foods commonly available at the household level. This information has been used to create a manual for the development of community- tailored interventions to improve infant feeding for Latin America. Funding: PAHO PO-12437 (SP)

505.15

CAN DRIED BLOOD SPOT RETINOL BE USED TO ASSESS VITAMIN A STATUS?

Neal E. Craft, Lisa K. Brindle, Yulang Li: Craft Technologies, Inc., 109 Park Ave., Wilson, NC 27893

The most widely accepted means of assessing vitamin A deficiency (VAD) is the measurement of serum retinol. The collection of blood samples for biochemical analysis can be very problematic in developing countries. The collection of dried blood spots (DBS) is simple, convenient, acceptable, and safer than drawing venous blood. The comparison and interchangeability of serum retinol with DBS retinol is described. The objectives were: 1) to correlate serum retinol concentrations to DBS retinol and 2) to establish the sensitivity and specificity of DBS retinol in various populations. DBS samples and matching sera were received from Nepal, Liberia, and Guatemala. Both serum retinol and DBS retinol were measured by HPLC. DBS retinol was correlated to serum retinol and the sensitivity and specificity for identifying VAD was established. DBS retinol values correlated well ($r = 0.8$-0.9) with serum retinol measured by HPLC. Compared to serum retinol cut-points, sensitivity (76-89%) and specificity (85-98%) were very good although they varied slightly between populations. In the subset of samples compared from different populations, DBS retinol correlated very well with serum retinol. Since both serum retinol and DBS retinol are a measure of the same indicator, DBS was highly predictive of VAD. Thus, to the extent that serum retinol is a predictor of VA status, DBS retinol can be used to assess VA status.

Funding provided by The Micronutrient Initiative/IDRC, Task Force Sight & Life, UNICEF.

505.16

Nutritional Programming of Hepatic IGF-I Gene Expression in Rat Pups

Ng Dakfilla, Lino Balonan, Hwai-Ping Sheng: The University of Hong Kong, Pokfulam Road, Hong Kong, Hong Kong

Postnatal nutritional environment has been shown to affect plasma levels of growth hormone (GH) and insulin-like growth factors (IGFs), thus affecting early growth. However, whether alterations of the GH-IGF-I axis persist into adulthood thus leading to metabolic abnormalities in later life is not known. Our objective is to investigate the short-term and long-term effects of early nutrition on plasma IGF-I levels and hepatic IGF-I gene expressions. Newborn SD rats were reared in litter sizes of 4 (Gp S), 10 (Gp M) and 16 (Gp L) pups to induce different rates of growth. Pups were then weaned at age 21 d to an ad libitum standard rat chow until age 90 d. Plasma IGF-I levels and hepatic IGF-I mRNA levels were measured at 20, 60 and 90 d of age. Our data showed that mean body weight of pups in Gp L lagged behind that of the other 2 groups at weaning and at 60 d of age. At 90 d of age, mean weight of Gp L pups was similar to that of Gp S and Gp M showing that catch-up growth had occurred. Mean plasma IGF-I levels in Gp L (165±39 ng/mL) was significantly lower than those in Gp S (270±35) at weaning, and it remained lower, though not significantly (L: 1120±90 and 1169±80; S: 1250±125 and 1331±72), at 60 and 90 d of age. However, mean hepatic IGF-I mRNA levels were higher in Gp L (142, 220±48 and 226±26) than those in group S (118±67, 132±33 and 170±3) at 20, 60 and 90 d respectively. Our data suggest that nutritional programming of IGF-I gene expression occurred and over-expression of hepatic IGF-I mRNA and low plasma IGF-I level induced by early undernutrition probably involved post-translational defects in hepatic IGF-I synthesis.

Acknowledgement: RGC: HKU 7190/99M and CRCG grants

000227.001

AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

Program number 20.9 Poster S77 is now scheduled in session 687, poster board B49A

Program number 6.14 Poster S14 is now scheduled in session 439, poster board B124A

Program number 27.1 Poster R1 is now scheduled in session 437, poster board B106A

Program number 6.37 Poster S37 is now scheduled in session 694, poster board B150A

Program number 27.6 Poster R6 is now scheduled in session 177, poster board B36A

The ASBMB Public Affairs Meeting scheduled to be held March 31 at 9:00 AM in the Rosen Centre has been cancelled.

