VII. HUMAN CLINICAL TRIALS

A. Introduction

Ross Products Division conducted two large, multicenter, prospective, randomized clinical trials to evaluate the addition of AA + DHA to infant formulas. The trials included a study of 470 preterm infants provided supplemented or control formula and/or human milk from the time of first enteral feeding through 12-months term corrected age (CA), and a study of 404 term infants provided AA + DHA supplemented or control formula (or human milk and formula) through the first 12 months of life. Eighty-two term infants and 158 preterm infants were randomized to formulas supplemented with an AA-rich oil (SUNTGA) and DHA-rich oil (from tuna oil), the sources of AA and DHA that are the subject of this GRAS determination. Reports of these clinical trials have been published (O'Connor et al. 2001; Auestad et al. 2001). Summaries of the trials are provided below; the summaries include information from both published and unpublished reports (O'Connor et al. 2001; Auestad et al. 2001; Ross Products Division 1998,1999a, b, 2000).

The trials were designed to determine if adding AA and DHA from different sources to formulas supported growth and enhanced selected developmental outcomes. Assessment of growth was a primary outcome variable in each of the studies. The visual and developmental tests in the trial with preterm infants and the trial with term infants were chosen after consultation with experts in the visual and behavioral sciences, many of whom also participated as investigators in these trials.

Establishing an association between an intervention, such as supplementation of formula with AA and DHA, and a specific developmental outcome, requires that a reliable association be demonstrated between the intervention and the outcome. Methodological and procedural considerations are important for obtaining reliable results from tests of infant development. The central nervous system develops rapidly during infancy and is determined in large part by precisely regulated genetic processes together with interactions with a variety of environmental influences. In these trials, therefore, extensive maternal and family demographic information was obtained to demonstrate comparability among groups and when appropriate, to include known influential demographic variables as covariates to enhance the sensitivity of specific comparisons.

Criteria for the assessment methods in these trials required that the tests be standardized (i.e. having a uniform set of procedures for administration and scoring), be reliable (measure a cohesive construct in a similar manner across multiple administrations and time), and be valid (i.e. measure the construct intended). Multiple assessments of development were recommended and within practical limitations, more than one time point was preferable. As indicated in the reports from these studies, reliability across multiple testers was established.

The Bayley Scales of Infant Development was included as this is the most common assessment of overall infant development. However, although this test has been standardized

and validated, it is 'broad-based' and assesses infant functioning across multiple abilities. Further, it does not provide quantifiable information on more discrete processing skills such as language, attention, or rate of information processing. Therefore, these two trials also employed the Fagan Test of Infant Intelligence to evaluate information processing, and the MacArthur Communicative Developmental Indices to evaluate early vocabulary (language) development. Many of the developmental tests, including the tests of visual development, rely on the attentiveness of the infant to the test. The Infant Behavior Questionnaire, which provides an assessment of infant temperament, therefore was also included.

The Teller Acuity Card Procedure was included as this is the most common assessment of visual acuity, and a reliable, standardized validated test instrument is available. However, although this test has been standardized and validated, it is thought to lack the sensitivity and specificity that the visual evoked potential (VEP) method of assessing visual acuity provides. The VEP method was also included in the preterm trial of AA and DHA assessment, and previously in a Ross study with term infants (Auestad et al. 1997).

B. Clinical Trial of Growth and Development of Preterm Infants Fed Formula Containing SUNTGA25 AA-Rich Oil and DHA-Rich Tuna Oil

Between October 24, 1996, and June 5, 1998, Ross Products Division conducted an intent-to-treat, prospective, randomized, multicenter, international, double-masked, collaborative feeding study to assess the growth and development of preterm infants fed formulas supplemented with different sources of AA and DHA (Ross Study AG38, Ross Products Division1998, 1999a,b). Infants consumed human milk and/or the assigned preterm formula from the time of first enteral feeding until term CA. From term CA until 12-months CA, the infants consumed a nutrient-enriched post-discharge formula with or without the same sources of AA and DHA as consumed until term CA. Multiple confirmatory safety assessments were made over the approximately 16-month study period, including evaluations of growth and development, and systematic monitoring of blood biochemistries, plasma and red blood cell fatty acids, tolerance, and adverse events.

1. Materials and Methods

Study Participants

A total of 470 premature infants were randomized into the study. Infants were recruited in a total of eight centers located in London, United Kingdom; Santiago, Chile; Little Rock, AR; Kansas City, MO; Portland, OR; Cleveland, OH; Louisville, KY; and New York, NY. The study was approved by the IRB of each study site.

Infants representative of the populations in the participating neonatal intensive care units (NICUs) were recruited for the study. Infants were eligible to enter the study if they met the following criteria: singleton or twin (both needed to meet inclusion criteria) infants born < 33 weeks gestational age; weighed 750 to 1800 g at birth; initiated enteral feeding by 28 days of life; English or Spanish was the primary language spoken at home; and had parents willing to provide the assigned formula as the sole milk feedings (other than human milk) continuing through 12-months CA. Infants born small-for-gestational age or infants with patent ductus arteriosis also were eligible to enroll in the study if they met the other criteria. The study was

designed to include a representative group of infants born prematurely, not only the largest and healthiest of premature infants.

Infants with the following known conditions or situations at the time of enrollment were not eligible for entry into the study: serious congenital abnormalities that could affect growth and development; surgery for necrotizing enterocolitis (NEC) prior to randomization; Grade III or IV periventricular/intraventricular hemorrhage; maternal incapacity including maternal cocaine or alcohol abuse during pregnancy or concurrent, or if the mother or infant tested positive for HIV prior to randomization; had undergone major surgery; extracorporeal membrane oxygenation; liquid ventilation; asphyxia (hypoxia or ischemia) as evident by severe and permanent neurological damage; uncontrolled systemic infection at enrollment; or infants enrolled in other studies (this was reviewed on a case-by-case basis).

A standardized SAS computer program was used to generate the randomization schedule for the study. The randomization was stratified by gender and birth weight group (750 to 1250 g and 1251 to 1800 g). A separate randomization schedule was generated for each stratum at each site. Subjects were randomized to formula groups using permuted blocks to maintain balance among groups. If a set of twins was enrolled into the study, both infants were assigned to the same formula group.

Study Design

The study consisted of two phases. The first phase of the study spanned the time of first enteral feeding until the post-discharge visit corresponding to term CA. The second phase of the study ran from term CA until 12-months CA. All infants were randomized into the study within 72 hours of initiation of enteral feeding (SDAY 1), including trophic feeds or water. At randomization, infants were assigned to one of three formula groups and were initially fed human milk and/or the assigned formula.

In recognition of the acknowledged health benefits in human milk, intake of human milk was encouraged prior to initiation of the formula feedings and throughout the study. Human milk-fed infants were also assigned at random to be fed one of the three study formulas if and when supplementation of human milk was initiated or weaning occurred. Given the encouragement of human milk feedings, infants consequently followed one of three feeding practices; infants could be 1) exclusively human milk-fed; 2) exclusively formula-fed; or 3) fed a combination of human milk and formula. When infants were fed formula (either exclusively or partially), the study protocol required that it be the study formula through term CA and the assigned formula after term CA, unless there was medical indication to do otherwise or the subject was removed from the feeding protocol portion of the study.

Once full feedings were established in-hospital, the enteral feeding goal was ≥120 kcal/kg/day; however, 100 kcal/kg/day was considered full enteral feeding. For infants not yet receiving full enteral feeding, parenteral nutrition was combined with enteral feedings according to the clinical judgment of the NICU's medial staff and/or the prescribed schedule of the NICU for feeding goals. The parenteral nutrition regimen, the time of first enteral feeding, and the advancement of enteral feedings were entirely at the discretion of the NICU medical staff and

were dependent on the ability of the infant to tolerate the enteral feeding assigned by randomization or human milk.

Enteral feedings were withheld at the discretion of the medical staff if the infant showed signs of intolerance to the formula and/or human milk. Possible reasons for withholding enteral feedings included gastric residuals, vomiting, abdominal distention, guaiac-positive stools, stool pH ≤ 5, pneumatosis, intestinalis, clinical suspicion of sepsis, or positive blood cultures. The assigned formula could be reintroduced after indicators of intolerance subsided, at the discretion of the medical staff.

It was recommended that all in-hospital expressed human milk be fortified at an energy density of 22 to 24 kcal/fl oz and a minimum protein intake of 2.8 g/kg/day. When the amount of human milk expressed by the study subject's mother was insufficient to meet the volume needs of the infant or the mother discontinued expressing human milk, research site teams were instructed to recommend supplementing available breastmilk with the assigned randomized formula.

After hospital discharge, the study sponsor provided no specific recommendations for feeding other than that study formulas and/or human milk should be the sole milk-based sources of nutrition for the infant. Water could be provided *ad libitum* throughout the study.

Parents or guardians were encouraged to delay the introduction of solids until infants were at least 2-months CA, unless the caretakers were advised to do otherwise by their primary-care physicians. It should be noted that in the U.S. and Chile, most parents were advised to delay solids until 4-months CA, whereas in the U.K. it is common practice to introduce solids soon after 2-months CA.

During the in-hospital and post-discharge phases of the study, multivitamin and/or mineral use was permitted as indicated by hospital protocol or advised by the infant's primary-care physician. Often during the in-hospital portion of the study and, on occasion, after hospital discharge when the infant was not growing well, or upon recommendation by the infant's primary care physician, the study formula or human milk was supplemented with any of a number of nutritional products including Similac NeoSure® powder, medium-chain triglyceride oil, term formula concentrate, powdered human milk fortifier, or vegetable oil.

Feeding protocol deviations did not necessarily result in early exit from the feeding protocol portion of the study. By definition, an infant exited the feeding protocol portion of the study if he/she no longer was consuming either human milk or the study formula. Any variations in feeding that occurred during the in-hospital phase or any feeding deviation that lasted 7 or more days after the infant was discharged from the hospital required documentation. In the event that an infant permanently exited the feeding protocol, every effort was made to continue collecting the primary outcome data for that infant throughout the study period to 12-months CA.

Formulas

At enrollment, infants were randomized to one of three formulas. The three formulas used in the first phase of the study included: 1) Similac® Special Care® (SSC) matrix without

added AA and DHA (control); 2) SSC matrix with a target level of 0.40 wt % AA from fungal oil (Suntory Ltd., Japan) and 0.25 wt % DHA from a fish oil source (Mochida International Co., Ltd., Japan) (fungal/fish), and; 3) SSC matrix with target levels of 0.40 wt % AA from an egg-derived triglyceride source and 0.25 wt % DHA from a combination of egg-derived triglyceride and fish oil sources (egg-DTG/fish). Eicosapentaenoic acid (EPA; 20:5n-3) was 0.09 wt % of fatty acids in the fungal/fish formula and was not detectable in the egg-DTG formula. All formulas provided the dietary essential fatty acids linoleic and alpha-linolenic acid (16.0-17.5% and 2.4-2.6% of the total fatty acids, respectively).

The control and base formulas used in the first phase of the study were a modified version of SSC, which is a proprietary milk-based formula designed to meet the unique nutrient needs of the premature infant and is typically fed during the initial in-hospital course, and for a duration of time after hospital discharge for very small premature infants. The modified version of SSC used in the study differed from the commercial version of the product in that it contained nucleotides, had a modified whey-to-casein ratio (50:50) and contained beta-carotene and natural vitamin E. The study formulas provided 24-kcal/fl oz and were packaged in ready-to-feed 4-fl oz bottles with labels identifying the study group by code, an expiration date and the batch number of the product. Three batches of each of the study formulas were manufactured during the course of the study.

The three formulas used in the second phase of the study included: 1) Similac NeoSure® matrix without added AA and DHA (control); 2) Similac NeoSure® matrix targeting 0.40 wt % AA from a fungal source (Suntory Ltd., Japan) and 0.15 wt % DHA from a fish oil source (Mochida International Co., Ltd., Japan) (fungal/fish), and; 3) Similac NeoSure® matrix targeting 0.40 wt % AA and 0.15 wt % DHA from an egg-derived triglyceride source (egg-DTG). Eicosapentaenoic acid (EPA; 20:5n-3) was not detectable in any of the formulas. All formulas provided the dietary essential fatty acids linoleic and alpha-linolenic acid (19.1-20.3% and 2.4% of the total fatty acids, respectively). Infants were to be transitioned to the Similac NeoSure® Powder formulas at term CA. At the discretion of the study site clinical team, the time at which the infant was switched from SSC to Similac NeoSure® may have been modified as nutritional and/or medical needs of the infant required.

Similac NeoSure® is a milk-based proprietary post-discharge nutrient enriched formula. The Similac NeoSure® used in this study had modifications to the lactose content and antioxidant system, and addition of nucleotides. The study formulas were packaged in 75.3 g powder packets and provided 22 kcal/fl oz as prepared. Sixteen batches of the formula were manufactured during the study.

Replicate batches of Similac® Special Care® 20, 24 Ready-To-Feed (RTF) and Similac NeoSure® and Powder containing Suntory/Mochida oils at the appropriate levels were manufactured and enrolled in a shelf life testing protocol. To date, no loss in AA or DHA has been noted based on fatty acid profile analysis at predetermined intervals over shelf life.

Study Outcomes Measured

Table VII-1 presents the study timeline and the time at which all study outcomes were assessed. Descriptions of the study outcomes are provided below. In this discussion of the clinical study, outcomes have been classified as one of three types of safety assessments: growth, development, and monitoring parameters including blood biochemistries, tolerance, morbidity, and adverse events.

Table VII-1
Study Variables and Data Collection (Preterm Infant Study)

		In-Hospital	Post Discharge [Post-conceptional age in wks [CA in months])[a]							
	Study Initiation	Daily (except as indicated)	At hospital discharge	40[b] (0)	48 (2)	57 (4)	66 (6)	79 (9)	92 (12)	100[c] (14)
Demographics	x		x							
HOME								x		
WAIS-R								x		
Growth:	x			x	x	x	x	x	x	
Weight		x	x							
Length		x (~ 1 x/wk)								
Head Circumference		x (~ 1 x/wk)								
Development: Visual Development, Acuity Card Procedure					x	x	x			
Development: VEP[d]						x	x			
Development: Paired Comparison Procedure							x	x		
Development: Bayley MDI, PDI									x	
Development: Language Development, MCDI								x		x
Development: Bayley BRS									x	
Infant Behavior Questionnaire							x		x	
Monitoring: Blood Chemistries		(see note)[e]								
Monitoring: Complete Blood Counts[f]						x			x	
Monitoring: Plasma antioxidants[g]						x			x	
Monitoring: Plasma and RBC Fatty Acids[f]	x		x			x			x	
Monitoring: Plasma cholesterol and triglyerides						x			x	
Monitoring: Parenteral Nutrition, HM or Formula Intake		x		x‡	x‡	x‡	x‡	x‡	x‡	
Monitoring: Tolerance (Stool Patterns)		x	x	x	x	x				
Monitoring: Ross Questionnaires										
Infant Feeding &					x					
Stool and Formula Satisfaction					x					
Monitoring: Tolerance and Symptoms of Illness		x								

[a] A window of ± 7 days for visits and sample collection was permitted to allow scheduling flexibility only when necessary for post-discharge visits up to and including the 9-month CA visit. Whenever possible, the 6-month CA visits were scheduled in the portion of the window 0 to 7 days after the infant's 6-month CA birth date. At the 12- and 14-month CA visits, a window of ± 10 days was permitted if necessary

[b] For the purpose of standardizing across sites, a term pregnancy was defined as 40 weeks of gestation.

[c] Subjects did not attend a clinic visit. The MCDI was completed by parents at home and mailed to the investigator except at the Chile site.

[d] VEP grating acuity, contrast sensitivity, VEP resolution acuity, latency of wave forms, and peak-to-peak amplitude were assessed in 157 infants enrolled in the study protocol at 3 sites (Kansas City, MO; New York, NY; Portland, OR). (Study AH95)

[e] The standard in-hospital values for blood chemistries were recorded as they became available in the medical chart.

[f] Analyses were determined on available blood samples at study initiation (± 5 days) and at hospital discharge (± 5 days). Except at Portland, blood samples were not drawn for the exclusive purpose of this study; rather, if blood was being drawn as part of routine clinical practice, Ross-Abbott asked for an additional 1.0 mL for fatty acid analyses and complete blood counts. At the 4- and 12-month CA visits, Ross-Abbott asked that 1.5 mL of blood be drawn except at a subset of sites where 2 5 mL was needed. Complete Blood Counts include hemoglobin, MCH, MCHC, and MCV.

[g] Antioxidants include vitamin E, α-tocopherol, total antioxidant capacity (isoprostanes), and vitamin A (retinol).

‡ 3-day diaries, 3 days prior to the term, 2-, 4-, 6-, 9-, and 12-month CA visits. Three-day diaries also were mailed to parents for them to complete approximately 3 days prior to their infant's 1-, 3-, and 5-month CA birth dates.

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Demographics

Demographic information collected for each infant included: race, gender, gestational age, 5-minute Apgar score, multiple birth status, birth weight, length and head circumference, biological mother's history of smoking, drug and alcohol use (prenatally), and age and education of parents. Data were obtained from medical records or parental report. Additionally, the HOME Environment Inventory was administered by means of an in-office questionnaire to the biological mother (if she was living in the home) in order to assess the quality and quantity of cognitive, social and emotional support available to each infant in their home environment. The verbal scale of the Wechsler Adult Intelligence Scale-revised (WAIS-R), serving as a proxy of maternal intelligence, was individually administered to the biological mother (if living in the home) or primary care giver. Both tests were completed at the 9-month CA visit.

Growth

Anthropometric measurements (weight, length, and head circumference) were primary outcome variables and were collected at predetermined times during the study as indices of growth. All anthropometric measurements were collected at study initiation. The weight of each infant was collected daily during the in-hospital phase of the study, on the day of hospital discharge, and at post-discharge visits corresponding to the infant's term, 2-, 4-, 6-, 9-, and 12-month CA birth dates. Crown-heel length and head circumference measurements were performed approximately once per week for the duration of the hospital stay and at the post-discharge visits. For those subjects who were not discharged from the hospital at the time they reached term CA, anthropometric data collected at term CA (± 7 days) on in-hospital records were used in the analyses. Weight, length, and head circumference were measured according to standardized procedures.

Growth rates for each of weight, length, and head circumference were calculated as gains per day for the intervals SDAY 1 to hospital discharge, SDAY 1 to term CA, SDAY 1 to 4-months CA, and SDAY 1 to 12-months CA. Weight gains per day were defined as the difference between the first and last values for the interval divided by the number of days in the interval; weight gain was then adjusted by the average weight for the interval. Length and head circumference gains per day were defined as the difference between the first and last values for the interval divided by the number of days in the interval. Weight gain (g/kg/day), length gain (mm/day), and head circumference gain (mm/day) were computed individually for each subject. Repeated measures analysis of variance was employed to analyze weight, length, and head circumference gains over time (term, 2-, 4-, 6-, 9-, and 12-months CA).

Development

Visual Development

Visual acuity is the ability to resolve fine spatial detail in the visual image. Two assessments of visual acuity were conducted in this study.

Visual Evoked Potential (VEP) assessments of visual acuity were completed at 4- and 6-months CA (visit window ± 7 days) in a subpopulation of the infants participating in the growth and safety trial. A total of 157 infants from 3 study sites (Kansas City, MO; New York, NY; Portland, OR) were enrolled into the VEP protocol (Study AH95). The point at which the

electrophysiologic visual signal can no longer be distinguished from the ongoing EEG activity determines the acuity threshold. The contrast sensitivity measurement measures the least amount of contrast between the black and white stripes that is necessary for an infant to resolve the stripes. VEP grating acuity and resolution acuity; contrast sensitivity; P60, N80, P100 latency; and N80-P100 peak-to-peak amplitude at multiple check sizes were assessed at 4- and 6-months CA.

Behavioral visual acuity was measured at 2-, 4-, and 6-months CA using the Teller Acuity Card Procedure. Infants were shown a series of cards with black and white stripes varying in spatial frequency (stripe width) by half-octave steps, where one octave represents a halving or doubling of spatial frequency. The finest grating (stripe width) to which the infant showed a consistent fixation response is the visual acuity threshold in cycles/degree with variance (SD) in octaves. One of every four study infants, plus a small cohort of non-study infants, was tested by two trained testers at each site to determine reliability. Tester agreement was within 0.50 octaves (1 line on a Snellen eye chart) for 78% of tests and less than 0.59 octaves for 95% of the tests.

Information Processing

The Fagan Test of Infant Intelligence was administered at 6- and 9-mo CA to infants who remained on the study feeding at the time of the clinic visits. During a familiarization period, a face stimulus was shown until the infant accumulated a predetermined amount of looking time; during the subsequent test period, the familiar face stimulus was shown concurrently with a novel face stimulus. Novelty preference, a measure of visual recognition memory, was computed by determining the percent of total looking time spent looking at novel face stimuli during the test phase. In addition, the mean duration of individual looks was computed for the familiarization period; this evaluation is regarded as a measure of efficiency of information processing.

General Development

The Psychomotor Developmental Index (PDI) and the Mental Developmental Index (MDI) of the Bayley Scales of Infant Development were administered at 12-months CA to assess motor and cognitive development, respectively. The PDI and MDI involve a series of tasks that are presented to the infant while the parent/guardian is present, beginning with a set of items corresponding to the infant's chronological age. The standardized tests are designed to assess development of infants from 1 to 42 months of age.

Language Development

The vocabulary checklist from the infant version of the MacArthur Communicative Development Inventories (MCDI), a standardized parent-report instrument, was completed at 9- and 14-months CA. This checklist of words was used to provide information about each child's vocabulary comprehension (words the child understands) at 9- and 14-months CA, and vocabulary production (words the child says) at 14-months CA. Percentile scores were computed from age- and gender-specific norms and transformed to standard scores.

Temperament

The Behavior Rating Scale (BRS) of the Bayley Scales of Infant Development was administered at 12-mo CA. This assessment provides an index of the examiner's overall impression of the child's behavior. The BRS is completed by the tester after the Bayley Scales test session, and involves ratings of the infant's behavior during the session. Based on these ratings, scores on three subscales (orientation/engagement, emotional regulation, motor quality) were calculated.

The Infant Behavior Questionnaire (Rothbart) was completed by the parent(s) at 6- and 12-months CA. The questionnaire is a standardized 94-item instrument that measures the following dimensions of infant temperament: activity level, distress to novel stimuli, distress to limitations, soothability, smiling and laughter, and duration of orientating.

Monitoring

Blood Biochemistries

Blood Analytes: In-Hospital

Biochemical data were obtained as available from medical records during the in-hospital phase for laboratory values of blood analytes. Blood data collected include: electrolytes (sodium, chloride, potassium, CO_2, BUN, albumin, creatinine), tests of liver status (SGOT, SGPT, bilirubin (total and direct)), chemistries (phosphorus, calcium, alkaline phosphatase, total protein), and hematology (hemoglobin, hematocrit, MCH, MCHC, MCV, total white blood cells, platelets). These data were obtained as available from the medical record for each study subject (primarily from laboratory reports). In the event that multiple lab values were available for a single day, values from the blood drawn closest to 6:00 a.m. or the beginning of the NICU's 24-hour clock were used.

The statistical plan originally stated that individual hospital normative laboratory values would be collected and used to ascertain the percentage of subjects with abnormal blood values. During the study, it became apparent that most of the participating NICUs did not have validated laboratory norms for premature infants. In most instances, the laboratory norms reflected those for adults, or, at best, the term infant. Hence, the study investigators developed a list of commonly accepted normal values from the published literature. Most of the values were extracted from the text, Neonatology-Pathophysiology and Management of the Newborn (Avery et al., 1994). When necessary, the blood analyte data reported by the laboratories were converted to common units to allow for comparison. The percentage of abnormal laboratory values that were below or above the normal range were determined *post-hoc*.

Blood Analytes: Post Discharge

Analyses of blood samples for laboratory values other than CBCs, fatty acid analysis, and plasma antioxidant status were not in the original study protocol. However, study investigators received approval from 6 of the study site IRBs or Human Ethics Committees (Santiago, Chile; London, UK (2 sites); Kansas City, KS: Cleveland, OH; Louisville, KY) to analyze blood samples collected during the post-discharge phase of the study for additional analytes including:

SGOT, SGPT, alkaline phosphatase, total protein, bilirubin (total, conjugated, unconjugated), and albumin. The data were summarized with descriptive statistics.

Complete Blood Counts

At the 4- and 12-month CA visits, study investigators requested the blood draws for the study. CBCs (hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV)) were completed on the samples.

Plasma Antioxidants

Plasma antioxidant analyses including vitamin E (α-tocopherol and γ-tocopherol) concentrations, retinol concentrations, and total antioxidant capacity (isoprostanes) were completed on the 4- and 12-month CA blood samples.

Plasma and Red Blood Cell Fatty Acids

The fatty acid composition of plasma and red blood cell fatty acids (phospholipids, RBC-phosphatidylcholine [PC], and RBC-phosphatidylethanolamine [PE]) was measured in blood samples collected at study initiation (± 5 days) and at hospital discharge, though samples were not drawn for the exclusive purpose of this study at these times. At the 4- and 12-month CA visits, samples were collected for plasma and RBC fatty acid analyses.

Plasma Cholesterol and Triglyceride

Plasma cholesterol and plasma triglyceride measurements were completed on the 4- and 12-month CA blood samples.

Tolerance

Parenteral and Enteral Nutrition

Data detailing parenteral (in hospital only) and enteral (human milk and/or formula) intakes were obtained for each study infant. Data were collected daily during the in-hospital phase from flow sheets in each infant's medical records. Post-discharge, intake data were obtained from 3-day dietary intake records completed by parents in the three days preceding their infant's term, 1-, 2-, 3-, 4-, 5-, 6-, 9- and 12-month CA birth dates. Parents were asked to record the volume of formula consumed and the number of times the infant was breastfed in the diaries; solid food intakes were to be recorded in the diaries at 0-, 1-, 2-, 3-, and 4-months CA.

Stool Characteristics

The stool characteristics of infants were monitored during the in-hospital phase. Information collected included occurrence of hard or watery stools, frequency of stools per day, mean rank stool consistency, and descriptions of consistency and color. Post-discharge, information on the number of stools and mean rank stool consistency was obtained from 3-day stool records completed by parents in the three days preceding the 2- and 4-month CA visits.

Infant Feeding and Stool Patterns and Formula Satisfaction 000193

Parents completed two questionnaires developed by Ross Products Division at the 2-month CA study visit. The Infant Feeding and Stool Patterns Questionnaire assesses parental

perceptions about their infant's reactions to the study feedings (formula or human milk feedings), including ratings of stool characteristics, incidence of spitting up and vomiting, frequency of burping, gassiness, and acceptance of the feedings. The Formula Satisfaction Questionnaire assessed parent's overall satisfaction with the formula, including what they liked most and least about the formula, and ratings of the organoleptic characteristics of the formula.

Morbidity

Morbidity and clinical problems were monitored in-hospital and post-discharge. Data were collected in-hospital from medical records for each study subject; post-discharge data were collected from Serious and/or Unexpected Adverse Event (SAE) forms.

Adverse Events

A serious and/or unexpected adverse (SAE) event as defined by the study protocol was any experience which occurred during the clinical trial that resulted in death or was life threatening, disabling, required hospital admission, or required intervention to prevent permanent impairment. This definition excluded non-life threatening emergency room visits. During initial hospitalization, the site research teams were required to report all serious adverse events which, in the opinion of the site investigator, could be or were associated with the use of the study product. Site research teams were instructed not to include those events which were expected in the natural history of the premature infant and included in the morbidity and clinical problems. This could have included, but was not limited to NEC, peneumatosis, sepsis, respiratory distress syndrome, bronchopulmonary dysplasia, apnea or bradycardia, intraventricular hemorrhage, respiratory syncytial virus, and retinopathy of prematurity. In addition, abnormalities in various laboratory tests were expected and the site research teams did not need to report these unless the investigator believed that they were attributable to the study product.

Data Quality Assurance

Independent internal reviews, investigator and study coordinator meetings, and monitoring of the study sites were incorporated into the study protocol as measures to ensure data quality. Additionally, testing and scoring for all developmental measures and clinical procedures were standardized across all sites, and adherence to testing and scoring procedures was monitored throughout the study to assure reliability. Administrators and coders for the developmental testing were trained in proper procedures, had extensive experience in child development, and met certification requirements. All testers were masked to the infant feeding groups.

Determination of Sample Size

Enrollment of 420 infants was planned, allowing for 140 infants in each of three treatment groups. This sample size was based on the number of subjects required to detect a specified 0.5 S.D. difference in the Bayley Scales of Infant Development measured at 12-months CA, allowing for attrition of subjects, a possible blunting effect of human milk intake on primary outcome variables, and formation of an exclusive human milk reference group. A minimum of 82 infants in each of the three randomized feeding groups was required to detect a 0.5 S.D. difference with a power of 80% and α=0.05. Estimating an attrition rate of 20% and incorporating a 25% increase in sample size to accommodate human milk use in the study, the plan was to enroll a total of 140 infants per group. This sample size is considerably larger than

would have been required to detect differences in growth, a primary outcome variable in each phase of the study.

Statistical Analyses

In general, two sets of statistical analyses were conducted: one set was conducted on the "intent-to-treat" population, and one set was conducted on the "evaluable" population of subjects. The intent-to-treat population included all subjects randomized into the study, regardless of compliance with the study protocol, including compliance with the feeding protocol. Data were categorized into one of four types of study variables: primary, secondary, exploratory, and descriptive (includes demographic information). Primary and demographic outcome variables were analyzed as intent-to-treat variables.

The evaluable population included those infants who had > 80% of their feedings from the assigned study formula and/or human milk at the time of the visit (and data collection) as documented on dietary intake records. Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

Categorical variables were analyzed using Chi-square or Cochran-Mantel-Haenszel tests and continuous variables using analysis of variance (ANOVA) and/or analysis of covariance (ANCOVA). Data obtained at more than one time point were analyzed using repeated-measures analyses which accommodates missing observations (SAS® PROC MIXED).

For each study population (intent-to-treat and evaluable), three levels of analyses were completed for all primary and secondary analyses. Study design strata used in the randomization were defined as the covariates for the Level 1 analyses (site, gender, birth weight stratum (750 to 1250 g, 1251 to 1800 g). Covariates used in the Level 2 analyses were in addition to those used for Level 1; additional Level 2 covariates included: gestational age, size for gestation, HOME and WAIS-R for developmental outcomes; and prenatal smoking, in-home smoking at hospital discharge, gestational age, size for gestation, HOME, and WAIS-R for vision outcomes. The Level 3 analyses added human milk (HM) as a covariate in addition to the Level 2 covariates.

Human milk intake was defined as a categorical variable based on the classification of infants at the term CA visit as EHM-T, exclusively formula fed, <50% of energy intake from study formula, and ≥50% of energy intake from the study formula. The average in-hospital daily enteral energy intake of exclusively formula-fed infants was calculated by dividing the total in-hospital enteral energy intake for each infant by the number of days the infant was hospitalized.

The New York and Little Rock sites were treated statistically as one site due to the small numbers of subjects at these two sites. In the analysis of the exploratory variables, the feeding group*site interaction was dropped from the model since it prevented estimation of the LS means in the solution. Separate ANOVAs for SDAY 1 and term CA were conducted rather than

repeated measures analyses, based on the disparity of observations available at the two time points.

No adjustment for the multiplicity of endpoints was made in this study. All statistical tests of hypotheses were two-tailed with α=0.05 for main effects and α=0.10 for interaction effects. When multiple comparisons were made by gender, visits, and/or birth weight stratum, Bonferroni-adjusted p-values were used. Missing data were not imputed.

Ross Products Division summarized the findings in three reports: the first report presents results from the beginning of the study to term corrected age, i.e., the SSC phase of the clinical trial (Ross Products Division, 1998); the second report presents findings from term CA to the end of the study, as well as results of longitudinal repeated measures analyses of growth, i.e., the Similac NeoSure® phase of the study (Ross Products Division, 1999a). The third report presents specific measures of visual development (Ross Products Division, 1999b).

2. Results

Study Population

A total of 470 infants were randomized into the study: 154 infants to the control formula group, 158 infants to the fungal/fish AA + DHA supplemented group, and 158 infants to the egg-DTG/fish AA + DHA supplemented group. Forty-three subjects identified at term CA were in the group of exclusively human milk fed infants. Table VII-2 presents a summary of the number of infants participating at each phase of the study.

Of the 470 subjects randomized to the study, a total of 414 (88%) and 311 (66%) completed the feeding protocol through term and 12-months CA, respectively. The percentage of subjects completing the feeding protocol during the SSC phase did not differ among the study formula feeding groups. Likewise, the percentage of subjects completing the feeding protocol to 12-months CA did not differ among subjects in the three groups.

At term CA, 35%, 28%, and 33% of infants in the control, AA + DHA (fungal/fish) and AA + DHA (egg-DTG/fish) groups, respectively, consumed human milk at least once per day (data not shown). By 4-mo CA, 14%, 12%, and 12% of infants in the control, AA + DHA (fungal/fish) and AA + DHA (egg-DTG/fish) groups, respectively, consumed human milk.

Nineteen (13%), 22 (15%), and 11 (8%) of the infants in the control, AA + DHA (fungal/fish) and AA + DHA (egg-DTG/fish) groups, respectively, discontinued study feeding due to symptoms typically associated with feeding intolerance identified as vomiting, spit up, refusal to drink formula, or diarrhea/watery stools (primary reason for discontinuation provided by site investigator) (Table VII-3). There were no statistically significant differences among the feeding groups with respect to the number of subjects removed from the feeding protocol or exiting the study early (either the primary reason cited by the study investigator or any reason cited for an early exit).

Infant and Family Demographics

In general, the study population (including EHM feeders) consisted of premature infants among whom 43% weighed ≤1250 g at birth. One subject had a birth weight greater than 1800 g (1805 g). Only 9.5% of the infants in the study were born SGA as classified by the Colorado Intrauterine Growth Charts (Lubchenco, 1963) and 21% of the study infants were twins. Fifty-four percent of the study population was male; 46% was female. The majority of infants (77%) had a 5-minute APGAR score of 8 or greater. There were no statistically significant differences among study subjects randomized to the control, AA + DHA (fungal/fish), or AA + DHA (egg-DTG/fish) study groups with respect to birth weight, gestational age at birth, size at birth, gender, 5-minute APGAR score, or ethnicity. No differences were found at SDAY 1 for weight, length or HC. A summary of the neonatal and perinatal characteristics of the study population is presented in Table VII-4.

Growth

Indices of growth, including weight gains (g/day), length gains (mm/day), and HC gains (mm/day) from SDAY 1 to hospital discharge, term CA, 4-months CA, and 12-months CA were collected and analyzed as primary outcome variables in the study. Analyses were completed for both the intent-to-treat and evaluable populations. Mean values of weight, length and head circumference throughout the study are presented in Figure VII-1. Results of the intent-to-treat analyses from both the SSC phase and the Similac NeoSure® phase are presented in Tables VII-5, VII-6 and VII-7.

In the intent-to-treat population, few and inconsistent differences were found in weight, length, or head circumference gains from SDAY 1 to term CA, SDAY 1 to 4-months CA, or SDAY 1 to 12-months CA or in repeated measures of analysis of absolute weight, length and head circumference measurements through 12-months CA. These differences were not seen when analysis of the intent-to-treat population excluded infants consuming > 50% of initial in-hospital energy from human milk. A summary of the statistically significant feeding effects in the analyses using the Bonferroni adjusted α-levels is presented in Table VII-8. One statistically significant difference using the Bonferroni adjusted α-levels existed between the control group and the AA + DHA (fungal/fish) group: in the intent-to-treat analyses of length gains from SDAY 1 to 12-months CA, infants in the ≤1250 g birth weight stratum who consumed the AA + DHA (fungal/fish) formula had lower length gain as compared to infants who consumed control formula.

Development

Visual Development

Visual Evoked Potential Resolution Acuity

A statistically significant interaction was found between study feeding group and visit in all analyses (intent-to-treat and evaluable) of the VEP grating acuity measurements ($p<0.0328$); results of the intent-to-treat analysis are presented in Table VII-9 and Figure VII-2. Mean VEP grating acuity of infants did not differ by study feeding group at 4-months CA. At 6-months CA, the mean VEP grating acuity of infants randomized to either AA + DHA supplemented formula group was statistically significantly greater than for infants randomized to the control formula ($p = 0.012$). The mean VEP grating acuity of infants fed the AA + DHA supplemented formulas

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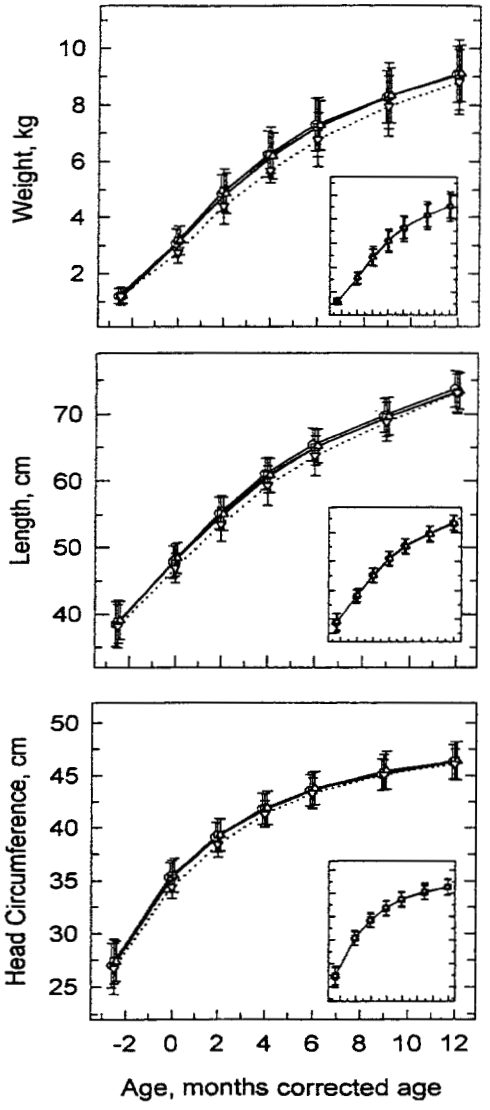
FIGURE VII-1
Growth Curves of Infants in Clinical Trial AG38



Weight, length, and head circumference of preterm infants fed human milk and/or nutrient-enriched formula with or without AA and DHA from Study Day 1 (median, 5 days of age) to 12-months CA. Values are presented as mean ± SD for the intent-to-treat population (large graph) and strict study feeding protocol followers (small graph). Hatched lines with triangle symbols denote data for infants who were exclusively human milk fed until term CA. Formula groups: 1) control [Circles], 2) AA + DHA (fungal/fish) [Squares], and 3) AA + DHA (egg-TG/fish) [Triangles with solid lines]. Repeated measures analysis demonstrated that the weight and lengths of AA + DHA (egg-TG/fish) infants were greater than control infants at term CA (2906 ± 48 g vs 2757 ± 50 g, p=0.03, 47 1 ± 0.2 cm vs 46.5 ± 0.02 cm, p=0.01; respectively). (O'Connor et al. 2001)

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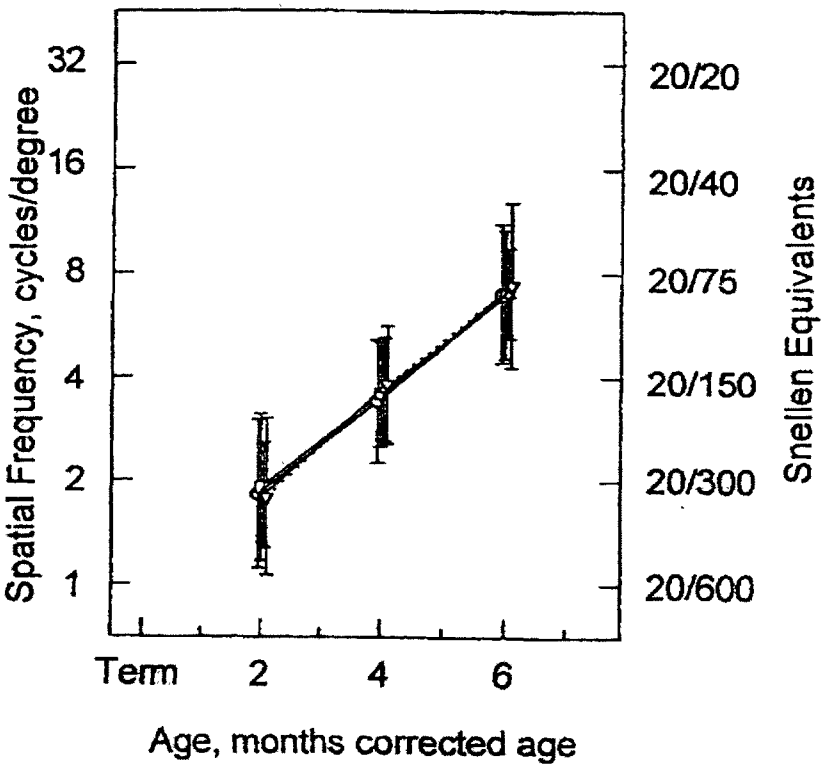
Figure VII-2

Visual Evoked Potential Visual Acuity

In Clinical Trial AG38



Visual Evoked Potential (VEP) visual acuity at 4- and 6-mos CA (± 7 days) of preterm infants at the Kansas City, New York, and Portland research sites fed human milk and/or nutrient-enriched formula with or without AA and DHA until 12-mos CA Data for the intent-to-treat population are shown on the left axis as mean (cycles/degree, cy/deg) ± SD (octaves). Hatched lines with triangle symbols denote data for infants who were exclusively human milk fed until term CA. Formula groups: 1) control [Circles], 2) AA + DHA (fungal/fish) [Squares], 3) AA + DHA (egg-TG/fish) [Triangles with solid lines]. At 6-mos CA, the mean VEP acuity of infants randomized to either AA + DHA (fungal/fish) (LS means ± SE, 11.4 ± 0.1) or AA + DHA (egg-TG/fish) (12.5 ± 0.1) was greater than that of infants randomized to the control (8.4 ± 0.1). (O'Connor et al. 2001).

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increased between 4- and 6-months CA; the mean VEP grating acuity of infants fed the control formula did not differ between visits. Analysis of the evaluable data set revealed that the VEP grating acuity of AA + DHA (egg-DTG/fish)-fed infants was greater than control-fed infants at 6-months CA (data not shown).

No differences in contrast sensitivity using a contrast sweep were found at the lower spatial frequencies of 1.5 and 3.0 cycles/degree among feeding group (Table VII-10). At a spatial frequency of 6.0 cycles/degree, more than one-half of the children in the intent-to-treat population were missing contrast sensitivity data for the 4-month CA visit due primarily to a low signal-to-noise ratio. Statistical analysis was completed using only the 6-month time point. No significant differences were found. Analysis of the evaluable data set yielded a significant feeding effect at 3.0 cycles/degree, though no significant differences among the formula groups were found (data not shown).

No significant feeding effects were found in the analysis of the latency and peak-to-peak amplitude measures at the 16x16 and 30x30 check sizes; statistical analyses of data collected at other check sizes was not completed due to missing data (Tables VII-11 to VII-13).

Teller Acuity Cards

The mean Teller acuity scores of infants did not vary across the formula groups at 2-, 4-, and 6-months CA in the intent-to-treat analysis (Table VII-14) or the evaluable data analysis (data not shown).

Information Processing

Analysis of the Fagan Test of Infant Intelligence revealed a statistically significant feeding by visit interaction for both novelty preference and average look duration during the familiarization period; pairwise comparisons of study feeding groups at each time-point yielded significant differences for novelty preference (Table VII-15). The mean novelty preference of AA + DHA (egg-DTG/fish)-fed infants was significantly greater than control formula- and AA + DHA (fungal/fish)-fed infants at 6-months CA. The difference between AA + DHA (egg-DTG/fish) and AA + DHA (fungal/fish) remained statistically significant using a Bonferroni adjusted alpha-level of 0.0083.

General Development

Regardless of whether the statistical analysis of the data included all infants randomized into the study (intent-to-treat analysis) or included only those infants who strictly adhered to the feeding protocol (evaluable analysis), no mean differences were found among study formula groups in the Bayley Metal Developmental Index (Table VII-16). Analysis of the Bayley Psychomotor Development Index (PDI) revealed a statistically significant feeding by birth weight stratum interaction among infants who consumed > 80% of their feeding as study formula and/or human milk (data not shown). The mean Bayley PDI score of infants in the 1250 g birth weight subgroup who strictly followed the feeding protocol was greater in infants fed AA + DHA (fungal/fish) (LS means ± SE, 90.6 ± 4.4) than control infants (81.8 ± 4.3; p=0.007). The Bayley PDI score of AA + DHA (egg-DTG/fish)-fed infants did not differ statistically from either the control or AA + DHA (fungal/fish) groups.

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The percentage of subjects in the intent-to-treat or subgroup populations who had significantly delayed mental or motor performance scores did not differ statistically by study formula group. Approximately 4% and 12% of all infants tested had Bayley mental and motor scores, respectively, below 70, a level indicative of significantly delayed performance (Table VII-17).

Language Development

Vocabulary comprehension did not differ among the three study formula groups at either 9- or 14-months CA in the intent-to-treat analysis (Table VII-18) or in the subgroup analysis (data not shown). No study feeding differences in vocabulary production or phrases understood at 14-months CA were found (Table VII-19). In view of the somewhat disproportionate distribution of twins among the three study formula groups, language outcomes were analyzed with and without twins. The validity of using percentile and gender-specific norms and standard score conversions established using English-speaking infants is not clearly established for Spanish speakers. When infants from Spanish-speaking families and twins were removed from the intent-to-treat analysis, infants randomized to the control group had lower vocabulary comprehension than infants randomized to the AA + DHA (egg-DTG/fish) or AA + DHA (fungal/fish) groups (LS means ± SE: 94.1 ± 2.9; 102.2 ± 2.8, p=0.0145; 100.6 ± 2.9, p=0.0422, respectively.) Likewise, in the comparable subgroup analysis, control-fed infants had lower vocabulary comprehension scores than AA + DHA (egg-DTG/fish)-fed infants.

Temperament

No statistically significant differences were found among study formula groups with respect to percentage of infants with questionable or nonoptimal scores on factors of the Behavior Rating Scale, a subscale of the Bayley Scales of Infant Development (Table VII-20). No statistically significant differences across study formula groups were found with respect to responses to the Infant Behavior Questionnaires (Table VII-21).

Monitoring

Blood Chemistries

Blood analytes: In-Hospital

During the in-hospital phase, the percentage of preterm infants with an abnormal blood value was analyzed by feeding group. Blood data collected and analyzed includes electrolytes (sodium, chloride, potassium), blood chemistries (phosphorus, calcium, alkaline phosphatase, total protein), tests of liver function (SGOT, SGPT, total and direct bilirubin), tests of kidney function (CO_2, BUN, creatinine, and albumin), and hematology (hemoglobin, hematocrit, MCH, MCHC, MCV, white blood count with differentials, platelet counts). The compilation of reference ranges for all laboratory values is presented in Table VII-22.

Results of the analyses indicate that the proportion of infants that had at least one abnormal blood analyte during the in-hospital phase of the study did not statistically differ among study formula group (Table VII-23). The percentage of infants in each group with abnormal laboratory values above or below the normal range was identified post-hoc. Within each analyte, the distribution of high and low values is similar across the feeding groups, though statistical analyses were not conducted (Table VII-24).

No statistically significant differences among study formula groups were found with respect to the proportion of subjects with abnormal hypersegmented neutrophils, basophils, eosinophils, monocytes, lymphocytes, and bands (data not shown).

The mean value and standard deviation of each of the blood analytes are presented in Tables VII-25 to VII-29. The results are presented for three time periods during the in-hospital phase: 0 to 30 days, 30 to 60 days, and greater than 60 days. Statistical analyses were not conducted on the data; no obvious differences among the data are apparent.