Protein Phosphorylation and Molecular Recognition Satellite Meeting - The oral presentation for M. Delhase, Program number 2.5 will now be made by J. D. Selengut, Program number 6.28.

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Session #67: Graduate Student Convocation: New Era in Academic and Industrial Partnership in Basic and Applied Research, and Funding the Modern Lab

The speakers' talk titles are as follows:

R.J. Shebuski: Bridging academic and industrial discoveries in the process of novel drug development.
B.R. Lucchesi: Academia, industry, training and research: a partnership with common goals.
M.W. Decker: The culture and science of drug discovery: negotiating the academic/industrial interface.
M.D. Johnson: Application of novel technologies in preclinical pharmaceutical development: integration of diverse skill sets, individual backgrounds and outlooks to meet a scientific challenge.

Session 455: Chemotherapy
Program #455.14 will not be presented.

Session 707: Education Workshop: Information Technology Acquisition of Integrative Pharmacology through the World Wide Web
S. Reents' talk title should read: Clinical Pharmacology 2000: a web-based clinical drug information resource.

Session 711: Drug Discovery and Development
Abstract 711.1 should have Scott Thomas listed as a co-author.

Session 715: Free Radicals and Antioxidants
Program #715.7 will not be presented.

The following abstract should appear in the late-breaking abstract poster session titled, "Neuropharmacology":

The interaction of morphine and GABAergic systems in anxiolytic behavior: using mu-opioid receptor knockout mice

K. Sasaki, L.-W. Fan, L.-T. Tien, T. Ma and I.K.Ho. Dept. of Pharmacol. and Toxicol., Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

This study was designed to investigate the involvement of mu-opioid receptor and GABA(A) receptor in anxiolytic action using mu-opioid receptor knockout and wild type female mice. Elevated plus-maze test was used to assess anxiolytic behavior. Both mu-opioid receptor knockout and wild type animals, 7-8 animals in each group, received a dose of muscimol, 0.5 mg/kg, s.c., 3 h after the pretreatment of morphine, 15 mg/kg, s.c.. The anti-anxietic activities were monitered 20 min after the muscimol administration. In another experiment, both groups received a dose of naloxone, 1.0 mg/kg, i.p., an opioid antagonist, or (+)bicucullin, 2.0 mg/kg, i.p., a GABA(A) receptor antagonist, 2.5 h after the morphine and 30 min before the muscimol injection. In each experiment the control mice received vehicle. The anxiety-related behaviors were recorded for a period of 5 min by VCR-recording system and were analyzed. The results show that morphine enhanced muscimol-induced staying time in open arms by 280% in wild type mice. Moreover, the effect of morphine in the wild type was inhibited by the pretreatment of either naloxone or (+)bicucullin. These effects were not observed in the mu-opioid receptor knockout mice. These results indicate that mu-opioid receptor plays an important role in the modification of anxiolytic behavior regulated by GABA-mediated synaptic transmission.

AMERICAN SOCIETY FOR NUTRITIONAL SCIENCES

Poster Session 505 - Maternal and Infant Nutrition 1
Mon., 7:30 AM - 8:30 AM – Orange County Convention Center, Hall B
Poster Board B487A

000228

Comparison of dietary sources of long-chain polyunsaturated fatty acids (LCPUFA) in the neonatal piglet: Impact on intestinal and liver parameters.