Blood Analytes: Post Discharge

Additional analytes were measured in blood samples collected at 4- and 12-months CA from infants at 6 of the study sites. Statistical analyses were not conducted on the data, though the mean, standard deviation, median and range were determined for each analyte and each study group. The results are presented in Table VII-30. The study formula groups were not distinguishable on the basis of the analytical results and the mean and median values for each group were within reference ranges for term infants as cited from Soldin et al. (1999).

Complete Blood Counts at 4- and 12-Months CA

No statistically significant differences existed among study formula groups with respect to mean hemoglobin concentrations and mean corpuscular hemoglobin concentration (Table VII-30). A statically significant feeding* birthweight stratum interaction was found for both MCV (p=0.0081) and MCH (p=0.0421). Pairwise comparison revealed that infants in the > 1250 g birth weight stratum fed AA + DHA (fungal/fish) had greater MCV and MCH values as compared to infants fed AA + DHA (egg-DTG/fish), though there were no statistically significant differences between infants fed the control formula and infants fed either of the supplemented formulas.

Plasma Antioxidants at 4- and 12-Months CA

The potential for increased oxidation of the supplemented dietary fatty acids was assessed by measuring plasma levels of isoprostanes. Mean levels of isoprostanes and vitamin E are presented in Table VII-32. No statistically significant effects were observed among the feeding groups for either of these measures. A statistically significant feeding*gender interaction was observed for plasma α-tocopherol; this interaction disappeared after normalizing the α-tocopherol data by log transformation or after adjustment of α-tocopherol levels for plasma cholesterol and triglyceride concentrations. A statistically significant interaction between study feeding and study visit was observed in the evaluable analysis of plasma retinol. At 12-months CA, infants fed the study formula containing AA + DHA (fungal/fish) had a statistically higher mean plasma retinol concentration as compared to infants fed formula containing AA + DHA from an egg-DTG source (p = 0.0021). There was no difference between the plasma retinol concentration of infants fed the AA + DHA (fungal/fish) formula and infants fed the control formula.

Plasma and Red Blood Cell Fatty Acids at 4- and 12-Months CA

Statistical analyses were performed only on fatty acid data from subjects remaining in the feeding protocol of the study at the time of the blood draw. A summary of the fatty acid levels

at SDAY 1, hospital discharge, 4- and 12-months CA in plasma and red blood cell phospholipids of infants who were following the feeding protocol at these time points is provided in the Tables VII-33 and VII-34.

At study initiation, the study formula groups did not differ significantly with respect to the level (g/100g) of AA, DHA, EPA or the ratios of EPA to AA and n-6 to n-3 fatty acids in the plasma or in the PE or PC fractions of the RBCs (with one exception (control < egg-DTG/fish in males only, p=0.003)). The plasma and RBC levels of the fatty acids in infants who strictly adhered to the feeding protocol were influenced by the type of formula consumed. The plasma levels of AA and DHA were significantly higher in infants fed the supplemented formulas as compared to levels in infants fed the control formula at hospital discharge, which was the end of the SSC phase of the study. EPA levels were lower in the group fed SSC with egg-DTG/fish than those fed the control or fungal/fish supplemented formula.

Levels of AA and DHA in RBC PE did not vary by feeding as compared at the time of hospital discharge, though differences were measured during the Similac NeoSure® phase of the study. Levels of AA and DHA in RBC PC were significantly higher in infants fed AA + DHA (fungal/fish) supplemented formula as compared to infants fed the control formula during both phases of the study. Levels of AA and DHA in RBC PC were higher in the AA + DHA (egg-DTG/fish) supplemented formula as compared to infants in the control group only during the Similac NeoSure® phase. EPA levels varied by formula group, with the egg-DTG/fish group tending to have lower levels than the control and fungal/fish group. The ratio of EPA to AA and n-6 to n-3 fatty acids also varied by formula group, with the control formula fed infants tending to have higher ratios as compared to the ratios measured in infants fed the AA + DHA supplemented formulas.

Plasma Cholesterol and Triglycerides

Results of the evaluable analysis of the plasma cholesterol and triglyceride concentrations at 4- and 12-months CA are presented in Table VII-35. There was a statistically significant main feeding effect of study feeding on plasma cholesterol. Infants randomized to the AA + DHA (fungal/fish) group had significantly higher plasma cholesterol concentrations than did infants in the control group and the AA + DHA (egg-DTG/fish) groups. The mean cholesterol concentrations of the control, AA + DHA (fungal/fish), and AA + DHA (egg-DTG/fish) groups at 4-months CA were 128.4 mg/dL, 146.5 mg/dL, and 132.9 mg/dL, respectively; mean concentrations at 12-months CA were 149.7 mg/dL, 171.8 mg/dL, and 153.4 mg/dL, respectively. Results of a *post-hoc* analysis are presented in Table VII-36.

There was a statistically significant interaction of study feeding and birth weight stratum on plasma triglyceride concentrations. However, pairwise comparison of plasma triglyceride concentrations across study formula groups within each birth weight stratum revealed no statistically significant differences.

Tolerance

Parenteral and Enteral Nutrition

Subjects were to be fed the assigned study formula or human milk from the time of first enteral feeding until 12-months CA. The mean number of days that infants consumed study

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formula from SDAY 1 to 12-months CA was 321 ± 12.8 for control; 322 ± 13.1 for AA + DHA (fungal/fish); 319 ± 13.2 AA + DHA (egg-DTG/fish); and 268 ± 18.7 for EHM. As shown in Table VII-37, no statistically significant differences existed among study formula groups with respect to the total amount of study formula consumed (kcal/day, kcal/kg/day, or kJ/day) during the SSC or Similac NeoSure® phase of the study.

Stool Characteristics

Information on infant's stool characteristics was collected both during the in-hospital phase of the study, at term CA, and at 2- and 4-months CA (Table VII-38). During the in-hospital phase of the study, the proportion of infants in each formula group experiencing a watery stool or a hard stool did not significantly differ. At 2-months CA, infants with birth weights > 1250 g fed AA + DHA (egg-DTG/fish) had a greater number of stools than did those infants fed AA + DHA (fungal/fish) ($p = 0.0042$).

Infant Feeding and Stool Patterns and Formula Satisfaction Questionnaires

Analyses of quantitative and qualitative data collected in 3-day stool diaries at term CA to characterize each infant's stool also indicate that no significant differences among the formula feeding groups were found with regard to number per day, consistency, and color. There were no significant differences between infants in the control group and infants fed the AA + DHA (fungal/fish) formula at 2- or 4-months CA with regard to the number of stools and mean rank stool consistency.

Parents/guardians' perceptions about formula tolerance and the acceptability of formula were assessed using questionnaires at each infant's 2-month CA visit (data not shown). At this juncture of the study protocol infants had been transitioned to Similac NeoSure® from SSC. Except for a statistically significant difference among study formula groups in response to the questions, "Did your baby seem to like the formula" (p=0.0490) and "My baby appeared to be constipated" (p=0.0380), no differences were found in the responses to questions about the infants' acceptance of formula and stool pattern characteristics. It appeared that more parents/guardians of infants in the AA + DHA (fungal/fish) group perceived that their babies liked the formula "very much" as compared to parents/guardians in the other two study formula groups. Parents/guardians of infants in the AA + DHA (egg-DTG/fish) group reported more often that their baby was rarely or never constipated (78%) versus the AA + DHA (fungal/fish) (62%) or control (65%) groups.

Morbidity

Throughout the study, safety of the study products was monitored and included reporting of in-hospital clinical problems/morbidity, post-discharge morbidity, serious and or unexpected adverse events (SAEs), and deaths.

In-hospital clinical problems/morbidity

During the in-hospital phase of the study, there were no significant differences among the feeding groups in the occurrence in any of the measures monitored for safety: percent of infants who had feedings withheld due to gastric residuals, days to full enteral feedings, percent of infants NPO for at least one day, suspected or confirmed NEC, suspected or confirmed systemic infection, or percent of infants with cases of chronic lung disease (Table VII-39). Prior to SDAY

1 and between SDAY 1 and hospital discharge, the incidence rate (% subjects) of respiratory support, corticosteroid therapy, antibiotic use and blood transfusions of the study infants also was monitored (Table VII-40). No statistically significant differences were found in any of these indices.

Additionally, the percent of infants who had a variety of clinical problems such as periventricular leucomalacia, retinopathy > Grade II, surgery (other than circumcision), or severe hypotension did not differ significantly among study formula groups (data not shown). Several of the clinical problems listed, including presence of congenital abnormality, shunting for hydrocephalus, intraventricular hemorrhage > Grade II, periventricular leucomalacia, and surgery, were in fact, exclusion criteria for the study, therefore the incidence of these conditions was expected to be low.

Post-Discharge Morbidity

The number of subjects who were re-hospitalized after the initial hospitalization period did not differ among study formula groups. Fifty-four of 143 (38%), 54 of 138 (39%), and 60 of 141 (43%) subjects randomized to the control, AA + DHA (fungal/fish) and AA + DHA (egg-DTG/fish) groups respectively, were rehospitalized at least once. These analyses were performed on the intent-to-treat population only. Additionally, examination of the number of infants within each birth weight stratum with at least one SAE rehospitalization did not statistically differ among study formula groups.

Serious Adverse Events

During the SSC Phase of the study, SAEs were categorized. There were no significant differences in the occurrence of these events by feeding group.

Each reported SAE was reviewed and assigned an alphanumeric organ-system and severity score by a neonatologist blinded to the study feeding programs. The five main categories included:

- Death;
- Pulmonary central, autonomic (e.g., apnea, sudden cyanosis, with or without associated reflux and bradycardia);
- Pulmonary parenchymal (e.g., pneumonia, RSV, asthma, wheezing);
- Other serious non-pulmonary diseases (e.g., diarrhea, dehydration, vomiting, fever, sepsis);
- Unrelated, irrespective of severity (e.g., laser therapy for retinopathy, hernia repairs, or repair for congenital hear defects).

The two subcategories included:

- Conditions that were associated with a presentation where immediate or near-immediate life saving measures were necessary. These included mechanical ventilation, pressors, fluid, and resuscitation; and
- Conditions requiring hospitalization and significant medical care but that did not have the potential for immediate threat to life.

Data were collected in-hospital from medical records for each study subject; post-discharge data were collected from Serious and/or Unexpected Adverse Event (SAE) forms.

During the study, a contingency table of SAEs using the 5 main categories and 2 subcategories for each event was constructed. The number of subjects who had at least one SAE did not differ among study formula groups. Sixty-three of 143 (44%), 63 of 138 (46%), and 66 of 141 (47%) subjects randomized to the control, AA + DHA (fungal/fish) and AA + DHA (egg-DTG/fish) groups respectively, had at least one SAE. Statistical analysis controlling for site yielded no differences across study formula groups in the number of SAEs by type.

Fifteen deaths occurred during the study of which 6, 3, and 6 were in the control, AA + DHA (fungal/fish), and AA + DHA (egg-DTG/fish) study formula groups, respectively. No infants died in the EHM feeding group.

In view of the long-term feeding nature of this study, a quarterly safety review was established. At the time the data were unblinded for the SSC phase of the study, six quarterly safety reviews had been completed by the study scientist and submitted to the Medical Director of Ross Products Division and the Director, Clinical Development, Nutritionals, International Medical Affairs of Abbott International. A seventh quarterly safety review was completed after filing the SSC Phase Final Report. The quarterly safety reviews were submitted to the individuals in a manner such that these data were reviewed in a blinded fashion.

The quarterly safety review involved the collection of the following variables: suspected and confirmed cases of systemic infection, suspected and confirmed cases of necrotizing enterocolitis (NEC), serious and/or adverse events as defined by the protocol, and deaths. Data were compiled for both the SSC and Similac NeoSure® feeding phases of the study.

On two occasions, these safety data were reviewed by an external panel of experts in an unblinded fashion. Panel members were selected for their known expertise in the areas of neonatology, nutrition, lipids, sudden infant death syndrome, toxicology, and epidemiology. Furthermore, data from safety reviews were shared with the Food and Drug Administration regulatory officials. At the request of FDA, an extensive review of SIDS was compiled from information collected in all clinical trials in preterm infants conducted by Ross and reviewed by the Panel members. A summary of the review of infant deaths is presented in Section VII-D. The complete review is contained in Attachment 2.

3. Discussion

This study represents the largest clinical assessment of AA + DHA supplemented formulas on preterm infants published to date. A total of 470 premature infants were enrolled in an intent-to-treat, prospective, randomized, multicenter, international, double-masked feeding study to evaluate growth and development of infants fed formulas with or without different sources of added AA and DHA. The sources of AA + DHA included a combination of AA-rich fungal oils and DHA-rich tuna oil, or a combination of egg-derived triglycerides and tuna oil. From first feedings to term CA, the AA- and DHA-rich oil formula provided 0.43% AA and 0.27% DHA; from term CA through 12-months CA, the oil-supplemented formula provided 0.43% AA and 0.16% DHA.

In the SSC feeding phase of Clinical Trial AG38, no consistent differences in growth of preterm infants were found in relation to AA + DHA supplementation of the SSC formulation, a formula designed for use until term CA, regardless of whether or not the analyses controlled for human milk intake. Growth was assessed by multiple parameters, including rate of weight, length, and head circumference gains as well as absolute weight, length and head circumference measurements from the time of first enteral feeding to term CA. Multiple measures of growth were also assessed in the post-term CA phase of this trial when infants were fed the AA and DHA supplemented formulation Similac NeoSure®. Again, no consistent differences in growth were found between infants who consumed the formula supplemented with AA- and DHA-rich oils as compared to infants consuming the control formula. These findings were irrespective of HM intake. Analysis of growth also included longitudinal assessments over both the in-hospital and post-discharge phases of the study.

Some statistically significant differences in the growth measures in both the SSC and Similac NeoSure® feeding phases of the trial were found. A total of more than 200 statistical analyses were conducted for the assessment of growth in this clinical trial. The statistical differences that were found were inconsistent among the different sets of analyses (intent-to-treat and evaluable datasets), and most differences were small in magnitude. It can be concluded that the few statistically significant differences that were found occurred by chance and are of no relevance on safety of the AA- and DHA-rich oil formula.

Development of infants through 14-months CA was assessed via multiple standard tests, including assessments of visual, cognitive, motor, and language development. Results of these standardized assessments consistently indicated that development of infants fed the supplemented formulas was comparable to or potentially enhanced as compared to infants fed the unsupplemented preterm formulas.

Specifically, results from the trial suggest that AA + DHA supplementation results in improved visual development of preterm infants at 6-months CA as assessed by VEP acuity. The magnitude of difference between the supplemented groups and the control group was approximately 0.3 – 0.4 octaves, which corresponds to approximately one line on a Snellen eye chart. Unlike AA + DHA supplemented infants whose VEP acuity improved between 4- and 6-months CA, the VEP acuity of infants randomized to the control formula did not improve, suggesting a slower rate of development of the visual system in this latter group of infants.

In addition to improved visual development in preterm infants, there was evidence of improved motor development among infants 1250 g birth weight who strictly adhered to the feeding protocol through 12-months CA. The Bayley motor index measures gross motor abilities such as sitting, walking, standing, stair climbing, and hand and finger fine motor skills.

Multiple assessments were monitored throughout the study to determine the effects of the enriched oils on study exits, morbidity, tolerance, and safety as assessed via blood chemistries, including indices of liver function, the immune system, and the kidneys. Effects on antioxidant status and hematology indices also were monitored.

Addition of AA and DHA did not affect multiple biochemical parameters monitored during the in-hospital phase. Comparison of the proportion of infants fed the AA + DHA supplemented preterm formula with at least one abnormal analyte to infants fed the control formula revealed no differences in tests of liver function (SGOT, SGPT, total and direct bilirubin), tests of kidney function (CO_2, BUN, creatinine, and albumin), electrolytes (sodium, chloride, potassium), and blood chemistries (phosphorus, calcium, alkaline phosphatase, total protein). Similarly, there were no differences in the proportions of infants with abnormal hematology measures, including hemoglobin, hematocrit, MCH, MCHC, MCV, white blood count with differentials, platelet counts, hypersegmented neutrophils, basophils, eosinophils, monocytes, lymphocytes, or bands. The lack of any significant differences in incidence of abnormal hematology measures among the formula groups, as well as the absence of differences in incidence of NEC, systemic infections, antibiotic use, or reports of bleeding problems, provides evidence that the addition of 0.43% AA and 0.27% DHA had no effects on the spleen health and immune function of these infants. A large percentage of the infants were found to have abnormal laboratory values during the in-hospital phase, though this was expected in this population of preterm infants and was not related to study formulas.

Clinical chemistries including SGOT, SGPT, alkaline phosphatase, total protein, bilirubin (total, conjugated, unconjugated), and albumin also were collected from approximately 25% of the study participants at 4- and 12-months CA. Although statistical analyses were not conducted on these indices, there are apparent differences among the mean values, suggesting that liver function, kidney function, and chemistries were not affected by the addition of 0.43% AA and 0.16% DHA to preterm infant formulas designed for intake after the infants reach term CA. Additionally, there were no differences in post-discharge morbidity which would suggest that the oils had no adverse effects on immune function or bleeding status.

Laboratory assessments can be useful for the evaluation of infant nutritional and clinical status in some situations. For most infants in each feeding group, the values for the analytes measured were within published reference ranges for term infants (Soldin et al., 1999). Some values were elevated, but this occurred sporadically and in each of the formula groups. It is not unusual for preterm infants to occasionally present with values outside of reference ranges for term infants on laboratory tests, even after hospital discharge, often reflecting chronic sequelae of their complicated hospital courses. Also, technical factors such as handling of the specimen (e.g., thawing, refreezing) may have introduced some variability.

Plasma levels of α-tocopherol and isoprostanes provide information about antioxidant status in relation to feeding AA + DHA supplemented formulas to preterm infants. The absence of differences in these markers among the AA + DHA supplemented and unsupplemented groups at 4- and 12- months CA in the Ross study provides evidence of stability of these fatty acids in plasma. Similarly, supplementation of preterm formulas with 0.43% AA and 0.16% DHA had no effects on iron status as assessed by indices including hemoglobin, MCHC, MCH, MCV, at these times.

Supplementation of the preterm infant formulas with the AA and DHA-rich oils predictably increased plasma and RBC phospholipid levels of AA and DHA as measured at hospital discharge and at the 4- and 12-months CA visits. Although statistical comparisons were

not conducted between data collected from any of the formula-fed infants and human milk-fed infants, the plasma and RBC levels of AA and DHA, as well as EPA and the EPA:AA and n-6:n-3 ratios, appeared to be most similar between infants fed formulas with AA + DHA or human milk versus the formula devoid of these fatty acids.

Supplementation of infant formula with AA and DHA (fungal/fish) had no effects on plasma triglyceride levels, however, the AA- and DHA-rich oil sources were found to have a significant effect on cholesterol levels as compared to infants fed the control or AA + DHA (egg-DTG/fish) source. This was an unexpected outcome in the study, and therefore has been reviewed and a manuscript is under development. An interpretation of the results and a discussion of the results in relation to the published literature are presented in Attachment 3. It can be concluded that AA and DHA supplementation in adults and infants has not been shown to have clinically adverse effects on total plasma/serum cholesterol levels. The slight increase observed in some infant and human studies is within the normal range. A relationship between cholesterol status in the first year and later susceptibility for vascular disease has not been established.

Assessments of formula intake, tolerance, stool patterns, and formula satisfaction also were consistent across the formula groups in revealing no untoward effects of the supplemented formulas on tolerance. Analysis of reported clinical problems, rehospitalizations, serious and or adverse events, and death revealed no differences among the feeding groups. Fifteen deaths occurred during the course of the study; however, following an extensive review of the deaths, it was determined that the deaths were not related to the study products. These results indicate that the AA- and DHA-rich oil formulas presented no increased risks of safety or tolerance for this vulnerable population of infants.

In conclusion, the addition of AA- and DHA-rich oils to Similac® Special Care®, a formula for premature, low birth weight babies, and to Similac NeoSure®, a post-discharge formula for preterm infants, caused no adverse events at the levels provided in this clinical trial. Similac® Special Care® was supplemented with 0.43% AA (wt % of fatty acids in the formula) from the AA-rich oil, and 0.27 % DHA from the DHA-rich oil. Similac NeoSure® was supplemented with 0.43 % AA from the AA-rich oil, and 0.16% DHA from the DHA-rich oil.

Consumption of these supplemented formulas was not related to any adverse effects on infant's growth, development, morbidity, incidence of serious and or adverse events, antibiotic use, clinical chemistries, or antioxidant status as compared to infants fed the unsupplemented formulas. The supplemented formulas were well tolerated and the infants had normal stool characteristics as compared to infants fed the control formulas. The AA + DHA supplemented Similac NeoSure® formula was associated with significantly higher plasma cholesterol levels as compared to infants fed the unsupplemented formula, though despite the statistically significant difference, the cholesterol levels of the infants consuming the supplemented formula were within normal ranges and the effect is not believed to be adverse. Finally, the AA + DHA supplemented preterm formulas were found to have beneficial effects on Visual Evoked Potential grating acuity at 6-months CA. The results of this clinical trial indicate that the addition of AA- and DHA-rich oils to SSC and Similac NeoSure® formulas at the levels and conditions provided in this study are safe for consumption by premature infants.

TABLE VII-2 Disposition of Study Infants Subjects Randomized to the Feeding Protocol (n = 470)				
		AA + DHA		
	Control	Fungal/fish	Egg-DTG/fish	Exclusive Human Milk [†]
Randomized to Study	**154**	**158**	**158**	—
Study Groups	**144**	**140**	**143**	**43**
Randomized but withdrew on or prior to SD1	2	2	3	0
Withdrew but baseline demographic data were available	1	0	1	**0**
Intent-to-treat population	**143**	**138**	**141**	**43**
Started Study	**142**	**138**	**140**	**43**
Withdrew from feeding protocol but completed entire study [a]	23	30	11	1
Lost to follow-up	28	19	38	2
Completed the SSC Phase feeding protocol [b] (% randomized to study)	**126 (88)**	**120 (86)**	**126 (88)**	**42 (98)**
Completed the entire study feeding protocol [a] (% randomized to study)	**91 (63)**	**89 (64)**	**91 (64)**	**40 (93)**

[†] Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

[a] Includes subjects who did not attend all study visits but continued to follow the feeding protocol.

[b] A total of seven infants who withdrew from the feeding protocol portion of the study after SDAY 1 never received a SSC study feeding: 4 control infants; 2 fungal/fish oil infants; and 1 egg-DTG/fish oil infant.

000210

Table VII-3
Primary Reason for Early Exit from the Feeding Protocol
SSC and Similac NeoSure® Phases Combined

		AA + DHA		
Primary Reason for Early Exit [a]	**Control**	**Fungal/fish**	**Egg-DTG/fish**	**Exclusive Human Milk [†]**
Noncompliance	6	0	5	1
Parental dissatisfaction	4*	9	5	0
Vomiting, spit up, refused to drink formula, diarrhea/watery stools	19	22	11	1
Serious medical condition	4*	5	2	0
Death	4	3	6	0
Failure to thrive	0	1	0	0
Removed by investigator	4	0	2	0
Other	10	9	18	1

* Two additional deaths occurred in infants exiting for other reasons.
[a] Primary reason was assessed by the investigator at each site.
[†] Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000211

TableVII-4
Neonatal and Perinatal Characteristics of the Total Study Population of Preterm Infants
Intent-to-Treat Analysis

Characteristics	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM[†]
Birthweight[*], g				
Mean ± SEM	1287 ± 23	1305 ± 25	1309 ± 24	1275 ± 47.6
n	142	138	140	43
Gestational Age at Birth[*], wks				
Mean ± SEM	29.6 ± 0.2	29.8 ± 0.2	29.7 ± 0.2	29.7 ± 0.3
n	143	138	141	43
Size at Birth[‡], n				
SGA	9	14	14	7
AGA	133	124	126	36
Unknown	1	0	1	0
Gender[‡], n				
Male	77	77	77	20
Female	66	61	64	23
Multiple Birth Status[‡], n				
Singleton	119	110	101	39
Twin	24	28	40	4
Apgar Score[‡]				
Mean ± SEM	8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	8.3 ± 0.2
Median	8.0	8.0	8.0	8.0
1st, 3rd quartile	7.0, 9.0	8.0, 9.0	8.0, 9.0	8.0, 9.0
n	143	136	141	43
Ethnicity, n				
US[‡]				
Caucasian	61	59	62	32
African	22	19	28	2
Hispanic/Latino	4	2	3	0
Asian	0	1	0	0
Native American	0	1	0	0
Biracial	15	15	6	0
Other	1	0	1	0
n	103	97	100	34
UK[‡]				

000212

TableVII-4
Neonatal and Perinatal Characteristics of the Total Study Population of Preterm Infants
Intent-to-Treat Analysis

Characteristics	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM[†]
Caucasian	20	21	23	9
African	0	1	0	0
Asian	1	0	1	0
Biracial	3	0	0	0
Other	0	0	1	0
n	24	22	25	9
Chile[‡]				
Hispanic/Latino	16	19	16	--
n	16	19	16	--
Study Day 1				
Weight[*], g				
Mean ± SEM	1207 ± 23.2	1208 ± 23.4	1219 ± 23.6	1198 ± 49.8
n	142	138	140	43
Length,[*] cm				
Mean ± SEM	38.6 ± 0.3	38.9 ± 0.3	39.1 ± 0.3	38.6 ± 0.5
n	131	130	132	41
Head Circumference,[*] cm				
Mean ± SEM	27.2 ± 0.2	27.3 ± 0.2	27.4 ± 0.2	27.0 ± 0.4
n	133	129	135	40
Postnatal Age[*], d				
Mean ± SEM	5.5 ± 0.3	5.0 ± 0.3	4.6 ± 0.2	5.5 ± 0.4
n	142	138	140	43
Human Milk Intake, n[¶]				
EHM	--	--	--	43
EFF	43	40	36	--
≥ 50% avg kcal/d from formula	64	66	73	--
< 50% avg kcal/d from formula	35	32	31	--

000213

TableVII-4
Neonatal and Perinatal Characteristics of the Total Study Population of Preterm Infants
Intent-to-Treat Analysis

Characteristics	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†

†Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.
* Differences across formula groups were assessed by ANCOVA controlling for site, gender, birth weight stratum, feeding*gender, and feeding*birth weight stratum. No statistically significant differences were found.
‡ Differences across formula groups were assessed using Chi-Square analyses controlling for site.
Abbreviations used: AA, arachidonic acid; DHA, docosahexaenoic acid; DTG, derived triglyceride; EFF, exclusively formula fed
¶ Determination was made at term CA.

000214

Table VII-5
Mean Weight Gains and Absolute Weight Measurements by Formula Group
Intent-to-Treat Analysis

	Control	AA + DHA (fungal/fish)	AA + DHA (egg-DTG/fish)	Exclusive Human Milk †
Weight Gains	g/kg body weight/day Mean ± SEM (n)			
SDAY 1 to hospital discharge	14.7 ± 0.3 (135)	14.9 ± 0.3 (133)	14.1 ± 0.3 (136)	12.3 ± 0.5 (41)
SDAY 1 to term CA	13.4 ± 0.2 (135)	13.7 ± 0.2 (134)	13.3 ± 0.2 (135)	12.0 ± 0.3 (42)
SDAY 1 to 4-months CA	7.3 ± 0.1 (127)	7.3 ± 0.1 (126)	7.2 ± 0.1 (121)	7.1 ± 0.1 (41)
SDAY 1 to 12-months CA	3.6 ± 0.0 (119)	3.6 ± 0.0 (123)	3.5 ± 0.0 (105)	3.5 ± 0.0 (41)
Absolute Weight	g Mean ± SEM (n)			
Hospital discharge	2207 ± 39 (135)	2238 ± 36 (134)	2267 ± 42 (137)	2021 ± 45 (41)
Term CA	3073 ± 43 (135)	3158 ± 45 (134)	3175 ± 44 (135)	2740 ± 58 (42)
2-months CA	4851 ± 58 (131)	4963 ± 67 (131)	4842 ± 65 (126)	4389 ± 102 (41)
4-months CA	6229 ± 72 (127)	6208 ± 87 (126)	6177 ± 74 (121)	5658 ± 132 (41)
6-months CA	7287 ± 86 (119)	7197 ± 93 (128)	7267 ± 81 (117)	6755 ± 152 (40)
9-months CA	8274± 88 (123)	8306 ± 104 (125)	8324 ± 93 (109)	7948 ± 167 (41)
12-months CA	9094 ± 91 (119)	9068 ± 111 (123)	9125 ± 98 (105)	8814 ± 180 (41)

Note: see Table VII-8 for a summary of statistically significant feeding effects on growth using Bonferroni adjusted α-levels.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000215

Table VII-6
Mean Length Gains and Absolute Length Measurements by Formula Group
Intent-to-Treat Analysis

	Control	AA + DHA (fungal/fish)	AA + DHA (egg-DTG/fish)	Exclusive Human Milk†
Length Gains	mm/day Mean ± SEM (n)			
SDAY 1 to hospital discharge	1.3 ± 0.1 (123)	1.3 ± 0.1 (125)	1.1 ± 0.1 (124)	1.3 ± 0.1 (39)
SDAY 1 to term CA	1.4 ± 0.0 (124)	1.4 ± 0.0 (124)	1.4 ± 0.0 (125)	1.3 ± 0.1 (40)
SDAY 1 to 4-months CA	1.2 ± 0.0 (120)	1.2 ± 0.0 (123)	1.2 ± 0.0 (113)	1.1 ± 0.0 (39)
SDAY 1 to 12-months CA	0.8 ± 0.0 (111)	0.8 ± 0.0 (118)	0.8 ± 0.0 (100)	0.8 ± 0.0 (39)
Absolute Length	cm Mean ± SEM (n)			
Hospital discharge	43.9 ± 0.2 (131)	44.1 ± 0.2 (133)	44.1 ± 0.2 (132)	43.2 ± 0.3 (40)
Term CA	47.8 ± 0.2[a] (134)	48.1 ± 0.2[b] (132)	48.4 ± 0.2[a, b] (131)	46.9 ± 0.3 (42)
2-months CA	55.2 ± 0.2 (130)	55.2 ± 0.2 (131)	55.1 ± 0.2 (123)	53.5 ± 0.4 (41)
4-months CA	61.0 ± 0.2 (127)	60.8 ± 0.2 (126)	60.8 ± 0.2 (120)	59.3 ± 0.5 (41)
6-months CA	65.4 ± 0.2 (119)	65.0 ± 0.2 (127)	65.2 ± 0.2 (116)	63.7 ± 0.5 (40)
9-months CA	69.8 ± 0.2 (122)	69.5 ± 0.3 (125)	69.6 ± 0.3 (108)	68.8 ± 0.5 (41)
12-months CA	73.7 ± 0.3 (119)	73.2 ± 0.3 (123)	73.4 ± 0.3 (105)	73.1 ± 0.4 (41)

Note: see Table VII-8 for a summary of statistically significant feeding effects on growth using Bonferroni adjusted α-levels.

[a, b] Unlike superscripts within each row denote statistically significant differences using Level 3 analyses at term CA only ($p = 0.0417$)

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

Table VII-7
Mean Head Circumference Gains and Absolute Head Circumference Measurements by Formula Group
Intent-to-Treat Analysis

	Control	AA + DHA (fungal/fish)	AA + DHA (egg-DTG/fish)	Exclusive Human Milk†
Head circumference gains	mm/day Mean ± SEM (n)			
SDAY 1 to hospital discharge	1.3 ± 0.0 (125)	1.3 ± 0.1 (123)	1.2 ± 0.0 (127)	1.2 ± 0.0 (38)
SDAY 1 to term CA	1.2 ± 0.0 (126)	1.2 ± 0.0 (122)	1.2 ± 0.0 (131)	1.1 ± 0.0 (39)
SDAY 1 to 4-months CA	0.8 ± 0.0 (121)	0.8 ± 0.0 (121)	0.8 ± 0.0 (116)	0.8 ± 0.0 (38)
SDAY 1 to 12-months CA	0.4 ± 0.0 (112)	0.4 ± 0.0 (118)	0.4 ± 0.0 (103)	0.4 ± 0.0 (38)
Absolute head circumference measurements	cm Mean ± SEM (n)			
Hospital discharge	32.2 ± 0.1 (131)	32.1 ± 0.2 (132)	32.3 ± 0.2 (132)	31.5 ± 0.2 (40)
Term CA	35.3 ± 0.1 (134)	35.4 ± 0.1 (131)	35.5 ± 0.1 (135)	34.4 ± 0.2 (42)
2-months CA	39.1 ± 0.1 (130)	39.3 ± 0.1 (130)	39.3 ± 0.1 (125)	38.4 ± 0.2 (41)
4-months CA	41.8 ± 0.1 (127)	41.7 ± 0.1 (126)	41.9 ± 0.2 (121)	41.3 ± 0.2 (41)
6-months CA	43.6 ± 0.1 (118)	43.6 ± 0.2 (128)	43.8 ± 0.2 (115)	43.3 ± 0.2 (40)
9-months CA	45.1 ± 0.1 (123)	45.3 ± 0.2 (125)	45.5 ± 0.2 (108)	45.1 ± 0.2 (41)
12-months CA	46.3 ± 0.2 (119)	46.4 ± 0.2 (123)	46.4 ± 0.2 (105)	46.1 ± 0.2 (41)

Note: see Table VII-8 for a summary of statistically significant feeding effects on growth using Bonferroni adjusted α-levels.
† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000217

Table VII-8 Summary of Statistically Significant Feeding Effects on Growth Using Bonferroni Adjusted α-Levels *Intent-to-Treat and Evaluable Analyses*	
Outcome variable/ Population	**Difference**
Absolute Weights	
Term CA *Intent-to-treat*	AA + DHA (egg-DTG) > Control
Length Gains	
SDAY 1 to 4-months CA *Evaluable*	Control > AA + DHA (egg-DTG)
SDAY 1 to 12-months CA, 1250 g birth weight stratum *Intent-to-treat*	Control > AA + DHA (fungal/fish)
Absolute Lengths	
Term CA *Intent-to-treat*	AA + DHA (egg-DTG) > Control
Head Circumference Gains	
SDAY 1 to term CA, Female infants *Intent-to-treat* *Evaluable*	 Control > AA + DHA (egg-DTG) Control > AA + DHA (egg-DTG)
SDAY 1 to term CA, > 1250 g birth weight stratum *Intent-to-treat*	AA + DHA (fungal/fish) > AA + DHA (egg-DTG)

000218

Table VII-9
Visual Development: Visual Evoked Potential Grating Acuity
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
4-months CA				
Mean ± SEM (SD) n	8.4 ± 0.1 (0.8) 39	8.6 ± 0.1 (0.8) 46	8.3 ± 0.2 (0.9) 33	8.6 ± 0.1 (0.7) 22
6-months CA				
Mean ± SEM (SD) n	8.5 ± 0.1 (0.8) [a] 39	11.4 ± 0.1 (0.6) [b] 50	12.1 ± 0.1 (0.9) [b] 37	15.3 ± 0.1 (0.7) 22

Mean values are cycles/degree. SEM and SD values are octaves.

* Differences in acuity measures across formula groups were determined using PROC MIXED analyses controlled for: Level 1- site, gender, & birthweight stratum; Level 2 - site, gender, birthweight stratum, maternal history of smoking during pregnancy, postnatal smoking in the home, and maternal education; or Level 3 - site, gender, birthweight stratum, maternal history of smoking during pregnancy, postnatal smoking in the home, maternal education, and human milk intake.

There was a statistically significant interaction between feeding and visit using all 3 levels: Level 1, p=0.0312; Level 2, p=0.0324; Level 3, p=0.0328.

[a, b] VEP grating acuity of AA + DHA (Fungal/Fish)- and AA + DHA (egg-DTG/fish)-fed infants was consistently greater than control-fed infants at 6 months CA:

Level 1, AA + DHA (Fungal/Fish) > control, p=0.0120; AA + DHA (egg-DTG/fish) > control, p=0.0030.
Level 2, AA + DHA (Fungal/Fish) > control, p=0.0096; AA + DHA (egg-DTG/fish) > control, p=0.0017.
Level 3; AA + DHA (Fungal/Fish) > control, p=0.0098; AA + DHA (egg-DTG/fish) > control, p=0.0018.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000219

Table VII-10
Visual Development: Contrast Sensitivity Using a Contrast Sweep (1.5, 3.0, and 6.0 cycles/degree)*
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
1.5 cycles/degree				
4-months CA				
Mean ± SEM (SD) n	11.0 ± 0.1 (0.4) 34	11.6 ± 0.1 (0.4) 38	14.9 ± 0.1 (0.4) 26	11.2 ± 0.1 (0.4) 22
6-months CA				
Mean ± SEM (SD) n	9.2 ± 0.1 (0.5) 31	13.1 ± 0.1 (0.3) 44	13.8 ± 0.1 (0.4) 32	16.5 ± 0.1 (0.4) 19
3.0 cycles/degree				
4-months CA				
Mean ± SEM (SD) n	7.2 ± 0.1 (0.4) 31	5.2 ± 0.1 (0.4) 39	9.0 ± 0.1 (0.4) 25	7.3 ± 0.1 (0.3) 20
6-months CA				
Mean ± SEM (SD) n	7.0 ± 0.1 (0.4) 32	7.8 ± 0.1 (0.4) 43	8.7 ± 0.1 (0.4) 30	10.8 ± 0.1 (0.4) 21
6.0 cycles/degree				
4-months CA				
Mean ± SEM (SD) n	5.1 ± 0.1 (0.4) 14	3.1 ± 0.1 (0.2) 16	3.9 ± 0.1 (0.4) 11	3.7 ± 0.1 (0.3) 7
6-months CA				
Mean ± SEM (SD) n	5.1 ± 0.1 (0.3) 21	4.2 ± 0.1 (0.3) 32	7.0 ± 0.1 (0.3) 22	4.8 ± 0.1 (0.4) 16

000220

Table VII-10
Visual Development: Contrast Sensitivity Using a Contrast Sweep (1.5, 3.0, and 6.0 cycles/degree)*
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†

* Contrast sensitivity is the reciprocal of the contrast threshold. These data were $\log^{10}$ transformed prior to statistical analysis. Mean values are the antilog (geometric mean). SEM and SD are in log units. Differences were determined using PROC MIXED analyses controlled for: Level 1- site, gender, & birthweight stratum; Level 2 - site, gender, birthweight stratum, maternal history of smoking during pregnancy, postnatal smoking in the home, and maternal education; or Level 3 - site, gender, birthweight stratum, maternal history of smoking during pregnancy, postnatal smoking in the home, maternal education, and human milk intake. As > 50% of the data were missing for contrast sensitivity at 6.0 cycles/degrees at 4-months CA, statistical analysis was performed only on 6-months CA data. For all levels of analysis, there was a marginally statistically significant feeding effect (main effect) at 6 months-CA for contrast sensitivity at 6.0 cycles/degree, $0.05 < p < 0.10$:

AA + DHA (egg-DTG/fish) > AA + DHA (fungal/fish): Level 1, p=0.0196; Level 2, p=0.0287; Level 3, p-0.0275.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000221

Table VII-11
Visual Development: P60, N80, P100 Latency and N80-P100 Peak-to-Peak Amplitude Measures Derived from a Transient Checkerboard Stimulus (16x16 Check Size)*
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
N80 - P100 (peak to peak amplitude)				
4-months CA				
Mean ± SEM (SD) n	20.1 ± 1.6 (8.9) 29	23.5 ± 1.8 (10.5) 36	21.6 ± 2.3 (12.4) 29	25.9 ± 3.6 (16.2) 20
6-months CA				
Mean ± SEM (SD) n	18.8 ± 1.8 (9.0) 25	20.2 ± 1.7 (11.2) 44	20.6 ± 2.2(12.3) 30	23.5 ± 2.3 (9.9) 19
P60 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	67.7 ± 2.6 (11.7) 20	66.9 ± 1.9 (8.4) 20	66.8 ± 2.2 (8.8) 16	65.6 ± 2.9 (9.2) 10
6-months CA				
Mean ± SEM (SD) n	61.4 ± 2.7 (12.1) 20	61.7 ± 2.0 (11.3) 31	63.0 ± 1.8 (8.3) 21	62.2 ± 1.0 (3.5) 12
N80 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	82.1 ± 2.0 (10.7) 29	80.5 ± 1.3 (8.0) 36	79.3 ± 1.2 (6.5) 29	80.9 ± 1.4 (6.3) 20
6-months CA				
Mean ± SEM (SD) n	79.3 ± 2.3 (11.4) 25	79.7 ± 1.6 (10.4) 44	80.1 ± 1.3 (7.4) 30	78.6 ± 1.1(5.0) 19

Table VII-11
Visual Development: P60, N80, P100 Latency and N80-P100 Peak-to-Peak Amplitude Measures Derived from a Transient Checkerboard Stimulus (16x16 Check Size)*
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
P100 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	121.5 ± 2.5 (14.7) 36	117.8 ± 1.9 (11.9) 40	118.1 ± 1.6 (9.0) 30	121.3 ± 2.3 (10.5) 21
6-months CA				
Mean ± SEM (SD) n	109.1 ± 2.1 (11.2) 29	107.5 ± 2.0 (13.8) 46	113.7 ± 2.1 (11.5) 31	111.4 ± 2.4 (10.4) 19

* As > 50% of the data were missing for contrast sensitivity at P60 latency at 4-months CA, statistical analysis was performed only on 6-months CA data. Differences among study feeding were determined using PROC MIXED analyses controlled for: Level 1- site, gender, & birthweight stratum; Level 2 - site, gender, birthweight stratum, maternal history of smoking during pregnancy, postnatal smoking in the home, and maternal education; or Level 3 - site, gender, birthweight stratum, maternal history of smoking during pregnancy, postnatal smoking in the home, maternal education, and human milk intake. No statistically significant feeding or feeding*visit effects were found.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000223

Table VII-12
Visual Development: P60, N80, P100 Latency and N80-P100 Peak-to-Peak Amplitude Measures Derived from a Transient Checkerboard Stimulus (30x30 Check Size)*
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
N80 - P100 (peak to peak amplitude)				
4-months CA, n				
Mean ± SEM (SD) n	17.2 ± 1.4 (7.3) 28	20.5 ± 1.4 (8.7) 38	19.0 ± 2.2 (10.4) 22	23.5 ± 3.3 (14.1) 18
6-months CA				
Mean ± SEM (SD) n	14.9 ± 1.7 (7.8) 22	16.7 ± 1.5 (9.5) 38	19.6 ± 2.3 (11.0) 22	21.1 ± 2.4 (9.7) 17
P60 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	73.1 ± 3.0 (12.3) 17	71.9 ± 2.3 (11.4) 24	72.0 ± 2.9 (11.8) 16	74.2 ± 3.2 (11.1) 12
6-months CA				
Mean ± SEM (SD) n	66.3 ± 2.3 (9.2) 16	65.5 ± 2.2 (11.6) 27	70.2 ± 3.0 (11.5) 15	68.5 ± 3.4 (10.1) 9
N80 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	87.5 ± 2.2 (11.6) 28	88.1 ± 1.6 (9.8) 38	89.4 ± 2.1 (10.0) 22	87.3 ± 2.4 (10.0) 18
6-months CA				
Mean ± SEM (SD) N	81.9 ± 2.1 (10.0) 22	82.9 ± 2.0 (12.4 38	83.8 ± 1.9 (9.1) 22	81.2 ± 1.7 (7.2) 17

000224

Table VII-12
Visual Development: P60, N80, P100 Latency and N80-P100 Peak-to-Peak Amplitude Measures Derived from a Transient Checkerboard Stimulus (30x30 Check Size)*
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
P100 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	130.0 ± 2.4 (13.1) 30	128.4 ± 2.2 (13.9) 39	131.4 ± 1.6 (8.2) 25	131.4 ± 2.8 (12.4) 19
6-months CA				
Mean ± SEM (SD) n	118.9 ± 2.5 (12.8) 26	119.3 ± 2.6 (16.9) 42	122.6 ± 2.5 (12.4) 25	120.6 ± 2.4 (10.2) 18

* As > 50% of the data were missing for contrast sensitivity at P60 latency at 4-months CA, statistical analysis was performed only on 6-months CA data. Differences among study feeding groups were determined using PROC MIXED analyses controlled for: Level 1- site, gender, & birthweight stratum; Level 2 - site, gender, birthweight stratum, maternal history of smoking during pregnancy, postnatal smoking in the home, and maternal education; or Level 3 - site, gender, birthweight stratum, maternal history of smoking during pregnancy, postnatal smoking in the home, maternal education, and human milk intake. No statistically significant feeding or feeding*visit effects were found.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000225

Table VII-13
Visual Development: P60, N80, P100 Latency and N80-P100 Peak-to-Peak Amplitude Measures Derived from a Transient Checkerboard Stimulus (60x60 Check Size)*
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
N80 - P100 (peak to peak amplitude)				
4-months CA				
Mean ± SEM (SD) n	17.4 ± 2.6 (8.8) 11	14.3 ± 1.6 (6.6) 18	15.1 ± 1.5 (5.6) 14	19.7 ± 2.8 (8.8) 10
6-months CA				
Mean ± SEM (SD) n	19.5 ± 3.2 (13.3) 17	18.5 ± 2.2 (10.6) 23	24.1 ± 3.5 (15.4) 19	22.3 ± 3.5 (11.8) 11
P60 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	82.6 ± 2.2 (6.1) 8	80.5 ± 2.5 (8.9) 13	84.2 ± 3.3 (10.4) 10	85.1 ± 3.7 (9.9) 7
6-months CA				
Mean ± SEM (SD) n	69.9 ± 2.5 (8.1) 11	76.7 ± 3.6 (15.6) 19	72.8 ± 3.6 (12.4) 12	74.7 ± 5.5 (13.4) 6
N80 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	100.2 ± 3.0 (10.1) 11	105.9 ± 1.9 (8.2) 18	104.1 ± 2.3 (8.7) 14	103.4 ± 3.0 (9.6) 10
6-Months CA				
Mean ± SEM (SD) n	87.4 ± 2.2 (9.1) 17	91.5 ± 2.6 (12.7) 23	89.4 ± 2.7 (11.7) 19	89.6 ± 2.9 (9.5) 11

000226

Table VII-13
Visual Development: P60, N80, P100 Latency and N80-P100 Peak-to-Peak Amplitude Measures Derived from a Transient Checkerboard Stimulus (60x60 Check Size)*
Intent-to-Treat Analysis

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
P100 Latency, msec				
4-months CA				
Mean ± SEM (SD) n	137.7 ± 2.9 (10.1) 12	141.9 ± 2.2 (9.6) 19	145.0 ± 3.8 (14.2) 14	138.8 ± 2.8 (8.9) 10
6-months CA				
Mean ± SEM (SD) n	131.4 ± 2.1 (9.1) 19	134.1 ± 1.6 (8.0) 25	132.7 ± 2.0 (9.0) 21	134.9 ± 1.5 (5.0) 11

* As > 50% of these measures were missing, statistical analysis were not performed on these data.
† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000227

Table VII-14
Visual Development: Teller Visual Acuity Procedure
Intent-to-Treat Analysis*

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
2-months CA				
Mean ± SEM (SD)	1.83 ± 0.06 (0.72)	1.91 ± 0.06 (0.71)	1.82 ± 0.06 (0.67)	1.80 ± 0.12 (0.76)
n	124	129	120	40
4-months CA				
Mean ± SEM (SD)	3.43 ± 0.05 (0.60)	3.58 ± 0.05 (0.51)	3.70 ± 0.05 (0.50)	3.82 ± 0.09 (0.57)
n	123	122	116	41
6-months CA				
Mean ± SEM (SD)	6.99 ± 0.06 (0.66)	6.95 ± 0.05 (0.61)	7.03 ± 0.05 (0.50)	7.39 ± 0.12 (0.79)
n	116	123	111	40