Susan A Mathews[1], Robert J Harrell[1], William T Oliver[1], Jack Odle[1], Deborah A Diersen-Schade[2]: [1]North Carolina State University, Box 7621, Raleigh, NC 27695-7621, [2]Mead-Johnson Nutritionals, 2400 West Lloyd Expressway, Evansville, IN 47721

Addition of arachidonic acid (AA) and docosahexaenoic acid (DHA) to infant formula promotes visual and neural development. This study was designed to determine if the source of dietary LCPUFA affected intestinal morphology (n=5), lactase activity (n=10), apparent dry matter digestibility (n=10), or liver histology (n=10). Piglets had ad libitum access from day 1 to 16 of age to a skim milk-based formula with different fat sources added to provide 50% of the energy. Formulas included LCPUFA as follows: single cell oil triglyceride (TG), egg phospholipid (EPL), TG with phospholipid and cholesterol added to match EPL (TG+PL), and a control with no added LCPUFA (CTL). Formulas with LCPUFA

provided 0.6% of fatty acids as AA and 0.3% as DHA. CTL piglets had 40% longer ileal villi than the EPL fed piglets (P<0.03), but the TG and TG+PL fed piglets were similar to CTL. No differences were detected in villi width, crypth depth, lactase specific activity, absolute liver weights, or gross liver histologic parameters (P>0.1). Apparent dry matter digestibility was 10% greater in the CTL, TG, and TG+PL fed piglets compared to the EPL fed piglets (P<0.002). These data demonstrate that the EPL source of preformed AA and DHA reduces villi length and apparent dry matter digestibility and suggest that the single cell TG source of LCPUFA may be preferred for supplementing infant formulas.

AMERICAN SOCIETY FOR NUTRITIONAL SCIENCES

Poster Session 496 - Dietary Intake and Clinical Dietetics
Mon., 7:30 AM - 8:30 AM – Orange Country Convention Center, Hall B
Poster Board B380A

Patterns of Use of Supplements containing Iron among Women of Reproductive Age in the United States.
Usha Ramakrishnan[1], Morgen Hickey[1], Laura Kettel Khan[2], Mary Cogswell[2]: [1]Emory University, Atlanta, GA 30322, [2]Centers for Disease Control and Prevention, Atlanta, GA
Introduction: The promotion of multivitamin mineral (MVTM) supplements containing iron (Fe) has been suggested for women of reproductive age who are at increased risk for iron deficiency. **Objective:** To describe and identify correlates of patterns of use of supplements containing Fe among women of reproductive age (12-49 y) in the United States. **Methods:** Details of supplement use including type, composition and frequency of use in the past month were obtained for 5924 women in the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. The overall patterns of use and socio-demographic correlates within race-ethnicity groups were estimated using SUDAAN. **Results:** Twenty-two percent consumed supplements containing Fe, of which 76.3% reported daily use. Among supplement users, 78.1% used multi-vitamin mineral combinations, 14.8% used Fe only supplements and 5.8% used both types and the mean amount of Fe ingested was 26 mg/day for MVTM and 69.6 mg/day for Fe only supplements. The use of supplements containing Fe differed significantly by race-ethnicity group; 24.1% for NHW, 17.2% for NHB, and 15.2% for MA women. Adolescents (12-19 y) were less likely to consume supplements compared to adult women (20-49 y) for NHW (O.R=0.38, 95% CI: 0.25, 0.58) and NHB (OR=0.47, 95% CI: 0.47, 0.26, 0.65). Post-partum status (<1y) was positively associated with supplement use among NHB and MA women after adjusting for age and other socio-demographic correlates. Education and income were positively associated with supplement use in NHW women.
Conclusions: Groups at higher risk of iron deficiency, e.g., Mexican American women and low-income women, are least likely to take supplements containing iron, suggesting supplement use is unrelated to actual need. (Supported by NIH: HD-34531).

AMERICAN ASSOCIATION OF IMMUNOLOGISTS

SUNDAY, April 01
Program Changes:

Session 261: Block Symposium: MHC Class I: Structure and Function
The following presentation, scheduled for 8:00 AM is cancelled.

Crystal Structures of Non Canonical Binding Peptides: Low Affinity Vs High Affinity Binding [Vasso Apostolopoulos[1], Minmin Yu[1], Geoffrey Pietersz[2], Wenjun Li[2], Luc Teyton[1], Ian McKenzie[2], Ian Wilson[1]]1The Scripps Research Institute, [2]The Austin Research Institute

Session 269: B Cell Development
The following paper will not be presented.