* Mean values are cyc/deg. SEM and SD values are in octaves. Differences in acuity measures across formula groups were determined using PROC MIXED for repeated measures analyses controlling for: Level 1) site, gender, visit, birth weight stratum, feeding*gender, feeding*visit, and feeding*birth weight stratum; Level 2) site, gender, visit, birth weight stratum, size for gestation, HOME, WAIS-R, maternal smoking, postnatal smoking in the home, gestational age, feeding*gender, feeding*visit, and feeding*birth weight stratum; and Level 3) site, gender, visit, birth weight stratum, size for gestation, HOME, WAIS-R, maternal smoking, postnatal smoking in the home, gestational age, feeding*gender, feeding*visit, feeding*birth weight stratum, and human milk intake. A statistically significant effect was not observed for study feeding, feeding* visit, feeding *gender, or feeding* birth weight stratum.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

000228

Table VII-15
Information Processing: Fagan Test of Infant Intelligence: *Evaluable Analysis**

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM[†]
% Novelty Preference				
6-months CA				
Mean ± SEM (SD)	57.5 ± 0.8 (7.4)[a, b]	57.0 ± 0.8 (7.5)[a]	59.4 ± 0.8 (7.7)[b]	57.9 ± 1.1 (7.0)
n	96	91	92	38
9-months CA				
Mean ± SEM (SD)	58.4 ± 0.8 (7.2)	59.0 ± 0.8 (7.4)	58.9 ± 0.8 (7.2)	58.9 ± 1.3 (7.7)
n	92	82	81	36
Average Look Duration During the Familiarization Phase , sec				
6-months CA				
Mean ± SEM (SD)	2.2 ± 0.1 (0.8)	2.2 ± 0.1 (1.0)	2.1 ± 0.1 (0.8)	2.2 ± 0.1 (0.8)
n	96	91	92	38
9-months CA				
Mean ± SEM (SD)	1.4 ± 0.0 (0.4)	1.4 ± 0.0 (0.4)	1.5 ± 0.1 (0.5)	1.6 ± 0.1 (0.4)
n	92	83	82	36
Average Look Duration During the Test Phase, sec				
6-months CA				
Mean ± SEM (SD)	1.9 ± 0.1 (0.6)	1.8 ± 0.1 (0.7)	1.9 ± 0.1 (0.6)	1.9 ± 0.1 (0.6)
n	96	91	92	38
9-months				
Mean ± SEM (SD)	1.3 ± 0.0 (0.3)	1.3 ± 0.0 (0.4)	1.3 ± 0.0 (0.4)	1.3 ± 0.0 (0.3)
n	92	83	82	36
Average Look Duration for An Abbreviated Time Period of Familiarization, sec[‡]				
6-months CA				
Mean ± SEM (SD)	1.7 ± 0.1 (0.7)	1.8 ± 0.1 (1.4)	1.6 ± 0.1 (0.8)	1.8 ± 0.1 (0.8)
n	95	93	92	38
9-months CA				
Mean ± SEM (SD)	1.3 ± 0.0 (0.4)	1.4 ± 0.1 (0.6)	1.5 ± 0.1 (0.7)	1.4 ± 0.1 (0.4)
n	92	83	83	36

000229

Table VII-15
Information Processing: Fagan Test of Infant Intelligence: *Evaluable Analysis**

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†

* These outcomes represent secondary and/or exploratory variables; an intent-to-treat analysis was not conducted, therefore results of the evaluable analysis are presented here. Differences across study formula groups were determined using PROC MIXED for repeated measures analysis controlling for: Level 1) site, gender, visit, birth weight stratum, feeding*visit, feeding*gender, and feeding*birth weight stratum; Level 2) site, gender, visit, birth weight stratum, feeding*visit, feeding*gender, and feeding*birth weight stratum, HOME, WAIS-R, gestational age, size for gestation; Level 3) site, gender, visit, birth weight stratum, HOME, WAIS-R, gestational age, size for gestation, parity, maternal age, human milk intake, feeding*visit, feeding*gender, and feeding*birth weight stratum. A statistically significant feeding*visit interaction was observed for novelty preference (Level 3 only, p=0.0992), average look duration during an abbreviated time period of familiarization (Level 1, p=0.0864; Level 2, p=0.0802; Level 3, p=0.0712). Unlike superscripts in each row denote statistically significant differences using Level 3 analyses (Bonferroni adjusted α-level of 0.0083).

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses

‡ During the first 10 (6-months CA) or 6 (9-months CA) seconds of the first three familiarization periods

Abbreviations: AA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; DTG, derived triglyceride.

0002230

Table VII-16
General Development: Mental and Psychomotor Index Scores of the Bayley Scales of Infant Development at 12-Months CA: *Intent-to-Treat Analysis**

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
Mental Development Index (MDI)				
Mean ± SEM (SD)	92.15 ± 1.12 (12.17)	92.76 ± 1.01 (11.21)	93.40 ± 1.27 (13.03)	93.10 ± 2.26 (14.49)
n	119	123	105	41
Psychomotor Development Index (PDI)				
Mean ± SEM (SD)	86.28 ± 1.49 (16.18)	87.22 ± 1.28 (14.15)	85.90 ± 1.40 (14.37)	86.76 ± 2.37 (15.18)
n	118	123	105	41

* Differences in MDI and PDI standard scores across formula groups were determined using ANCOVA controlling for: Level 1) site, gender, birth weight stratum, feeding*gender, feeding*birth weight stratum; Level 2) site, gender, birth weight stratum, feeding*gender, feeding*birth weight stratum, size for gestation, HOME, WAIS-R, gestational age; and Level 3) site, gender, birth weight stratum, feeding*gender, feeding*birth weight stratum, HOME, WAIS-R, gestational age, size for gestation, human milk intake, birth order, and the first language of the biological mother. MDI and PDI scores < 50 were excluded from the statistical analyses but are included in the data presented in this Table. No statistically significant effects of study formula feeding, feeding*gender, or feeding*birth weight stratum were found.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses

Abbreviations: AA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; DTG, derived triglyceride; HOME, WAIS-R.

0002231

Table VII-17
General Development: Bayley Scales of Infant Development at 12-Months CA: *Intent-to-Treat Analysis**

	Control (n=119)	AA + DHA (Fungal/Fish) (n=123)	AA + DHA (Egg-DTG/Fish) (n=105)	EHM† (n=43)
Infants with MDI < 70, n (%)	6 (5.0)	2 (1.6)	5 (4.8)	3 (7.3)
Infants with PDI < 70, n (%)	15 (12.7)	14 (11.4)	12 (11.4)	5 (12.2)
Infants with MDI < 50, n (%)	1 (0.8)	1 (0.8)	2 (1.9)	0 (0.0)
Infants with PDI < 50, n (%)	4 (3.4)	3 (2.4)	4 (3.8)	2 (4.9)

* Standard scores. Differences across study formula groups were evaluated by Cochran-Mantel-Haenszel statistics. No statistically significant differences were found.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

Abbreviations: AA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; DTG, derived triglyceride; MDI, mental development index; PDI, psychomotor development index.

000232

Table VII-18
Language Development: Vocabulary Comprehension Scores
from the MacArthur Communicative Development Inventories: *Intent-to-Treat Analysis**

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
9-months CA				
Mean ± SEM (SD)	103.7 ± 2.0 (21.9)	104.2 ± 1.7 (19.1)	101.7 ± 1.9 (19.4)	96.2 ± 2.8 (17.6)
n	122	122	102	39
14-months CA				
Mean ± SEM (SD)	99.9 ± 1.7 (17.1)	101.6 ± 1.6 (16.4)	101.2 ± 2.0 (18.8)	97.0 ± 2.4 (14.5)
n	98	101	93	38

* Differences in the standard comprehension scores across study formula groups were determined using PROC MIXED for repeated measures analysis controlling for Level 1) site, gender, birth weight stratum, visit, feeding*visit, feeding*gender, and feeding*birth weight stratum; Level 2) site, visit, gender, birth weight stratum, size for gestation, gestational age, HOME, WAIS-R; feeding*visit, feeding*gender, and feeding*birth weight stratum; Level 3) site, visit, gender, birth weight stratum, size for gestation, gestational age, HOME, WAIS-R, feeding*visit, and human milk intake. No statistically significant effects of feeding, feeding*visit, feeding*gender, or feeding*birth weight stratum were found.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses

Abbreviations: AA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; DTG, derived triglyceride.

000233

Table VII-19
Language Development: Vocabulary Production Scores and Phrases Understood from the MacArthur Communicative Development Inventories at 14 Months CA: *Intent-to-Treat Analysis**

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
Vocabulary Production				
Mean ± SEM (SD)	97.8 ± 1.9 (18.4)	96.6 ± 1.7 (17.2)	98.3 ± 1.9 (18.1)	96.6 ± 3.1 (18.9)
n	98	102	93	38
Phrases Understood				
Mean ± SEM (SD)	100.4 ± 1.7 (17.3)	103.5 ± 1.8 (17.8)	102.8 ± 2.1 (20.0)	97.0 ± 2.2 (13.7)
n	99	102	93	38

* Differences in the standard comprehension scores across study formula groups were determined using ANCOVA controlling for Level 1) site, visit, gender, birthweight stratum, feeding*gender, and feeding*birth weight stratum; Level 2) site, gender, birth weight stratum, size for gestation, HOME, WAIS-R; feeding*gender, and feeding*birth weight stratum; Level 3) site, gender, birth weight stratum, size for gestation, HOME, WAIS-R, feeding*gender, feeding*birth weight stratum, and human milk intake. No statistically significant effects of feeding, feeding*gender, or feeding*birth weight stratum were found.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

Abbreviations: AA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; DTG, derived triglyceride

000234

Table VII-20
Temperament: Factors of the Behavior Rating Scale (Orientation/Engagement, Emotional Regulation, Motor Quality), Scores in Questionable and Nonoptimal Percentiles: *Evaluable Analysis**

	Control (n=79)	AA + DHA (Fungal/Fish) (n=77)	AA + DHA (Egg-DTG/Fish) (n=76)	EHM† (n=35)
Orientation/Engagement Factor, n (%)				
Questionable	25 (31.7)	14 (18.2)	12 (15.8)	6 (17.1)
Nonoptimal	5 (6.3)	10 (13.0)	14 (18.4)	6 (17.1)
Emotional Regulation Factor, n (%)				
Questionable	15 (19.0)	20 (26.0)	12 (15.8)	5 (14.3)
Nonoptimal	11 (13.9)	8 (10.4)	14 (18.4)	4 (11.4)
Motor Quality Factor, n (%)				
Questionable	12 (15.2)	8 (10.4)	8 (10.5)	4 (11.4)
Nonoptimal	13 (16.5)	7 (9.1)	11 (14.5)	3 (8.6)

* These outcomes represent secondary and/or exploratory variables; an intent-to-treat analysis was not conducted, therefore results of the evaluable analysis are presented here.
Standard scores. Data across formula groups were evaluated by Cocharan-Mantal-Haenszel statistics controlling for site. No statistically significant differences were found. Each factor can fall into one of three descriptive categories:
Within normal limits (≥ 26th percentile for age)
Questionable (11th < 25th percentile for age)
Nonoptimal (≤ 10th percentile for age)
† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.
Abbreviations: AA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; DTG, derived triglyceride

Table VII-21
Temperament: Infant Behavior Questionnaire at 6- and 12-Months CA: *Evaluable Analysis**

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
Activity Level				
6-months CA				
Mean ± SEM (SD) n	4.4 ± 0.1 (0.9) 95	4.2 ± 0.1 (0.9) 92	4.2 ± 0.1 (0.9) 87	4.3 ± 0.1 (0.8) 38
12-months CA				
Mean ± SEM (SD) n	4.4 ± 0.1 (0.9) 78	4.6 ± 0.1 (0.9) 78	4.5 ± 0.1 (1.0) 75	4.8 ± 0.1 (0.8) 35
Distress to Novel Stimuli				
6-months CA				
Mean ± SEM (SD) n	2.8 ± 0.1 (1.0) 95	2.8 ± 0.1 (1.0) 92	2.9 ± 0.1 (0.9) 87	2.6 ± 0.1 (0.8) 38
12-months CA				
Mean ± SEM (SD) n	3.0 ± 0.1 (0.8) 78	3.0 ± 0.1 (0.8) 78	3.0 ± 0.1 (0.9) 75	2.9 ± 0.1 (0.8) 35
Soothability				
6-months CA				
Mean ± SEM (SD) n	5.5 ± 0.1 (1.0) 95	5.5 ± 0.1 (0.9) 91	5.6 ± 0.1 (1.0) 87	5.0 ± 0.1 (0.8) 38
12 Months CA				
Mean ± SEM (SD) Median 1st, 3rd quartile n	5.4 ± 0.1 (1.1) 5.6 4.9, 6.1 78	5.4 ± 0.1 (0.9) 5.3 4.6, 6.0 78	5.5 ± 0.1 (1.0) 5.5 4.8, 6.3 75	5.0 ± 0.2 (0.9) 5.1 4.4, 5.6 35
Distress to Limitations				
6-months CA				
Mean ± SEM (SD) n	3.3 ± 0.1 (0.8) 95	3.2 ± 0.1 (0.8) 92	3.2 ± 0.1 (0.8) 87	3.2 ± 0.1 (0.8) 38
12-months CA				
Mean ± SEM (SD) n	3.7 ± 0.1 (0.8) 78	3.7 ± 0.1 (0.8) 78	3.7 ± 0.1 (0.9) 75	3.6 ± 0.1 (0.8) 35

000236

Table VII-21
Temperament: Infant Behavior Questionnaire at 6- and 12-Months CA: *Evaluable Analysis**

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
Smiling and Laughter				
6-months CA				
Mean ± SEM (SD)	5.5 ± 0.1 (0.9)	5.4 ± 0.1 (0.9)	5.5 ± 0.1 (0.8)	5.0 ± 0.2 (1.0)
n	95	92	87	38
12-months CA				
Mean ± SEM (SD)	5.3 ± 0.1 (0.9)	5.2 ± 0.1 (0.7)	5.5 ± 0.1 (0.7)	5.0 ± 0.1 (0.7)
n	78	78	75	35
Duration of Orienting				
6-months CA				
Mean ± SEM (SD)	4.5 ± 0.1 (1.2)	4.5 ± 0.1 (1.0)	4.5 ± 0.1 (1.0)	3.8 ± 0.2 (1.0)
n	95	92	87	38
12-months CA				
Mean ± SEM (SD)	3.8 ± 0.1 (1.2)	3.7 ± 0.1 (1.1)	3.9 ± 0.1 (1.1)	3.3 ± 0.2 (1.0)
n	78	78	75	35

* These outcomes represent secondary and/or exploratory variables; an intent-to-treat analysis was not conducted, therefore results of the evaluable analysis are presented here.

Values are based on a 7-point scale; 1 = never, 7 = always. Descriptions of Categories:

Activity: Gross motor and locomotor activity.

Distress to Novel Stimuli: Distress and extended latency to approach intense or novel stimuli.

Soothability: Reduction in fussiness when soothing techniques are used.

Distress to Limitations: Crying or fussiness while waiting for food or when prevented access to a toy.

Smiling and Laughter:

Duration of Orienting: Vocalizing, playing with, or looking at object for an extended time period.

Differences across study formula groups were determined using PROC MIXED for repeated measures analysis controlling for site, gender, visit, birth weight stratum, feeding*visit, feeding*gender, and feeding*birth weight stratum. Statistically significant feeding*visit ($p=0.0583$) and feeding*birth weight stratum ($p=0.0306$) interaction was found for activity level. Further, a statistically significant interaction for feeding*gender ($p=0.0568$) on soothability was found. However, pairwise comparison of study formula groups at or within the appropriate visit, birth weight stratum, or gender did not yield any statistically significant differences using a Bonferroni adjusted α-level.

† Exclusive Human Milk (EHM) infants are defined as those infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from time of hospital discharge to term corrected age. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.

Abbreviations: AA, arachidonic acid; CA, corrected age; DHA, docosahexaenoic acid; DTG, derived triglyceride.

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Table VII-22
Reference Ranges for Blood Analytes in Infants[a]

Sodium (mM/L)		**Chloride (mM/L)**	
1 to 7 days	133-146	1 to 7 days	100-117
8 to 21 days	129-142	8 to 21 days	102-116
22 to 35 days	133-148	22 to 35 days	110-115
> 35 days	133-142	> 35 days	101-115
Common conversions: 1 mM/L or 1 mmol/L = 1 mEq/L		*Common conversions:* 1 mM/L or 1 mmol/L = 1 mEq/L	
Potassium (mM/L)		**CO2 (mM/L)**	
1 to 7 days	4.6-6.7	1 to 7 days	13.8-27.1
8 to 21 days	4.5-7.1	8 to 21 days	12.4-26.2
22 to 35 days	4.5-6.6	22 to 35 days	12.5-26.1
> 35 days	4.6-7.1	> 35 days	13.7-26.9
Common conversions: 1 mM/L or 1 mmol/L = 1 mEq/L		*Common Conversions:* 1 mM/L or 1 mmol/L = 1 mEq/L	
Phosphorus (mg/dL)		**Calcium (mg/dL)**	
1 to 7 days	5.4-10.9	1 to 7 days	6.1-11.6
8 to 21 days	6.2-8.7	8 to 21 days	8.1-11
22 to 35 days	5.6-7.9	22 to 35 days	8.6-10.5
> 35 days	4.2-8.2	> 35 days	8.6-10.8
Common conversions: mg/dL x 0.3229 = mmol/L 1 mg/dL = 1 mg%		*Common conversions:* mg/dL x 0.2495 = mmol/L 1 mg/dL = 1 mg%	
Alkaline Phosphatase (U/L)	150-400		
BUN (mg/dL)		**Albumin (g/dL)**	
1 to 7 days	3.1-25.5	1 to 7 days	3.28-4.5
8 to 21 days	2.1-31.4	8 to 21 days	3.16-5.26
22 to 35 days	2-26.5	22 to 35 days	3.2-4.34
> 35 days	2.5-30.5	> 35 days	3.4-4.6
Common conversions: mg/dL x 0.3570 = mmol/L		*Common conversions:* G/dL x 10 = g/L 1 mg/dL x 1000 = 1 g/dL	
Creatinine* (mg/dL)		**Total Protein (g/dL)**	
1-4 days	0.3-1.0	1 to 7 days	4.4-6.26
> 4 days	0.2-0.4	8 to 21 days	4.28-6.7
Common conversions: mg/dL x 88.40 = µmol/L 1 mg/dL = 1 mg%		22 to 35 days 35 days *Common conversions:* G/L x 0.1 = g/dL 1 g/L = 1 g%	4.14-6.9 4.02-5.86
SGOT (AST) (IU/L) ≤ 54		**SGPT (ALT) (IU/L)** ≤ 50	
Total Bilirubin* (mg/dL)		**Direct Bilirubin* (mg/dL)**	<0.2
Day 1 (Birthday) Day 2 3 to 5 days 5 days *Common conversions:* mg/dL x 17.1 = µmol/L 1 mg/dL = 1 mg%	<8.0 <12.0 <16.0 <2.0	*Common conversions:* mg/dL x 17.1 = µmol/L 1 mg/dL = 1 mg%	

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Table VII-22 Reference Ranges for Blood Analytes in Infants[a]			
Hemoglobin (g/dL)		**Hematocrit (%)**	
1-3 days	<14.5	1-3 days	<45
4 days-7 days	<13.5	4 days-7 days	<42
8 days-14 days	<12.5	8 days-14 days	<39
15 days-30 days	<10	15 days-30 days	<31
31 days-60 days	<9	31 days-60 days	<28
61 days-180 days	<9.5	61 days-180 days	<29
> 180 days	<10.5	> 180 days	<33
Common conversions: g/dL x 10 = g/L		*Common conversions:* % divided by 100 = 1	
Platelets (x 1,000 mm^3)		**MCV (fl)**	
1-2 days	80-356	1-3 days	<95
3-4 days	61-335	4 days-7 days	<88
5-6 days	100-502	8 days-14 days	<86
7-9 days	124-678	15 days-30 days	<85
10-13 days	172-680	31 days-60 days	<77
14-20 days	147-670	61 days-180 days	<74
21-27 days	201-720	> 180 days	<70
> 27 days	212-625		
MCH (pg)		**MCHC (g/dL)**	
1-3 days	<31	1-3 days	<29
4 days-7 days	<28	4 days-7 days	<28
8 days-14 days	<28	8 days-14 days	<28
15 days-30 days	<28	15 days-30 days	<29
31 days-60 days	<26	31 days-60 days	<29
61 days-180 days	<25	61 days-180 days	<30
> 180 days	<23	> 180 days	<30
White Blood cells (number/mm^3)			
Day 1 (birthday)	9,000-30,000		
2-7 days	5,000-21,000		
> 7 days	5,000-20,000		
Polys or Segs† (103 cells/µl)		**(%)‡**	
<12days	2.0-9.0	days 1 to 3	32.2-69.8
12-71 days	3.0-11.0	days 4 to 7	21.6-67.7
72-143 days	3.0-7.0	days 8-14	15.8-65.9
> 143 days	2.0-7.0	days 15-30	17.3-61.0
		> day 30	16.0-58.9
Basophils (number/mm^3)		**(%)‡**	
Day 1 (birthday)	0-640	days 1 to 3	0-2.3
2-7 days	0-250	days 4 to 7	0-2.7
> 7 days	0-230	days 8-14	0-1.8
		days 15-30	0-1.2
		> day 30	0-1.0
Eosinophils (number/mm^3)		**(%)‡**	
Day 1 (birthday)	20-850	days 1 to 3	0-2.9
2-7 days	70-1100	days 4 to 7	0-7.5
> 7 days	70-1000	days 8-14	0-5.0
		days 15-30	0-7.0
		> day 30	0-5.4

Table VII-22 Reference Ranges for Blood Analytes in Infants[a]			
Monocytes (number/mm^3)		**(%)**[‡]	
Day 1 (birthday)	400-3,100	days 1 to 3	0.8-16.3
2-7 days	300-2,700	days 4 to 7	1.3-18.7
> 7 days	200-2,400	days 8-14	1.9-22.2
		days 15-30	1.0-22.9
		> day 30	2.4-19.6
Lymphocytes (number/mm^3)		**(%)**[‡]	
Day 1 (birthday)	2,000-11,000	days 1 to 3	13.4-46.4
2-7 days	2,000-17,000	days 4 to 7	18.4-52.9
> 7 days	2,000-17,000	days 8 to 14	13.0-54.7
		days 15 to 30	10.4-58.7
		> day 30	21.3-63.6
Bands* (x10^3 cells/µl)		**C-reactive protein*** (mg/dL)	<1.0
Day 1 of life	0-1.4		
2 to 3 days	0-0.8	*Common conversions:* mg/dL x 10 = mg/L	
4 days	0-0.6		
5-28 days	0-0.5		
> 28 days	0-1.0		
Common conversions: x10^3 cells/µl = 10^9 cell/L			

[a] Primary source for reference values: Neonatology-Pathophysiology and Management of the Newborn, Avery 1994.
[b] Tietz, NW et al 1990
[c] Klaus et al 1993
[d] Soldin et al 1997

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Table VII-23
Percentage of Preterm Infants with an Abnormal Blood Value Fed Human Milk and/or a Preterm Formula with or without AA and DHA or Exclusively Human Milk

Blood Analytes[a]	Control (n=138)	Fungal/Fish (n=133)	Egg-DTG/Fish (n=136)	EHM† (n=54)
Electrolytes				
Sodium[†], %	42	38	47	48
Potassium, %	59	62	60	59
Chloride, %	48	44	51	37
CO_2, %	22	28	29	13
Kidney Function				
Blood urea nitrogen, %	20	21	21	11
Creatinine, %	68	75	74	74
Albumin, %	57	45	55	57
Liver Function				
SGOT, %	16	16	19	28
SGPT, %	6	5	4	7
Bilirubin (total), %	83	91	89	91
Bilirubin (direct), %	46	46	55	30
Chemistries				
Calcium, %	32	32	36	28
Phosphorus, %	51	51	49	59
Alkaline phosphatase, %	36	36	39	31
Total protein, %	12	11	13	7
Complete Blood Counts				
Hemoglobin, %	52	51	58	44
Hematocrit, %	64	68	65	57
MCH, %	2	1	1	2
MCHC, %	0	2	0	2
Mean corpuscular volume, %	4	3	2	2
Total white blood cells, %	33	31	27	33
Platelets, %	41	38	43	31

[†] Exclusive Human Milk (EHM) infants are defined as those infants receiving exclusively human milk at the time of these assessments. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.
[a] The normative ranges for each blood analyte are found in Table VII-22.
Differences across study formula groups were evaluated using Chi-square association analyses. No statistically significant differences were found.

Table VII-24
Percentage of Preterm Infants with an Abnormal Blood Value Fed Human Milk and/or a Preterm Formula with or without AA and DHA or Exclusively Human Milk
Percent Above or Below Normal (Post-hoc assessment)

Blood Analytes [a]	Control (n=138)	Fungal/Fish (n=133)	Egg-DTG/Fish (n=136)	EHM† (n=54)
Electrolytes				
Sodium[T]				
% below normal	28	21	28	33
% above normal	25	26	24	22
Potassium				
% below normal	51	51	50	54
% above normal	17	20	16	15
Chloride				
% below normal	46	38	45	37
% above normal	9	8	9	9
CO_2				
% below normal	4	5	7	0
% above normal	18	26	25	13
Kidney Function				
Blood urea nitrogen				
% below normal	14	15	10	7
% above normal	5	6	10	6
Creatinine				
% below normal	4	2	1	0
% above normal	68	75	73	74
Albumin				
% below normal	57	44	54	57
% above normal	0	1	0	0
Liver Function				
SGOT				
% below normal	0	0	0	0
% above normal	9	8	9	13
SGPT				
% below normal	0	0	0	0
% above normal	2	2	2	2
Bilirubin (total)				
% below normal	0	0	0	0
% above normal	83	91	88	91
Bilirubin (direct)				
% below normal	0	0	0	0
% above normal	46	46	54	30

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Table VII-24
Percentage of Preterm Infants with an Abnormal Blood Value Fed Human Milk and/or a Preterm Formula with or without AA and DHA or Exclusively Human Milk
Percent Above or Below Normal (Post-hoc assessment)

Blood Analytes [a]	Control (n=138)	Fungal/Fish (n=133)	Egg-DTG/Fish (n=136)	EHM† (n=54)
Chemistries				
Calcium				
% below normal	13	14	16	2
% above normal	20	19	20	28
Phosphorus				
% below normal	46	44	45	52
% above normal	7	8	6	7
Alkaline phosphatase				
% below normal	10	6	8	4
% above normal	27	31	32	28
Total protein				
% below normal	9	11	12	7
% above normal	2	0	0	0
Complete Blood Counts				
Hemoglobin				
% below normal	52	50	56	44
% above normal	0	0	0	0
Hematocrit				
% below normal	64	68	64	57
% above normal	0	0	0	0
MCH				
% below normal	2	1	1	2
% above normal	0	0	0	0
MCHC				
% below normal	0	2	0	2
% above normal	0	0	0	0
Mean corpuscular volume				
% below normal	4	2	2	0
% above normal	0	0	0	0
Total white blood cells				
% below normal	4	4	4	6
% above normal	30	29	25	30
Platelets				
% below normal	22	20	25	22
% above normal	22	17	20	11

† Exclusive Human Milk (EHM) infants are defined as those infants receiving exclusively human milk at the time of these assessments. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses.
[a] The normative ranges for each blood analyte are found in Table VII-22.
The percent below and above normal was identified *post-hoc*; no statistical comparisons among groups were conducted.

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Table VII-25 Blood Chemistries In-Hospital: Electrolytes				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
Sodium (mEq/L)				
0 to 30 days				
Mean ± SD	137.8 ± 2.9	138 ± 3.3	138 ± 3.1	137.4 ± 3.0
Median	137.5	138	137.7	136.9
1st , 3rd quartile	136 , 139.9	136.1 , 140.2	135.6 , 140.0	135.3 , 138.8
Range	131.3 - 147.0	129.1 - 147.0	130.7 - 145.6	131.5 – 144.5
n	129	129	133	51
31 to 60 days				
Mean ± SD	137.6 ± 2.5	137.3 ± 2.3	137.0 ± 2.5	136.6 ± 2.2
Median	137.6	137.0	137.0	136.7
1st , 3rd quartile	136.0 , 139.3	136.0 , 138.0	135.5 , 139.0	135.0 , 137.5
Range	132.0 - 143.0	132.0 - 142.0	128.0 - 142.0	131.8 – 141.5
n	45	42	52	19
>60 days				
Mean ± SD	138.8 ± 2.8	139.0 ± 2.4	137.9 ± 2.4	-
Median	138.5	138.6	137.8	138.0
1st , 3rd quartile	137.0 , 140.4	137.0 , 141.0	137.0 , 140.0	-
Range	134.0 – 145.0	136.0 - 143.2	132.3 – 141.0	134.0 – 139.5
n	12	15	14	5
Chloride (mEq/L)				
0 to 30 days				
Mean ± SD	107.1 ± 4.3	107.0 ± 5.2	106.6 ± 4.8	107.2 ± 4.4
Median	107.5	106.9	106.5	107.3
1st , 3rd quartile	104.2 , 109.8	104.6 , 110.2	104.1 , 110.0	104.5 , 109.7
Range	95.7 – 120.0	91.7 – 119.0	88.9 – 116.0	96.0 – 119.0
n	100	104	108	38
31 to 60 days				
Mean ± SD	106.3 ± 4.5	104.9 ± 5.0	106.6 ± 4.8	105.2 ± 2.8
Median	107.3	105.0	107.0	105.5
1st , 3rd quartile	104.2 , 109.4	100.9 , 109.5	105.0 , 110.0	103.4 , 108.0
Range	95.8 – 112.5	97.9 – 113.0	90.5 – 113.5	101.4 – 108.6
n	30	25	35	9
>60 days				
Mean ± SD	105.8 ± 4.8	105.2 ± 4.1	106.4 ± 3.6	-
Median	107.5	150.4	105.0	104.5
1st , 3rd quartile	102.1 , 109.3	102.9 , 108.0	103.3 , 110.2	-
Range	97.5 – 111.0	98.5 – 112.0	103.0 – 111.0	104.5 – 116.0
n	8	9	7	3
Potassium (mEq/L)				
0 to 30 days				
Mean ± SD	5.2 ± 0.6	5.2 ± 0.7	5.3 ± 0.7	5.2 ± 0.6
Median	5.1	5.2	5.2	5.0
1st , 3rd quartile	4.8 , 5.5	4.8 , 5.6	4.9 , 5.6	4.8 , 5.5
Range	4.0 – 7.3	3.5 – 7.9	3.9 – 8.0	3.5 – 6.9
n	117	122	124	48

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Table VII-25 Blood Chemistries In-Hospital: Electrolytes				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
31 to 60 days				
Mean ± SD	4.9 ± 0.6	5.0 ± 0.7	4.9 ± 0.7	4.6 ± 0.4
Median	5.0	5.0	5.0	4.8
1st , 3rd quartile	4.6 , 5.3	4.4 , 5.4	4.6 , 5.4	4.2 , 4.9
Range	3.5 – 6.2	4.0 – 6.7	3.4 – 6.0	3.9 – 5.1
n	38	33	41	13
>60 days				
Mean ± SD	5.1 ± 0.7	5.1 ± 0.8	4.9 ± 1.0	-
Median	5.0	5.2	4.8	5.2
1st , 3rd quartile	4.6 , 5.8	4.5 , 5.5	4.4 , 5.9	-
Range	4.1 – 6.4	3.5 – 6.1	3.3 – 6.3	4.4 – 5.5
n	10	13	9	4
CO_2 (mEq/L)				
0 to 30 days				
Mean ± SD	21.0 ± 3.0	21.8 ± 3.2	21.8 ± 3.1	21.4 ± 2.9
Median	20.9	22.0	21.9	21.4
1st , 3rd quartile	19.1 , 22.9	19.3 , 24.0	19.6 , 24.0	19.4 , 23.0
Range	14.0 – 30.7	15.0 – 28.5	15.1 – 31.0	15.0 – 28.1
n	76	91	88	30
31 to 60 days				
Mean ± SD	23.0 ± 3.0	26.5 ± 2.9	25.3 ± 6.0	22.7 ± 2.8
Median	23.0	26.0	23.5	23.0
1st , 3rd quartile	20.4 , 25.5	25.3 , 28.3	21.3 , 28.0	21.9 , 24.5
Range	17.3 – 28.0	20.25 – 32.1	18.5 – 41.5	18.0 – 26.0
n	21	16	22	6
>60 days				
Mean ± SD	23.7 ± 4.2	-	-	-
Median	24.0	24.4	29.6	20.0
1st , 3rd quartile	21.0 , 27.1	-	-	-
Range	18.0 – 28.0	23.0 – 30.5	24.0 – 37.5	17.0 – 23.0
n	6	5	4	2

† Exclusive Human Milk (EHM) infants are defined as those infants receiving exclusively human milk at the time of these assessments. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses. When the sample size was 5 or less, only the median and range of values are shown.

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Table VII-26 Blood Chemistries In-Hospital: Kidney Status				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
BUN (mg/dL)				
0 to 30 days				
Mean ± SD	9.5 ± 6.6	8.9 ± 6.1	10.3 ± 6.8	10.2 ± 7.3
Median	7.7	7.2	8.5	7.0
1st , 3rd quartile	5.0 , 12.5	5.0 , 11.4	5.7 , 13.3	5.0 , 15.0
Range	2.0 – 48.5	2.0 – 52.4	1.5 – 46.3	3.0 – 36.8
n	102	110	115	43
31 to 60 days				
Mean ± SD	5.0 ± 3.0	5.5 ± 3.1	4.9 ± 3.0	5.8 ± 3.0
Median	4.0	4.8	4.1	4.4
1st , 3rd quartile	3.3 , 5.9	3.4 , 7.3	3.2 , 5.0	3.8 , 7.5
Range	1.0 – 15.4	1.7 – 14.0	2.0 – 19.5	2.7 – 11.6
n	41	38	43	13
>60 days				
Mean ± SD	5.6 ± 3.2	6.4 ± 4.2	5.7 ± 2.3	-
Median	4.0	4.3	5.1	4.6
1st , 3rd quartile	3.5 , 9.0	3.4 , 8.9	4.0 , 7.0	-
Range	2.5 – 11.4	2.3 – 15.4	3.0 – 10.5	3.0 – 7.5
n	10	12	14	4
Creatinine (mg/dL)				
0 to 30 days				
Mean ± SD	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Median	0.7	0.7	0.7	0.6
1st , 3rd quartile	0.6 , 0.8	0.6 , 0.8	0.5 , 0.8	0.5 , 0.8
Range	0.3 – 1.6	0.3 – 1.3	0.3 – 1.6	0.3 – 1.0
n	96	102	109	44
31 to 60 days				
Mean ± SD	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Median	0.4	0.4	0.5	0.5
1st , 3rd quartile	0.4 , 0.5	0.3 , 0.5	0.4 , 0.5	0.4 , 0.5
Range	0.3 – 0.7	0.2 – 0.7	0.2 – 0.7	0.3 – 0.6
n	38	34	35	15
>60 days				
Mean ± SD	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	-
Median	0.4	0.4	0.4	0.4
1st , 3rd quartile	0.3 , 0.5	0.3 , 0.4	0.3 , 0.4	-
Range	0.2 – 0.5	0.2 – 0.6	0.3 – 0.5	0.2 – 0.6
n	11	12	12	4
Albumin (g/dL)				
0 to 30 days				
Mean ± SD	3.0 ± 0.6	3.1 ± 0.5	3.1 ± 0.4	3.1 ± 0.4
Median	3.0	3.0	3.1	3.1
1st , 3rd quartile	2.6 , 3.4	2.7 , 3.4	2.8 , 3.4	2.7 , 3.4
Range	0.4 – 4.7	1.9 – 4.4	2.1 – 4.1	2.0 – 3.9
N	90	81	93	41

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Table VII-26 Blood Chemistries In-Hospital: Kidney Status				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
31 to 60 days				
Mean ± SD	3.0 ± 0.5	3.1 ± 0.5	3.0 ± 0.5	3.0 ± 0.5
Median	3.0	3.1	3.1	3.0
1st , 3rd quartile	2.7 , 3.4	2.8 , 3.4	2.6 , 3.4	2.8 , 3.4
Range	2.0 – 4.1	1.8 – 4.1	1.9 – 4.2	2.4 – 3.7
N	33	33	35	13
>60 days				
Mean ± SD	3.2 ± 0.5	3.3 ± 0.3	3.0 ± 0.3	-
Median	3.4	3.4	3.1	3.0
1st , 3rd quartile	3.1 , 3.5	3.0 , 3.6	2.9 , 3.2	-
Range	2.0 – 3.6	2.9 – 3.7	2.4 – 3.3	2.4 – 3.3
N	9	10	12	3

† Exclusive Human Milk (EHM) infants are defined as those infants receiving exclusively human milk at the time of these assessments. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses. When the sample size was 5 or less, only the median and range of values are shown.

000247

Table VII-27 Blood Chemistries In-Hospital: Liver Function				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
SGOT (IU/L)				
0 to 30 days				
Mean ± SD	36.5 ± 18.0	32.9 ± 15.0	39.1 ± 22.6	39.0 ± 22.2
Median	32.5	30.0	30.2	28.0
1st , 3rd quartile	22.8 , 49.0	22.0 , 44.0	25.0 , 47.8	25.0 , 47.0
Range	5.0 – 76.0	9.0 – 76.0	15.0 – 129.0	18.0 – 93.0
n	48	45	48	23
31 to 60 days				
Mean ± SD	41.9 ± 25.5	44.9 ± 21.4	35.8 ± 12.5	37.9 ± 16.9
Median	29.7	37.5	33.0	31.5
1st , 3rd quartile	27.0 , 51.3	30.0 , 61.0	25.0 , 49.0	26.0 , 57.0
Range	15.0 – 102.0	18.0 – 88.0	19.0 – 61.0	19.3 – 64.5
n	17	18	15	7
>60 days				
Mean ± SD	-	-	54.6 ± 24.0	-
Median	33.0	36.0	53.0	45.5
1st , 3rd quartile	-	-	40.0 , 59.5	-
Range	26.0 – 77.4	24.0 – 68.0	29.2 – 110.0	37.0 – 54.0
n	5	5	9	2
SGPT (IU/L)				
0 to 30 days				
Mean ± SD	12.5 ± 8.7	10.2 ± 5.3	16.6 ± 22.8	12.2 ± 9.5
Median	11.0	9.8	9.0	9.0
1st , 3rd quartile	8.0 , 16.8	7.0 , 12.0	6.7 , 14.0	7.7 , 12.7
Range	1.0 – 56.0	1.0 – 24.4	1.0 – 112.0	6.0 – 47.6
n	42	41	47	19
31 to 60 days				
Mean ± SD	15.2 ± 12.2	15.8 ± 13.0	11.1 ± 4.8	11.5 ± 3.5
Median	10.6	12.0	12.3	10.0
1st , 3rd quartile	9.0 , 15.7	11.0 , 14.0	7.0 , 13.8	9.0 , 15.0
Range	6.0 – 57.2	8.0 – 64.0	3.0 – 22.0	8.3 – 16.8
n	18	17	14	6
>60 days				
Mean ± SD	-	22.0 ± 21.6	-	
Median	32.0	13.0	12.0	14.3
1st , 3rd quartile	-	9.0 , 32.3	-	-
Range	9.0 – 35.6	6.0 – 67.0	4.0 – 27.5	13.0 – 15.5
n	5	7	5	2
Total Bilirubin (mg/dL)				
0 to 30 days				
Mean ± SD	5.8 ± 2.7	6.2 ± 2.7	6.0 ± 2.6	6.2 ± 2.5
Median	5.8	6.1	5.8	6.2
1st , 3rd quartile	3.8 , 7.8	4.1 , 7.7	4.0 , 8.0	4.4 , 7.6
Range	0.5 – 12.6	0.2 – 12.2	1.1 – 12.2	0.75 – 11.0
n	129	129	131	51

000248

Table VII-27 Blood Chemistries In-Hospital: Liver Function				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
31 to 60 days				
Mean ± SD	1.9 ± 2.5	1.2 ± 1.3	1.0 ± 0.8	2.3 ± 2.7
Median	0.7	0.6	0.7	1.4
1st , 3rd quartile	0.5 , 1.2	0.5 , 1.5	0.5 , 1.3	0.75 , 2.5
Range	0.2 – 8.5	0.2 – 5.4	0.2 – 3.3	0.3 – 10.5
n	29	31	29	14
>60 days				
Mean ± SD	1.0 ± 1.2	0.5 ± 0.2	1.3 ± 2.1	-
Median	0.6	0.5	0.5	0.8
1st , 3rd quartile	0.5 , 0.9	0.4 , 0.7	0.4, ,0.8	-
Range	0.3 – 4.4	0.2 – 0.8	0.2 – 7.4	0.7 – 1.0
n	10	10	12	3
Direct Bilirubin (mg/dL)				
0 to 30 days				
Mean ± SD	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.5	0.4 ± 0.2
Median	0.3	0.4	0.3	0.4
1st , 3rd quartile	0.3 , 0.4	0.3 , 0.5	0.2 , 0.5	0.3 , 0.4
Range	0 – 1.5	0.1 – 1.3	0 – 4.2	0.1 – 1.1
n	64	61	78	17
31 to 60 days				
Mean ± SD	0.3 ± 0.5	0.5 ± 0.7	0.6 ± 0.4	-
Median	0.2	0.2	0.5	0.3
1st , 3rd quartile	0.2 , 0.2	0.2 , 0.7	0.3 , 0.9	-
Range	0.1 – 1.6	0.1 – 1.8	0.2 – 1.1	0.2 – 0.4
n	9	6	6	4
>60 days				
Mean ± SD	-	-	-	
Median	0.2	0.2	1.8	
1st , 3rd quartile	-	-	-	
Range	0.2 – 2.0	0.2	0.1 – 5.2	
n	4	1	3	0

† Exclusive Human Milk (EHM) infants are defined as those infants receiving exclusively human milk at the time of these assessments. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses. When the sample size was 5 or less, only the median and range of values are shown.

000249

Table VII-28 Blood Chemistries In-Hospital: Clinical Chemistries				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
Calcium (mg/dL)				
0 to 30 days				
Mean ± SD	9.8 ± 1.1	9.9 ± 0.6	9.8 ± 0.8	10.1 ± 0.5
Median	9.8	10.0	9.9	10.1
1st , 3rd quartile	9.3 , 10.3	9.6 , 10.3	9.6 , 10.3	9.7 , 10.4
Range	1.4 – 13.7	7.9 – 11.1	5.4 – 11.0	8.8 – 11.0
n	111	118	119	44
31 to 60 days				
Mean ± SD	9.8 ± 0.6	10.0 ± 0.7	9.7 ± 0.5	9.9 ± 0.4
Median	9.8	9.9	9.8	9.8
1st , 3rd quartile	9.4 , 10.0	9.5 , 10.3	9.3 , 10.1	9.7 , 10.0
Range	8.6 – 11.1	8.3 – 11.5	8.3 – 10.8	9.6 – 10.7
n	39	42	44	16
>60 days				
Mean ± SD	9.9 ± 0.6	10.1 ± 0.7	9.4 ± 0.6	-
Median	9.7	9.9	9.5	9.7
1st , 3rd quartile	9.5 , 9.9	9.5 , 10.6	9.3 , 9.7	-
Range	9.1 – 11.2	9.2 – 11.4	8.1 – 10.6	9.3 – 9.9
n	11	12	14	3
Phosphorus (mg/dL)				
0 to 30 days				
Mean ± SD	6.1 ± 1.3	6.3 ± 1.2	6.5 ± 1.1	6.0 ± 1.2
Median	6.1	6.5	6.5	6.2
1st , 3rd quartile	5.3 , 6.9	5.6 , 7.2	5.8 , 7.3	5.1 , 6.6
Range	1.9 – 9.0	2.9 – 9.0	3.9 – 10.7	3.7 – 8.3
n	101	103	105	43
31 to 60 days				
Mean ± SD	6.6 ± 0.8	6.5 ± 0.7	6.7 ± 0.7	6.3 ± 0.7
Median	6.8	6.6	6.8	6.1
1st , 3rd quartile	6.3 , 7.2	6.1 , 7.1	6.4 , 7.3	5.9 , 6.5
Range	4.5 – 8.4	5.2 – 7.6	4.9 – 8.1	5.1 – 7.7
n	38	41	45	16
>60 days				
Mean ± SD	6.3 ± 0.7	6.4 ± 1.1	6.5 ± 0.8	-
Median	6.35	6.3	6.5	5.5
1st , 3rd quartile	5.8 , 6.7	5.8 , 7.5	5.9 , 6.9	-
Range	5.0 – 7.8	4.4 – 8.1	5.1 – 7.7	5.2 – 6.4
n	11	11	14	4
Alkaline Phosphatase (U/L)				
0 to 30 days				
Mean ± SD	369.9 ± 247.6	386.7 ± 225.8	374.4 ± 215.9	421.2 ± 290.5
Median	288.0	309.8	306.0	317.5
1st , 3rd quartile	192.0 , 457.0	215.8 , 526.5	222.5 , 422.0	240.3 , 404.0
Range	42.9 – 1102.4	119.0 – 1139.0	107.0 – 1067.3	166.0 – 1333.3
N	99	96	100	41

000250

Table VII-28 Blood Chemistries In-Hospital: Clinical Chemistries				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
31 to 60 days				
Mean ± SD	373.5 ± 219.6	380.12 ± 219.7	395.2 ± 250.2	442.3 ± 344.2
Median	300.0	276.0	298.5	316.3
1st , 3rd quartile	225.0 , 455.0	228.3 , 503.5	217.0 , 583.0	237.7 , 574.5
Range	142.0 – 1101.2	120.0 – 983.0	57 – 1174.3	137.0 – 1444.4
N	37	40	43	16
>60 days				
Mean ± SD	406.0 ± 277.1	459.5 ± 304	472.3 ± 345.0	-
Median	306.0	346.5	307.5	314.0
1st , 3rd quartile	248.0, 408.6	250.5 , 578.5	222.0 , 600.0	-
Range	180.0 – 1040.0	183.5 – 1061.0	135.0 – 1261.0	199.0 – 845.3
n	11	12	14	4
Total Protein (g/dL)				
0 to 30 days				
Mean ± SD	5.1 ± 0.7	5.0 ± 0.6	4.9 ± 0.5	5.1 ± 0.5
Median	5.1	5.0	5.0	5.0
1st , 3rd quartile	4.7 , 5.4	4.5 , 5.3	4.6 , 5.2	4.6 , 5.5
Range	3.5 – 7.5	3.8 – 6.3	3.3 – 5.8	4.2 – 6.0
n	61	58	69	24
31 to 60 days				
Mean ± SD	4.4 ± 0.6	4.7 ± 0.5	4.3 ± 0.5	4.7 ± 0.4
Median	4.5	4.5	4.4	4.7
1st , 3rd quartile	3.9 , 5.0	4.3 , 5.0	4.1 , 4.7	4.4 , 4.9
Range	3.1 – 5.5	3.6 – 5.8	3.0 – 5.0	4.1 – 5.3
n	22	23	26	8
>60 days				
Mean ± SD	4.8 ± 0.5	4.9 ± 0.4	4.4 ± 0.6	-
Median	4.9	4.9	4.4	4.2
1st , 3rd quartile	4.5 , 5.0	4.7 , 5.1	4.0 , 4.8	-
Range	4.0 – 5.3	4.4 – 5.5	3.4 – 5.3	4.2
n	6	6	9	2

† Exclusive Human Milk (EHM) infants are defined as those infants receiving exclusively human milk at the time of these assessments. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses. When the sample size was 5 or less, only the median and range of values are shown.