Adenovirus Transfection with Inducible Nitric Oxide Synthase (iNOS) Decreases Arginase Activity in Bovine Pulmonary Arterial Endothelial Cells (bPAEC) [Kate P Stanley, Louis G Chicoine, Leif D Nelin] University of New Mexico

Session 270: MHC Class I: Structure and Function
The following presentation is cancelled.

Crystal Structures of Non Canonical Binding Peptides: Low Affinity Vs High Affinity Binding [Vasso Apostolopoulos[1], Minmin Yu[1], Geoffrey Pietersz[2], Wenjun Li[2], Luc Teyton[1], Ian McKenzie[2], Ian Wilson[1]]1The Scripps Research Institute, [2]The Austin Research Institute

The following presentation has been added.

Aminopeptidase Trimming of Peptides in the ER is Required for Proper Loading of MHC I Molecules [Thomas Serwold, Stephanie Gaw, Nilabh Shastri] University of California-Berkeley

Session 273: Mediators of Inflammation and Leukocyte Migration
The following presentation is cancelled.

Tumor Necrosis Factor a-induced Activation of Downstream NF-κB site of the Promoter mediates Epithelial ICAM-1 Expression and Neutrophil Adhesion :Involvement of PKCa, Tyrosine Kinase and IKK2, but not MAPKs, pathway [Ching-Chow Chen, Chian-Yin Chou: Pharmacology, No.1, Jen-Ai Rd, 1st Section, Taipei, 10018 Taiwan]

Session 275: Immune System Regulation and Tolerance I: Mechanisms of Costimulation
The following presentation is cancelled.

B7-dependent costimulation present in mice lacking CD28 and CTLA-4 [Mariette A. Oosterwegel[1], Didier A. Mandelbrot[2], Gordon J. Freeman[3], Arlene H. Sharpe[4]] [1]UMC Utrecht, [2]Brigham and Women's hospital, [3]Dana Farber Cancer Institute, [4]Brigham and Women

Experimental Biology

2001

March 31 - April 4, 2001

Orlando, FL

Program Addendum

000230

Effect of increasing dietary long chain polyunsaturates (LCP) with high vitamin E on lipid peroxidation and susceptibility to oxidation of piglet erythrocytes, plasma, and liver

E. Sarkadi Nagy, M.C. Huang, R. Kirwan, A. Chao, C. Tschanz, J.T. Brenna*
Cornell University, Ithaca, NY; *<jtb4@cornell.edu>

SUMMARY. The purpose of this study was to determine the effects of increasing amounts of dietary docosahexaenoic acid (DHA) and arachidonic acid (AA) on lipid peroxidation and in vitro susceptibility to oxidation in piglet tissues. Twenty four one-day old piglets (n=6 per group) were bottle-fed a sow milk replacement formula containing one of four experimental treatments: no long chain fatty acid ("Diet 0") and three different levels of DHA/AA at 1-fold (0.3%/0.6% FA; "Diet 1") 2-fold (0.6%/1.2% FA; "Diet 2") and 5-fold (1.5%/3% FA; "Diet 5") concentration of human infant formula, and with equal amounts of vitamin E (260 mg/kg diet) for four weeks.

Red blood cell (RBC) membrane total lipid DHA and AA increased in an approximately dose-dependent manner, while 18:2n-6 (linoleic acid) and 18:3n-3 (linolenic acid) concentrations decreased. These modifications resulted in an increase in the membrane unsaturation index from Diet 0 to Diet 2, but not for Diet 5. Lipid peroxidation was assessed by measuring conjugated diene and glutathione (GSH) levels in the liver, and thiobarbituric acid-reactive substance (TBARS) in plasma. There were no significant differences between the groups in any of these parameters. Oxidative stability was assessed by determination of TBARS concentration after exposure of RBCs to 10 mM H_2O_2, and liver homogenates to various concentrations of H_2O_2 (0,1,5,10 mM) for 30 minutes. TBARS levels of the erythrocyte membranes increased in a dose-dependent manner when in vitro oxidation was induced. The TBARS levels of the liver homogenates of the Diet 5 group were significantly different than those of the membranes of the Diet 0 group when the in vitro oxidation was induced with only the highest concentration of H_2O_2 (10 mM).