000251

Table VII-29 Blood Chemistries In-Hospital: Hematology				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
Hemoglobin (g/dL)				
0 to 30 days				
Mean ± SD	13.0 ± 2.1	12.5 ± 1.9	12.8 ± 1.9	12.9 ± 1.5
Median	12.7	12.3	12.6	12.9
1st , 3rd quartile	11.7 , 14.3	11.1 , 13.5	11.9 , 13.7	11.8 , 13.8
Range	9.3 – 21.6	8.2 – 19.9	7.5 – 19.8	9.6 – 16.2
n	128	118	125	52
31 to 60 days				
Mean ± SD	10.1 ± 2.0	10.3 ± 1.8	10.2 ± 1.6	10.0 ± 1.6
Median	9.9	10.5	10.0	10.2
1st , 3rd quartile	8.9 , 11.0	8.8 , 11.7	8.9 , 11.5	8.8 , 11.5
Range	6.5 – 16.6	6.8 – 12.8	7.1 – 14.3	7.0 – 13.1
n	61	53	64	25
>60 days				
Mean ± SD	10.6 ± 2.0	10.2 ± 1.7	10.1 ± 1.6	-
Median	9.8	10.8	10.4	10.1
1st , 3rd quartile	9.3 , 12.4	9.1 , 11.0	8.5 , 10.9	-
Range	7.0 – 14.1	6.6 – 12.5	7.5 – 13.1	7.2 – 14.5
n	15	16	19	5
Hematocrits (%)				
0 to 30 days				
Mean ± SD	38.9 ± 6.6	37.5 ± 5.1	38.7 ±4.8	38.0 ± 4.5
Median	38.0	37.1	38.1	38.4
1st , 3rd quartile	34.9 , 42.5	34.1 , 40.0	35.9 , 41.3	34.2 , 40.4
Range	25.7 – 63.5	25.8 – 53.4	28.3 – 52.0	29.9 – 48.2
n	127	115	122	48
31 to 60 days				
Mean ± SD	29.9 ± 4.8	30.6 ± 5.1	30.2 ± 4.7	29.8 ± 4.9
Median	29.2	31.3	30.0	29.3
1st , 3rd quartile	26.3 , 33.4	26.6 , 34.9	26.5 , 33.3	27.1 , 34.0
Range	21.8 – 45.2	21.9 – 40.6	21.7 – 43.0	20.7 – 40.7
n	64	60	63	26
>60 days				
Mean ± SD	30.8 ± 5.8	32.1 ± 4.4	30.0 ± 4.8	-
Median	28.8	32.0	31.0	30.5
1st , 3rd quartile	27.7 , 36.8	29.4 , 36.0	26.0 , 33.0	-
Range	21.5 – 40.5	22.7 – 38.8	22.6 – 39.3	26.6 – 32.3
n	13	17	17	5
MCH (pg)				
0 to 30 days				
Mean ± SD	34.6 ± 2.8	34.6 ± 2.4	34.5 ± 2.7	34.3 ± 2.5
Median	34.6	34.6	34.6	33.7
1st , 3rd quartile	32.2 , 36.8	32.9 , 36.1	32.8 , 36.4	32.5 , 36.0
Range	27.7 – 41.3	29.8 – 40.8	27.9 – 40.5	30.1 – 39.7
n	112	108	108	49

000252

Table VII-29 Blood Chemistries In-Hospital: Hematology				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
31 to 60 days				
Mean ± SD	31.3 ± 1.8	30.8 ± 2.1	30.5 ± 1.9	30.6 ± 2.2
Median	31.0	30.5	30.2	30.7
1st , 3rd quartile	30.0 , 32.3	29.8 , 31.7	29.4 , 31.5	28.9 , 32.0
Range	27.7 – 35.4	26.9 – 36.0	26.8 – 35.8	26.8 – 35.0
n	51	45	52	22
>60 days				
Mean ± SD	29.6 ± 1.4	29.8 ± 0.9	29.6 ± 1.6	-
Median	29.2	29.8	29.5	29.3
1st , 3rd quartile	28.7 , 30.4	29.0 , 30.4	28.6 , 30.5	-
Range	27.6 – 32.6	28.5 – 31.5	26.4 – 32.7	28.2 – 31.0
n	12	13	17	4
MCHC (g/dL)				
0 to 30 days				
Mean ± SD	34.2 ± 0.9	34.1 ± 1.0	34.2 ± 0.9	34.2 ± 0.8
Median	34.2	34.1	34.3	34.0
1st , 3rd quartile	33.7 , 35.0	33.7 , 34.8	33.6 , 34.9	33.7 , 34.9
Range	30.1 – 36.0	30.4 – 36.6	31.3 – 36.0	31.9 – 35.7
n	107	105	106	42
31 to 60 days				
Mean ± SD	33.9 ± 0.9	33.4 ± 1.3	33.5 ± 0.9	33.5 ± 1.2
Median	34.0	33.5	33.6	33.5
1st , 3rd quartile	33.4 , 34.3	32.8 , 34.1	33.0 , 34.05	33.0 , 34.0
Range	31.7 – 35.9	29.9 – 36.0	31.3 – 35.0	30.0 – 35.7
n	48	42	53	21
>60 days				
Mean ± SD	33.5 ± 1.0	33.1 ± 0.8	33.6 ± 1.4	-
Median	33.4	33.3	33.4	33.2
1st , 3rd quartile	32.9 , 33.5	32.9 , 33.5	32.9 , 34.3	-
Range	32.3 – 35.8	31.1 – 34.0	30.8 – 36.7	32.9 – 34.1
n	12	12	16	4
MCV (fl)				
0 to 30 days				
Mean ± SD	101.5 ± 7.8	101.5 ± 7.0	101.4 ± 6.9	100.8 ± 6.6
Median	101.6	101.1	101.1	100.6
1st , 3rd quartile	96.0 – 107.6	96.5 , 106.2	96.6 , 105.6	95.9 , 105.6
Range	82.5 – 122.0	88.6 – 118.4	85.4 – 118.1	88.7 – 114.0
n	113	108	108	49
31 to 60 days				
Mean ± SD	91.9 ± 5.1	92.5 ± 4.9	91.6 ± 5.2	91.4 ± 5.0
Median	91.1	92.6	90.8	91.8
1st , 3rd quartile	88.9 , 94.3	90.4 , 95.0	87.8 , 94.3	87.2 , 94.5
Range	83.3 – 102.7	81.0 – 105.3	83.45 – 103.0	81.3 – 101.0
n	50	45	53	22

Table VII-29 Blood Chemistries In-Hospital: Hematology				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**
>60 days				
Mean ± SD	88.4 ± 3.2	90.7 ± 3.8	88.5 ± 3.6	-
Median	87.5	90.7	88.3	88.3
1st , 3rd quartile	86.9 , 89.1	88.0 , 93.0	86.0 , 91.0	-
Range	84.8 – 97.6	85.2 – 99.8	83.5 – 97.0	85.9 – 90.7
n	12	14	17	4
WBC (number/mm^3)				
0 to 30 days				
Mean ± SD	14133 ± 5371	14394 ± 5862	13900 ± 5285	14193 ± 4675
Median	13050	13367	12680	12796
1st , 3rd quartile	10347 , 16833	10533 , 16810	10610 , 16404	10739 , 16500
Range	4100 – 37888	6543 – 41250	4500 – 35872	8308 – 26200
n	124	117	118	50
31 to 60 days				
Mean ± SD	10356 ± 3103	11194 ± 3861	10660 ± 2873	10610 ± 2628
Median	10186	10400	10683	10150
1st , 3rd quartile	8500 , 11990	8700 , 12400	9000 , 11750	9000 , 12200
Range	5600 – 23875	6350 – 27650	6110 – 24100	5760 – 18800
n	59	47	61	23
>60 days				
Mean ± SD	9391 ± 2920	9728 ± 2680	9548 ± 2798	-
Median	8590	9300	8715	11468
1st , 3rd quartile	7800 , 10543	8250 , 10450	7501 , 11625	-
Range	4900 – 14900	5600 – 16670	5850 – 15200	6600 – 12900
n	13	15	16	5
Platelets (x 1000/mm^3)				
0 to 30 days				
Mean ± SD	351.4 ± 132.5	360.8 ± 132.5	358.2 ± 144.4	377.7 ± 127.4
Median	345.0	339.0	339.8	362.3
1st , 3rd quartile	264.4 , 429.3	281.0 , 442.0	252.2 , 435.8	268.1 , 449.4
Range	96.3 – 818.0	114.0 – 781.0	56.2 – 998.0	183.8 – 785.6
n	121	115	118	50
31 to 60 days				
Mean ± SD	409.9 ± 151.7	397.3 ± 152.9	421.3 ± 192.6	410.9 ± 124.8
Median	396.0	370.0	374.3	383.0
1st , 3rd quartile	300.8 , 505.1	298.0 , 488.6	308.3 , 475.8	334.0 , 469.7
Range	163.4 – 825.0	143.0 – 753.0	152.0 – 1405.5	172.0 – 732.3
n	59	47	56	23
>60 days				
Mean ± SD	301.7 ± 136.4	336.5 ± 125.5	366.2 ± 181.8	-
Median	252.7	301.0	329.7	441.0
1st , 3rd quartile	181.5 , 418.0	233.4 , 403.5	218.0 , 470.0	-
Range	152.0 – 593.0	194.0 – 602.0	137.0 – 834.0	174.5 – 731.0
n	13	15	17	5

000254

Table VII-29 Blood Chemistries In-Hospital: Hematology				
Analyte	**Control**	**AA + DHA (Fungal/fish)**	**AA + DHA (Egg-DTG/Fish)**	**Exclusively Human Milk†**

† Exclusive Human Milk (EHM) infants are defined as those infants receiving exclusively human milk at the time of these assessments. Infants in the EHM group served as a reference only, as data from these infants were not included in any of the statistical analyses. When the sample size was 5 or less, only the median and range of values are shown.

000255

Table VII-30
Post-Discharge Blood Chemistries
***Post-Hoc Assessment*[a]**

Analyte	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM at Term CA; Mixed at 4-, 12-m CA[†]	Pediatric Reference Range *
Alanine aminotransferase (ALT, SGPT), U/L					Term Infants: 1 d - 12 mos (2.5 - 97.5th percentiles)
Hospital Discharge					
Mean ± SD	14.8 ± 6.5	13.5 ± 4.0	20.6 ± 14.7	-	
Median	13.0	13.0	14.0	15.0	6 - 47 U/L
1st , 3rd quartile	11.0 , 16.0	10.0 , 14.0	12.0 , 31.0	-	
Range	8.0 - 35.0	8.0 - 24.0	9.0 - 65.0	9.0 - 29.0	
n	17	21	19	3	
4-months CA					
Mean ± SD	25.2 ± 13.5	24.5 ± 16.0	23.3 ± 12.5	-	
Median	24.0	21.0	19.0	38.0	
1st , 3rd quartile	15.0 , 34.0	15.0 , 28.0	15.0 , 30.0	-	
Range	5.0 - 58.0	7.0 - 84.0	11.0 - 64.0	27.0 - 95.0	
n	33	38	27	5	
12-months CA					
Mean ± SD	25.3 ± 18.1	21.3 ± 8.2	28.0 ± 34.0	50.2 ± 71.5	
Median	21.0	19.0	24.0	27.0	
1st , 3rd quartile	15.0 , 29.0	16.0 , 26.0	15.0 , 29.0	24.0 , 35.0	
Range	8.0 - 110.0	9.0 - 48.0	11.0 - 213.0	21.0 - 253.0	
n	34	34	33	10	
Total Bilirubin, mg/dL					1 mo - adult, < 1.0 mg/dL
Hospital Discharge					
Mean ± SD	1.8 ± 1.5	1.7 ± 1.2	1.4 ± 1.5	-	
Median	1.6	1.6	1.0	2.1	
1st , 3rd quartile	1.0 , 2.1	0.7 , 2.4	0.6 , 1.6	-	
Range	0.4 - 5.8	0.5 - 4.7	0.4 - 6.6	0.5 - 7.7	
n	17	15	15	3	

000256

Table VII-30
Post-Discharge Blood Chemistries
***Post-Hoc Assessment*[a]**

Analyte	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM at Term CA; Mixed at 4-, 12-m CA[†]	Pediatric Reference Range *
4-months CA					
Mean ± SD	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	-	
Median	0.4	0.3	0.4	0.4	
1st , 3rd quartile	0.4 , 0.6	0.3 , 0.5	0.3 , 0.4	-	
Range	0.2 - 1.3	0.2 - 1.3	0.3 - 0.7	0.3 - 1.0	
n	29	36	26	5	
12-months CA					
Mean ± SD	0.4 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	
Median	0.4	0.4	0.4	0.5	
1st , 3rd quartile	0.3 , 0.4	0.3 , 0.5	0.3 , 0.5	0.4 , 0.5	
Range	0.3 - 1.8	0.2 - 0.7	0.2 - 1.2	0.3 - 0.7	
n	32	34	34	9	
Conjugated Bilirubin, mg/dL					Neonate < 0.6 mg/dL Post-neonatal < 0.1 mg/dL
Hospital Discharge					
Mean ± SD	0.1 ± 0.2	0.2 ± 0.3	0.4 ± 1.1	-	
Median	0.1	0.1	0.1	0.1	
1st , 3rd quartile	0.0 , 0.2	0.0 , 0.2	0.0 , 0.2	-	
Range	0.0 - 0.9	0.0 -1.1	0.0 - 4.5	0.0 - 0.4	
n	16	14	15	3	
4-months CA					
Mean ± SD	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	-	
Median	0.2	0.3	0.3	0.2	
1st , 3rd quartile	0.1 , 0.4	0.2 , 0.3	0.2 , 0.4	-	
Range	0.1 - 0.8	0.1 - 0.7	0.1 - 0.5	0.1 - 0.4	
n	29	35	25	5	
12-months CA					
Mean ± SD	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	
Median	0.3	0.3	0.2	0.3	
1st , 3rd quartile	0.2 , 0.4	0.1 , 0.3	0.1 , 0.4	0.2 , 0.4	
Range	0.0 - 0.5	0.0 - 0.4	0.1 - 1.0	0.2 - 0.4	
n	32	34	33	9	

0002257

Table VII-30
Post-Discharge Blood Chemistries
***Post-Hoc Assessment*[a]**

Analyte	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM at Term CA; Mixed at 4-, 12-m CA[†]	Pediatric Reference Range *
Unconjugated Bilirubin, mg/dL					
Hospital Discharge					
Mean ± SD	1.4 ± 1.2	1.5 ± 1.4	1.0 ± 0.6	-	
Median	1.4	1.0	1.0	1.7	
1st , 3rd quartile	0.4 , 2.0	0.5 , 2.1	0.5 , 1.6	-	
Range	0.2 - 4.7	0.2 - 4.7	0.1 - 2.1	0.4 - 7.7	
n	16	14	15	3	
4-months CA					
Mean ± SD	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	-	
Median	0.2	0.1	0.1	0.3	
1st , 3rd quartile	0.1 , 0.3	0.0 , 0.2	0.0 , 0.2	-	
Range	0.0 - 1.2	0.0 - 1.0	0.0 - 0.2	0.0 - 0.8	
n	29	35	25	5	
12-months CA					
Mean ± SD	0.2 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	
Median	0.1	0.2	0.1	0.2	
1st , 3rd quartile	0.0 , 0.2	0.1 , 0.3	0.0 , 0.2	0.1 , 0.2	
Range	0.0 - 1.6	0.0 - 0.6	0.0 - 0.5	0.0 - 0.3	
n	32	34	33	9	
Alkaline Phosphatase, U/L					Term Infants: 1 d - 12 mos (2.5 - 97.5th percentiles)
Hospital Discharge					
Mean ± SD	280 ± 114	219 ± 64	221 ± 90	-	
Median	266	221	185	202	55 - 425 U/L
1st , 3rd quartile	203 , 337	181 , 255	170 , 242	-	
Range	113 - 594	110 - 330	155 - 505	129 - 235	
n	16	14	15	3	
4-months CA					
Mean ± SD	280 ± 79	237 ± 50	238 ± 52	-	
Median	268	228	228	243	
1st , 3rd quartile	231 , 308	196 , 283	217 , 260	-	
Range	123 - 467	159 - 351	126 - 400	199 - 756	
n	29	33	24	5	

000258

Table VII-30
Post-Discharge Blood Chemistries
***Post-Hoc Assessment*[a]**

Analyte	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM at Term CA; Mixed at 4-, 12-m CA[†]	Pediatric Reference Range *
12-months CA					
Mean ± SD	212 ± 41	306 ± 383	207 ± 34	229 ± 62	
Median	205	228	201	216	
1st , 3rd quartile	185 , 244	178 , 251	183 , 233	204 , 267	
Range	141 - 321	140 - 2164	130 -286	123 - 343	
n	31	33	29	9	
Albumin, g/dL					Term Infants: 1 d - 12 mos (2.5 - 97.5th percentiles)
Hospital Discharge					
Mean ± SD	2.6 ± 0.3	2.7 ± 0.3	2.5 ± 0.2	-	1.8 - 4.9 g/dL
Median	2.6	2.7	2.5	2.7	
1st , 3rd quartile	2.4 , 2.8	2.4 , 2.9	2.3 , 2.7	-	
Range	2.2 - 3.1	2.0 - 3.3	2.1 - 2.8	2.7 - 2.7	
n	16	14	15	3	
4-months CA					
Mean ± SD	3.9 ± 0.3	4.0 ± 0.3	3.9 ± 0.2	-	
Median	3.9	4.1	3.9	4.1	
1st , 3rd quartile	3.8 , 4.1	3.8 , 4.2	3.7 , 4.1	-	
Range	3.3 - 4.6	3.2 - 4.7	3.6 - 4.2	3.6 - 4.5	
n	29	30	22	5	
12-months CA					
Mean ± SD	4.1 ± 0.3	4.2 ± 0.4	4.2 ± 0.3	4.2 ± 0.3	
Median	4.1	4.2	4.1	4.3	
1st , 3rd quartile	3.9 , 4.3	4.0 , 4.3	3.9 , 4.3	4.0 , 4.4	
Range	3.5 - 5.0	3.6 - 5.0	3.6 - 4.7	3.9 - 4.7	
n	30	33	30	8	
Aspartate Aminotransferase (AST, SGOT), U/L					Term Infants: 1 d - 12 mos (2.5 - 97.5th percentiles)
Hospital Discharge					
Mean ± SD	29.1 ± 8.8	33.3 ± 10.6	43.0 ± 46.6	-	13 - 100 U/L
Median	26.5	31.0	30.0	35.0	
1st , 3rd quartile	24.0 , 31.0	25.0 , 39.0	22.0 , 38.0	-	
Range	21.0 - 57.0	22.0 - 60.0	21.0 - 208.0	29.0 - 42.0	
n	16	14	15	3	

000259

Table VII-30
Post-Discharge Blood Chemistries
Post-Hoc Assessment [a]

Analyte	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM at Term CA; Mixed at 4-, 12-m CA [†]	Pediatric Reference Range *
4-months CA					
Mean ± SD	47.3 ± 12.9	44.7 ± 20.9	42.7 ± 9.2	-	
Median	47.0	40.0	41.0	56.0	
1st , 3rd quartile	39.0 , 52.0	35.0 , 45.0	39.0 , 44.0	-	
Range	29.0 - 94.0	26.0 - 117.0	30.0 - 70.0	43.0 - 73.0	
n	29	29	22	5	
12-months CA					
Mean ± SD	45.7 ± 12.4	44.6 ± 11.5	47.9 ± 16.8	67.0 ± 53.3	
Median	41.0	42.0	46.5	50.5	
1st , 3rd quartile	36.0 , 54.0	36.0 , 51.0	37.0 , 57.0	44.0 , 55.0	
Range	28.0 - 68.0	30.0 - 73.0	7.0 - 107.0	39.0 - 198.0	
n	30	31	30	8	
Total Protein, g/dL					Term Infants: 1 d - 12 mos (2.5 - 97.5th percentiles)
Hospital Discharge					
Mean ± SD	4.4 ± 0.4	4.6 ± 0.4	4.2 ± 0.5	-	
Median	4.4	4.6	4.0	5.2	3.4 - 7.9 g/dL
1st , 3rd quartile	4.0 , 4.7	4.5 , 4.8	3.9 , 4.5	-	
Range	3.8 - 4.9	3.6 - 5.1	3.8 - 5.3	5.2 - 5.6	
n	16	14	15	3	
4-months CA					
Mean ± SD	6.5 ± 0.5	6.5 ± 0.5	6.2 ± 0.4	-	
Median	6.3	6.5	6.2	6.3	
1st , 3rd quartile	6.1 , 6.8	6.2 , 6.7	6.0 , 6.4	-	
Range	5.5 - 7.8	5.3 - 8.1	5.6 - 7.3	6.0 - 7.1	
n	28	28	22	5	
12-months CA					
Mean ± SD	6.7 ± 0.4	6.7 ± 0.3	6.7 ± 0.3	6.8 ± 0.4	
Median	6.7	6.7	6.7	6.7	
1st , 3rd quartile	6.4 , 6.9	6.5 , 7.0	6.4 , 6.9	6.5 , 7.0	
Range	6.1- 7.5	6.1 - 7.7	6.0 - 7.2	6.4 - 7.6	
n	30	31	30	8	

002260

Table VII-30
Post-Discharge Blood Chemistries
Post-Hoc Analysis of All Infants [a]

Analyte	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM at Term CA; Mixed at 4,12 m*	Pediatric Reference Range

† The Exclusive Human Milk group includes infants who consumed > 80% of their feedings from human milk from SDAY 1 to the term CA visit. After the term CA visit, infants in this group many have consumed a study formula (each infant was assigned a study formula at randomization) and/or human milk. Infants in the Exclusive Human Milk group served as a reference only; the data from these infants were not included in any of the statistical analyses.
When the sample size was 5 or less, only the median and range of values are shown.
* From: Soldin SJ, Brugnara C, Hicks JM: *Pediatric Reference Ranges* ed 3. Washington, DC: AACC Press, 1999.
Abbreviations: AA, Arachidonic acid; DHA, docosahexaenoic acid.

000261

Table VII-31
Measures of Iron Status at 4- and 12-Months CA
Evaluable Analysis*

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	Exclusive Human Milk†
Hemoglobin, g/dL				
4-months CA (mean ± SEM (SD))	12.0 ± 0.2 (1.0)	11.8 ± 0.2 (1.1)	12.1 ± 0.2 (1.1)	11.1 ± 0.6 (1.5)
n	49	43	37	7
12-months CA (mean ± SEM (SD))	12.5 ± 0.2 (1.1)	12.6 ± 0.1 (0.8)	12.4 ± 0.1 (0.8)	12.3 ± 0.3 (1.2)
n	48	48	40	22
Mean Corpuscular Volume (MCV), fl				
4-months CA (mean ± SEM (SD))	80.4 ± 0.6 (4.0)	80.5 ± 0.6 (3.6)	79.6 ± 0.9 (5.5)	73.6 ± 3.5 (9.3)
n	46	43	36	7
12-months CA (mean ± SEM (SD))	79.1 ± 0.7 (4.5)	80.2 ± 0.7 (4.6)	78.5 ± 0.7 (4.7)	79.3 ± 1.3 (6.3)
n	47	49	40	22
Mean Corpuscular Hemoglobin (MCH), pg				
4-months CA (mean ± SEM (SD))	27.2 ± 0.3 (2.1)	27.1 ± 0.2 (1.6)	26.6 ± 0.4 (2.3)	24.5 ± 1.4 (3.7)
n	47	43	36	7
12-months CA (mean ± SEM (SD))	26.7 ± 0.3 (1.9)	27.1 ± 0.3 (2.4)	26.5 ± 0.3 (1.8)	27.2 ± 0.5 (2.5)
n	47	49	39	22
Mean Corpuscular Hemoglobin Concentration (MCHC), pg				
4-months CA (mean ± SEM (SD))	33.9 ± 0.1 (0.7)	33.6 ± 0.1 (0.9)	33.5 ± 0.2 (1.2)	33.2 ± 0.4 (1.0)
n	46	39	36	7
12-months CA (mean ± SEM (SD))	33.8 ± 0.2 (1.1)	33.9 ± 0.2 (1.3)	33.8 ± 0.1 (0.9)	34.3 ± 0.2 (0.9)
n	47	48	39	22

Table VII-31
Measures of Iron Status at 4- and 12-Months CA
*Evaluable Analysis**

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	Exclusive Human Milk[1]

* These outcomes represent secondary and/or exploratory variables; an intent-to-intent analysis was not conducted, therefore results of the evaluation analysis are presented here. Differences across study formula groups were determined using PROC MIXED for repeated measures analysis controlling for site, visit, gender, birth weight stratum, feeding*visit, feeding*gender, and feeding*birth weight stratum. A statistically significant feeding*birthweight interaction was found for both MCV (p=0.0081) and MCH (p=0.0421). Pairwise comparison revealed that infants > 1250 g birth weight stratum fed AA + DHA (fungal/fish) had > MCV and MCH values than infants fed AA + DHA (egg-DTG/fish) using a Bonferroni adjusted α-level of 0.0083.