We conclude that there was no increase in oxidation due to high dietary and tissue LCP with this relatively high vitamin E concentration. The susceptibility of RBC and liver of piglets in the Diet 5 group to in vitro oxidation was increased compared to the Diet 0 group only under extreme oxidative conditions. Overall, the results suggest that the dietary vitamin E effectively prevented oxidation at the LCP concentrations investigated.

PAGES 000232-000239 HAVE BEEN REMOVED IN ACCORDANCE WITH COPYRIGHT LAWS
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HAVE BEEN REMOVED FROM THIS REQUEST

End Submission

[redacted]

Reference List for Industry Submission, GRN 000080

Pages	Author	Title	Publish Date	Publisher	BIB Info
000094-000102	Arterburn, L. M., Boswell, K.D., Henwood, S.M., Kyle, D.J.	A developmental safety study in rats using DHA- and ARA-rich single cell oils	2000	Food and Chemical Toxicology	Vol. 38, pp. 763-771
000103-000110	Birch, Eileen, E., Garfield, Sharon., Hoffman, Dennis, R., Uauy, Ricardo, Birch, David, G.	A randomized controlled trial of early dietary supply of long chain polyunsaturated fatty acids and mental development in term infants	2000	Developmental Medicine & Child Neurology	Vol. 42, pp. 174-181
000111-000119	Birch, Eileen, E., Hoffman, Dennis, R., Uauy, Ricardo., Birch, David, G., Prestidge, Claude	Visual Acuity and the Essentiality of Docosahexaenoic Acid and Arachidonic Acid in the Diet of Term Infants	1998	Pediatric Research	Vol. 44, No. 2, pp. 201-209
000120-000128	Boswell, K., Koskelo, E.K., Carl, L., Glaza, S., Hensen, D.J., Williams, K.D., Kyle, D.J.	Preclinical Evaluation of Single-cell Oils that are Highly Enriched with Arachidonic Acid and Docasahexaenoic Acid	1996	Food and Chemical Toxicology	Vol. 34, pp. 585-593
000129-000142	Burns, R.A., Wibert, G.J., Diersen-Schade, D.A., Kelly, C.M.	Evaluation of Single-cell Sources of Docosahexaenoic Acid and Arachidonic Acid: 3-Month Rat Oral Safety Study with An In Utero Phase	1999	Food and Chemical Toxicology	Vol. 37, pp. 23-36
000145-000149	Diersen-Schade, Deborah, A., Hansen, James, W., Harris, Cheryl, L., Merkel, Kimberly, L., Wisont, Karen, D., Boettcher, Julia, A.	Docosahexaenoic Acid Plus Arachidonic Acid Enhance Preterm Infant Growth	1998	Essential Fatty Acids and Eicosanoids: Invited Papers from the Fourth International Congress	pp. 123-127
000150-000157	NA	Lipids in early development	1994	FAO Food and Nutrition Paper 57	pp. 49-55
000158-000158	Hansen, J., Schade, D., Harris, C., Merkel, K., Adamkin, D., Hall, R., Lim, M., Moya, F., Stevens, D., Twist, P.,	Docosahexaenoic Acid plus Arachidonic Acid Enhance Preterm Infant Growth	1997	Prostaglandins Leukotrienes and Essential Fatty Acids	Vol. 57, pp. 196

NA- Not applicable

Pages	Author	Title	Publish Date	Publisher	BIB Info
000159-000172	Hoffman, Dennis, R., Eileen, E.Birch., Birch, David G., Uauy, Ricardo., Castaneda, Yolanda, S., Lapus, Maia, G., Wheaton, Dianna, H.	Impact of Early Dietary Intake and Blood Lipid Composition of Long-Chain Polyunsaturated Fatty Acids on Later Visual Development	November 2000	Journal of Pediatric Gastroenteriology and Nutrition	Vol. 31, No. 5, pp. 540-553
000177-000202	Huang, Meng-Chuan., Chao, Angela., Kirwan, Ryan., Tschanz, Carolyn., Peralta, Jose, M., Diersen-Schade, Deborah, A., Cha, Susan., Brenna, J, Thomas	Dietary Single Cell Long Chain Polyunsaturated Oils At Physiological Levels Do Not Alter Serum Clinical Indicators or Organ Weights in Piglets Fed From Birth To One Month	March 2001	Food and Chemical Toxicology	pp. 2-26
000203-000208	Innis, Sheila, M.	Human milk and formula fatty acids	April 1992	The Journal of Pediatrics	Vol. 120, No. 4, Part 2, pp. S56-S61
000209-000217	Koletxo, Berthold., Thiel, Iris., Abiodun, Philip O.	The fatty acid composition of human milk in Europe and Africa	April 1992	The Journal of Pediatrics	Vol. 120, No. 4, Part 2, pp. S62-S70
000218-000226	Koskelo, E.K., Boswell, K., Carl, L., Lanoue, S., Kelly, C. Kyle, D.	High Levels of Dietary Arachidonic Acid Triglyceride Exhibit No Subchronic Toxicity in Rats	1997	Lipids	vol. 32, No. 4, pp. 397-405
000232-000239	Wibert, G.J., Burns, R.A., Diersen-Schade, D.A., Kelly, C.M.	Evaluation of Single Cell Sources of Docosahexaenoic Acid and Arachidonic Acid: a 4-Week Oral Safety Study in Rats	1997	Food and Chemical Toxicology	Vol. 35, pp. 967-974

NA- Not applicable

Thomas L. Ferguson
Director, Regulatory Affairs
(812) 429-7886
(812) 429-5054 (fax)

Mead Johnson Nutritionals

Fax

To:	Dr. Linda Kahl	**From:**	Tom Ferguson
Fax:	(202) 418-3131	**Pages:**	2 (including cover memo)
Phone:	(202) 418-3101	**Date:**	November 14, 2001
Re:	FoxKiser	**CC:**	Diane Robertson [Fax: (202) 778-2330]

☒ **Urgent** ☐ **For Review** ☐ **Please Comment** ☐ **Please Reply** ☐ **Please Recycle**

• **Comments:**

Dr. Kahl,

Attached is a letter confirming that Diane is representing Mead Johnson Nutritionals. I will mail the original copy to you for your files. Please call me at (812) 429-7886 if you have any questions.

Thanks,

Tom Ferguson

Mead Johnson Nutritionals

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

November 14, 2001

PROPRIETARY

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

Dear Dr. Kahl:

This letter is to confirm that FoxKiser and, specifically, Diane E. Robertson are representatives of Mead Johnson Nutritionals.

Sincerely,

Thomas L. Ferguson
Director, Regulatory Affairs
Mead Johnson Nutritionals

Enclosures

cc: Diane E. Robertson (FoxKiser)

000255

AM

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Sent by Facsimile - 1-202-418-3131

November 15, 2001

Linda S. Kahl, Ph.D.
HFS-206
200 C Street, SW
Washington, DC 20204

RE: Our letter dated July 11, 2001, to Mead Johnson re the use of ARASCO and DHASCO in infant formula

Dear Dr. Kahl:

In response to your facsimile letter to me of earlier today, please be advised that your suggested sentence is appropriate, with one suggested change. To further capture the concept set forth in the sentence, I would suggest the insertion of the word "term" at one additional place in the sentence, as indicated by underlining below:

> "The July letter from Martek to Mead Johnson informs Mead Johnson that Martek had intended that its GRAS notice for use of ARASCO and DHASCO in term infant formula cover all such term infant formulas that were being developed in the U.S."

Please let me know if I can provide you with any further information or explanation with respect to Martek's July 11 letter pertaining to Mead Johnson's GRAS notification.

Sincerely,

Henry Linder, Jr.
Chief Executive Officer

000258

6480 Dobbin Road
Columbia, Maryland 21045

(410) 740-0081
Fax (410) 740-2985
www.martekbio.com