MCV: LS means ± SE, AA + DHA (fungal/fish) (81.2 ± 0.7) > AA + DHA (egg-DTG/fish) (78.0 ± 0.8), p=0.0013.

MCH: LS means ± SE, AA + DHA (fungal/fish) (27.5 ± 0.3) > AA + DHA (egg-DTG/fish) (26.9 ± 0.4), p=0.0069.

[1] The Exclusive Human Milk group includes infants who consumed > 80% of their feedings from human milk from SDAY 1 to the term CA visit. After the term CA visit, infants in this group many have consumed a study formula (each infant was assigned a study formula at randomization) and/or human milk. Infants in the Exclusive Human Milk group served as a reference only; the data from these infants were not included in any of the statistical analyses.

Table VII-32
Plasma Vitamin E, Retinol, and Isoprostanes at 4- and 12-Months CA
Evaluable Analysis*

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
α-Tocopherol, μmol/L				
4-months CA (mean ± SEM (SD))	25.9 ± 1.1 (7.8)	29.5 ± 1.3 (9.6)	28.4 ± 1.4 (8.9)	24.5 ± 2.7 (9.4)
n	55	58	42	12
12-months CA (mean ± SEM (SD))	27.5 ± 2.3 (11.3)	31.6 ± 2.1 (11.0)	26.1 ± 2.2 (9.1)	26.1 ± 2.3 (7.7)
n	25	28	18	11
γ-Tocopherol, μmol/L				
4-months CA (mean ± SEM (SD))	2.4 ± 0.1 (1.0)	2.6 ± 0.2 (1.4)	2.9 ± 0.2 (1.6)	2.1 ± 0.3 (0.9)
n	55	58	42	12
12-months CA (mean ± SEM (SD))	2.4 ± 0.2 (1.2)	2.3 ± 0.3 (1.5)	2.5 ± 0.2 (0.9)	2.7 ± 0.5 (1.7)
n	25	28	18	11
Isoprostanes, pg/mL				
12-months CA (mean ± SEM (SD))	903.2 ± 77.7 (549.5)	826.6 ± 65.9 (451.9)	853.9 ± 74.7 (484.1)	857.4 ± 89.2 (418.6)
n	50	47	42	22
Retinol, μmol/L				
4-months CA (mean ± SEM (SD))	1.0 ± 0.0 (0.2)	1.0 ± 0.0 (0.3)	1.0 ± 0.1 (0.3)	0.9 ± 0.1 (0.4)
n	55	58	42	12
12-months CA (mean ± SEM (SD))	1.2 ± 0.1 (0.4)[a, b]	1.4 ± 0.1 (0.4)[a]	1.0 ± 0.0 (0.2)[b]	1.1 ± 0.1 (0.2)
n	25	28	18	11

* These outcomes represent secondary and/or exploratory variables; intent-to-intent analysis was not conducted, therefore results of the evaluation analysis are presented here. Differences across study groups were determined using PROC MIXED for repeated measures analysis controlling for site, gender, visit, birth weight stratum, feeding*visit, feeding*gender, and feeding*birth weight stratum. A statistically significant feeding*gender interaction (p=0.0297) was observed for α-tocopherol, a feeding*visit interaction for plasma retinol (p=0.0312). Female infants fed AA + DHA (fungal/fish) (LS means ± SE, 35.7 ± 1.7 mg/L) had > plasma α-tocopherol levels than females in the control (27.4 ± 1.7 mg/L, p=0.0003) or the AA + DHA (egg-DTG/fish) (29.4 ± 2.0 mg/L, p=0.0105) groups. Unlike superscripts within each row denote statistically significant differences (Bonferroni adjusted α-level of 0.0167).

† The Exclusive Human Milk group includes infants who consumed > 80% of their feedings from human milk from SDAY 1 to the term CA visit. After the term CA visit, infants in this group many have consumed a study formula (each infant was assigned a study formula at randomization) and/or human milk. Infants in the Exclusive Human Milk group served as a reference only; the data from these infants were not included in any of the statistical analyses.

000264

Table VII-33
Fatty Acid Levels (wt%) in the Plasma and Red Blood Cell Phospholipids on Study Day 1 and at Hospital Discharge

	Control	AA + DHA Fungal/Fish	AA + DHA Egg-DTG/Fish	EHM†
	Mean ± SEM			
Study Day I				
Plasma (n)	(64)	(66)	(58)	(23)
20:4n6‡	14.4 ± 0.4	15.0 ± 0.4	14.6 ± 0.4	14.4 ± 0.6
20:5n3	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
22:6n3‡	3.2 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	2.9 ± 0.1
20:5n3/20:4n6	0.036 ± 0.002	0.036 ± 0.003	0.032 ± 0.002	0.031 ± 0.003
n6/n3‡	8.8 ± 0.3	8.6 ± 0.3	8.4 ± 0.3	9.5 ± 0.4
RBC PE (n)	(66)	(66)	(58)	(24)
20:4n6‡	24.2 ± 0.7	24.6 ± 0.6	24.5 ± 0.7	27.6 ± 0.7
20:5n3	0.4 ± 0.0[a]	0.4 ± 0.0[a,b]	0.5 ± 0.1[a]	0.4 ± 0.0
22:6n3‡	6.9 ± 0.3	6.8 ± 0.3	7.2 ± 0.3	7.5 ± 0.3
20:5n3/20:4n6	0.017 ± 0.002	0.017 ± 0.001	0.021 ± 0.003	0.015 ± 0.002
n6/n3‡	4.8 ± 0.1	4.8 ± 0.1	4.6 ± 0.1	4.8 ± 0.1
RBC PC (n)	(65)	(65)	(59)	(23)
20:4n6‡	10.6 ±0.5	10.4 ± 0.3	10.7 ± 0.5	11.1 ± 0.5
20:5n3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:6n3	1.8 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.7 ± 0.1
20:5n3/20:4n6	0.024 ± 0.002	0.023 ± 0.002	0.024 ± 0.002	0.024 ± 0.003
n6/n3‡	11.3 ± 0.4	11.8 ± 0.4	11.2 ± 0.5	12.3 ± 0.7
Hospital Discharge				
Plasma (n)	(48)	(44)	(36)	(15)
20:4n6‡	10.1 ± 0.3[a]	12.1 ± 0.3[b]	12.3 ± 0.3[b]	14.1 ± 0.6
20:5n3	0.5 ± 0.0[a]	0.6 ± 0.0[a]	0.4 ± 0.0[b]	0.4 ± 0.0
22:6n3‡	2.7 ± 0.1[a]	3.4 ± 0.1[b]	3.3 ± 0.1[b]	3.5 ± 0.2
20:5n3/20:4n6	0.054 ± 0.003[a]	0.048 ± 0.003[b]	0.036 ± 0.002[c]	0.030 ± 0.004
n6/n3‡	10.5 ± 0.3[a]	8.9 ± 0.3[b]	9.5 ± 0.3[a,b]	8.7 ± 0.4

000265

Table VII-33
Fatty Acid Levels (wt%) in the Plasma and Red Blood Cell Phospholipids on Study Day 1 and at Hospital Discharge

	Control	AA + DHA Fungal/Fish	AA + DHA Egg-DTG/Fish	EHM†
	Mean ± SEM			
RBC PE (n)	(46)	(43)	(37)	(15)
20:4n6‡	23.2 ± 0.6	22.7 ± 0.5	23.4 ± 0.6	26.9 ± 1.1
20:5n3	0.6 ± 0.0[a]	0.7 ± 0.0[b]	0.6 ± 0.0[a]	0.4 ± 0.0
22:6n3‡	5.9 ± 0.2	6.3 ± 0.2	6.2 ± 0.2	6.9 ± 0.4
20:5n3/20:4n6	0.025 ± 0.002[a]	0.031 ± 0.002[b]	0.025 ± 0.002[a,b]	0.017 ± 0.002
n6/n3‡	5.0 ± 0.2	4.5 ± 0.1	4.7 ± 0.1	4.7 ± 0.1
RBC PC (n)	(48)	(42)	(37)	(15)
20:4n6‡	6.8 ± 0.3[a]	7.4 ± 0.2[b]	7.4 ± 0.2[a, b]	8.7 ± 0.4
20:5n3	0.2 ± 0.0[a]	0.3 ± 0.0[b]	0.2 ± 0.0[a,b]	0.2 ± 0.0
22:6n3	1.3 ± 0.1[a]	1.5 ± 0.1[b]	1.4 ± 0.1[a, b]	1.5 ± 0.1
20:5n3/20:4n6	0.034 ± 0.002[a]	0.040 ± 0.002[b]	0.027 ± 0.002[c]	0.021 ± 0.003
n6/n3‡	15.6 ± 0.7[a]	12.8 ± 0.5[b]	14.4 ± 0.6[a, b]	13.3 ± 0.7

* ANOVA statistics controlling for site, gender, birth weight stratum, and interactions (feeding*gender, feeding*birth weight stratum) were performed on fatty acid data generated from blood samples collected from subjects in the feeding protocol only. Unlike superscripts within each row denote a statistically significant differences between study formula groups. The data and statistical analyses summarized in this table were generated from blood samples collected from evaluable subjects only.

† EHM = Exclusive Human milk reference group represents infants fed exclusively human milk at the time of the blood sample was taken. Not included in the statistical analyses

‡ Denotes an important site effect in the model.

No differences were found at study day 1 except for the following:

RBC-PE EPA: Control < egg DTG/fish, Males, p=0.003.

The following differences were found at hospital discharge:

Plasma-PL AA (Control < Fungal/Fish, females, p<0.0001; males, p=0.002).§
(Control < Egg-DTG/Fish, p<0.0001).§

Plasma-PL EPA (Control > Egg-DTG/Fish, p=0.004).§
(Fungal/Fish > Egg DTG/Fish, p=0.0009).§

Plasma-PL DHA (Control < Fungal/Fish, females, p<0.0001).§
(Control < Egg-DTG/Fish, males, p=0.003).§

0002266

Table VII-33
Fatty Acid Levels (wt%) in the Plasma and Red Blood Cell Phospholipids on Study Day 1 and at Hospital Discharge

	Control	AA + DHA Fungal/Fish	AA + DHA Egg-DTG/Fish	EHM†
	Mean ± SEM			
Plasma-PL EPA/AA	(Control > Fungal/Fish, p=0.01).§ (Control > Egg-DTG/Fish, p<0.0001). § (Fungal/Fish > Egg DTG/Fish, p=0.002). §			
Plasma-PL n6/n3	(Control > Fungal/Fish, p<0.0001).§			
RBC-PE EPA	(Control < Fungal/Fish, males, p=0.001). § (Egg DTG/Fish < Fungal/Fish, males, p=0.004). §			
RBC-PE EPA/AA	(Control < Fungal/Fish, males, p=0.002). §			
RBC-PC AA	(Control < Fungal/Fish, p=0.009).§			
RBC-PC EPA	(Control < Fungal/Fish, p=0.005).§ (Egg-DTG/Fish < Fungal/Fish, p=0.0002).§			
RBC-PC DHA	(Control < Fungal/Fish, p=0.02).§			
RBC-PC EPA/AA	(Control < Fungal/Fish, males, p=0.006).§ (Control > Egg-DTG/Fish, females, p=0.002).§ (Fungal/Fish > Egg DTG/Fish, males, p=0.003). §			
RBC-PC n6/n3	(Control > Fungal/Fish, p=0.004).§			

§ p-values adjusted for multiple comparisons using Bonferroni procedure.

000267

Table VII-34
Fatty Acid Levels (wt%) in Plasma and Red Blood Cell Phospholipids at 4- and 12-Months CA
Evaluable Analysis*

	Control	AA + DHA Fungal/Fish	AA + DHA Egg-DTG/Fish	EHM†
	Mean ± SEM			
4-months CA				
Plasma (n)	(63)	(64)	(53)	(10)
20:4n6‡	8.3 ± 0.2	12.1 ± 0.2	11.8 ± 0.3	13.0 ± 0.5
20:5n3‡	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
22:6n3‡	2.2 ± 0.1	3.9 ± 0.1	3.5 ± 0.1	4.1 ± 0.3
20:5n3/20:4n6‡	0.043 ± 0.002	0.029 ± 0.001	0.023 ± 0.001	0.028 ± 0.005
n6/n3‡	12.5 ± 0.5	8.4 ± 0.2	9.4 ± 0.3	8.0 ± 0.6
RBC PE (n)	(67)	(67)	(55)	(15)
20:4n6‡	17.5 ± 0.9	18.7 ± 0.9	17.9 ± 0.9	18.0 ± 1.8
20:5n3	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
22:6n3‡	3.1 ± 0.2	4.7 ± 0.3	4.3 ± 0.3	4.1 ± 0.7
20:5n3/20:4n6	0.032 ± 0.005	0.030 ± 0.005	0.034 ± 0.011	0.016 ± 0.004
n6/n3‡	6.9 ± 0.5	5.1 ± 0.2	5.6 ± 0.2	5.3 ± 0.3
RBC PC (n)	(65)	(65)	(56)	(15)
20:4n6‡	4.4 ± 0.2	5.8 ± 0.3	5.6 ± 0.3	6.5 ± 0.6
20:5n3‡	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:6n3‡	0.9 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.2
20:5n3/20:4n6‡	0.071 ± 0.019	0.051 ± 0.015	0.029 ± 0.005	0.019 ± 0.005
n6/n3‡	16.0 ± 0.7	12.9 ± 0.4	14.3 ± 0.8	13.4 ± 1.1
12-months CA				
Plasma (n)	(61)	(58)	(51)	(22)
20:4n6‡	8.6 ± 0.3	11.3 ± 0.3	11.1 ± 0.3	10.6 ± 0.5
20:5n3‡	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
22:6n3‡	1.8 ± 0.1	3.4 ± 0.1	3.1 ± 0.1	2.6 ± 0.2
20:5n3/20:4n6‡	0.040 ± 0.003	0.030 ± 0.001	0.024 ± 0.002	0.028 ± 0.005
n6/n3‡	13.1 ± 0.5	8.5 ± 0.2	9.7 ± 0.3	11.5 ± 0.8

000268

Table VII-34
Fatty Acid Levels (wt%) in Plasma and Red Blood Cell Phospholipids at 4- and 12-Months CA
Evaluable Analysis*

	Control	AA + DHA Fungal/Fish	AA + DHA Egg-DTG/Fish	EHM†
RBC PE (n)	(61)	(58)	(56)	(26)
20:4n6‡	17.3 ± 0.8	18.7 ± 0.8	18.5 ± 0.9	19.9 ± 1.1
20:5n3	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.0
22:6n3‡	2.4 ± 0.1	4.2 ± 0.3	4.0 ± 0.3	4.1 ± 0.4
20:5n3/20:4n6	0.025 ± 0.003	0.033 ± 0.008	0.031 ± 0.007	0.016 ± 0.002
n6/n3‡	6.8 ± 0.3	5.2 ± 0.1	5.8 ± 0.2	6.0 ± 0.4
RBC PC (n)	(61)	(58)	(56)	(26)
20:4n6‡	4.2 ± 0.2	5.4 ± 0.3	5.5 ± 0.3	6.0 ± 0.4
20:5n3‡	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:6n3‡	0.7 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
20:5n3/20:4n6‡	0.041 ± 0.008	0.041 ± 0.009	0.022 ± 0.003	0.022 ± 0.004
n6/n3‡	22.4 ± 1.3	15.4 ± 0.7	16.8 ± 0.8	18.4 ± 1.7

* The data and statistical analyses summarized in this table were generated from blood samples collected from evaluable subjects only. Differences in blood fatty acids across study formula groups were determined using PROC MIXED for repeated measures analysis controlling for site, visit, gender, birth weight stratum, study formula group, feeding*visit, feeding*gender, and feeding*birth weight stratum.

† The Exclusive Human Milk group is defined as infants receiving less than 100 mL/kg of birth weight of total in-hospital formula intake, and human milk more than 80% of the times they were fed daily from hospital discharge to term CA. Infants in the Exclusive Human Milk group served as a reference only; the data from these infants were not included in any of the statistical analyses.

‡ A main effect of feeding was found in each fatty acid variable listed and as shown below.

Plasma-PL AA: Control < AA + DHA (Fungal/Fish), Control < AA + DHA (Egg-DTG /Fish), $p < 0.0001$.§

Plasma-PL EPA: Control < AA + DHA (Egg DTG/Fish), $p<0.0001$.§
AA + DHA (Egg DTG/Fish) > AA + DHA (Fungal/Fish), $p<0.0001$.§

Plasma-PL DHA: Control < AA + DHA (Fungal/Fish), Control < AA + DHA (Egg-DTG/Fish), $p < 0.0001$.§
AA + DHA (Egg-DTG/Fish) < AA + DHA (Fungal/Fish), $p = 0.004$.§

0002692

Table VII-34
Fatty Acid Levels (wt%) in Plasma and Red Blood Cell Phospholipids at 4- and 12-Months CA
Evaluable Analysis*

	Control	AA + DHA Fungal/Fish	AA + DHA Egg-DTG/Fish	EHM†
Plasma-PL EPA/AA	Control > AA + DHA (Fungal/Fish), Control > AA + DHA (Egg-DTG/Fish), $p < 0.0001$.§ AA + DHA (Egg-DTG/Fish) < AA + DHA (Fungal/Fish), $p = 0.0007$.§			
Plasma-PL n6/n3:	Control > AA + DHA (Fungal/Fish), Control > AA + DHA (Egg-DTG/Fish), $p < 0.0001$.§ AA + DHA (Egg-DTG/Fish) > AA + DHA (Fungal/Fish), $p = 0.006$.§			
RBC-PE AA:	Control < AA + DHA (Fungal/Fish), females, $p = 0.001$.§			
RBC-PE DHA:	Control < AA + DHA (Fungal/Fish), Control < AA + DHA (Egg-DTG/Fish), $p < 0.0001$.§ AA + DHA(Egg-DTG/Fish) < AA + DHA (Fungal/Fish), females, $p = 0.006$.§			
RBC-PE n6/n3:	Control > AA + DHA (Fungal/Fish), $p < 0.0001$.§ Control > AA + DHA (Egg-DTG/Fish), $p = 0.0002$. §			
RBC-PC AA:	Control < AA + DHA (Fungal/Fish), Control < AA + DHA (Egg-DTG/Fish), $p < 0.0001$.§			
RBC-PC DHA:	Control < AA + DHA (Fungal/Fish), Control < AA + DHA (Egg-DTG/Fish), $p < 0.0001$. §			
RBC-PC EPA	Control > AA + DHA (Egg DTG/Fish), 4 months, $p = 0.0006$. §			
RBC-PC EPA/AA	Control > AA + DHA (Egg DTG/Fish), $p = 0.009$. §			
RBC-PC n6/n3:	Control > AA + DHA (Fungal/Fish), $p < 0.0001$. § Control > AA + DHA (Fungal/Fish), 12 months, $p < 0.0001$. §			

§ p-values adjusted for multiple comparisons using Bonferroni procedure.

000270

Table VII-35
Cholesterol and Triglycerides at 4- and 12-Months CA
Evaluable Analysis*

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	EHM†
Cholesterol, mg/dL				
4-months CA (mean ± SEM (SD))	128.4 ± 3.3 (25.0)	146.5 ± 3.8 (27.3)	132.9 ± 3.8 (25.1)	137.1 ± 7.2 (21.7)
n	56	53	43	9
12-months CA (mean ± SEM (SD))	149.7 ± 6.3 (45.3)	171.8 ± 4.8 (34.1)	153.4 ± 3.8 (26.0)	153.6 ± 7.7 (33.6)
n	52	50	46	19
Triglycerides, mg/dL				
4-months CA (mean ± SEM (SD))	152.5 ± 9.1 (67.8)	164.7 ± 10.8 (79.7)	146.1 ± 9.6 (63.3)	199.8 ± 37.4 (105.8)
n	56	54	43	8
12-months CA (mean ± SEM (SD))	141.0 ± 9.3 (67.9)	142.2 ± 12.3 (86.8)	139.2 ± 12.1 (82.1)	178.3 ± 17.8 (77.4)
n	53	50	46	19

* These outcomes represent secondary and/or exploratory variables; intent-to-intent analysis was not conducted, therefore results of the evaluation analysis are presented here. Differences across study groups were determined using PROC MIXED for repeated measures analysis controlling for site, gender, visit, birth weight stratum, feeding*visit, feeding*gender, and feeding*birth weight stratum. A statistically significant feeding*birth weight stratum interaction for serum triglycerides (p=0.0081). Pairwise comparison of plasma triglyceride concentrations across study formula groups within each birth weight stratum revealed no statistically significant differences.

A statistically significant main effect of feeding ($p < 0.0001$) was found for serum cholesterol levels:

AA + DHA (fungal/fish) > control, p=0.0001

AA + DHA (fungal/fish) > AA + DHA (egg-DTG/fish), p=0.0004.

Unlike superscripts within each row denote statistically significant differences (Bonferroni adjusted α level of 0.0167).

† The Exclusive Human Milk group includes infants who consumed > 80% of their feedings from human milk from SDAY 1 to the term CA visit. After the term CA visit, infants in this group many have consumed a study formula (each infant was assigned a study formula at randomization) and/or human milk. Infants in the Exclusive Human Milk group served as a reference only; the data from these infants were not included in any of the statistical analyses.

000271

Table VII-36
Plasma Cholesterol at 4- and 12-months CA: *Post hoc* analysis by ANCOVA*

	Control	AA + DHA Fungal/Fish	AA + DHA Egg-DTG
4-months CA			
LS mean (95% CI)	131[a] (124-138)	149[b] (141-156)	135[a] (126-143)
(n)	(56)	(53)	(43)
12-months CA			
LS mean (95% CI)	152[a] (141-163)	178[b] (166-190)	158[a] (147-170)
(n)	(52)	(50)	(46)

* Differences across study groups at each time point were determined using ANCOVA controlling for site, gender, visit, birth weight stratum, feeding*visit, feeding*gender, feeding*birth weight stratum, and human milk intake.

A statistically significant main effect of feeding ($p < 0.0016$) was found for serum cholesterol levels:
AA + DHA (Fungal/Fish) > control, p=0.0005
AA + DHA (Fungal/Fish) > AA + DHA (egg-DTG/fish), p=0.01.
Unlike superscripts within each row denote statistically significant differences (Bonferroni adjusted a-level of 0.0167).

000272

Table VII-37
Mean Reported Energy Intake from Study Formula
Evaluable Analysis*

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	Exclusive Human Milk †
	kcal/day			
Study Day 1 to Hospital Discharge†				
Total Energy Intake (enteral + parenteral sources)				
Mean ± SEM	167 ± 4	173 ± 4	174 ± 3	---
n	138	131	136	
Total Energy Intake (enteral sources only)*†				
Mean ± SEM	148 ± 4	155 ± 5	156 ± 4	---
n	138	131	136	
Term CA**				
Mean ± SEM (SD)	372 ± 16 (170)	388 ± 18 (177)	386 ± 15 (150)	228 ± 76 (152)
n	107	99	104	4
1-month CA**				
Mean ± SEM (SD)	467 ± 18 (172)	507 ± 17 (164)	495 ± 24 (227)	235± 63 (244)
n	93	91	91	15
2-months CA**				
Mean ± SEM (SD)	498 ± 20 (195)	528 ± 21 (203)	553 ± 22 (214)	304 ± 54 (229)
n	99	94	98	18
3-months CA**				
Mean ± SEM (SD)	553 ± 22 (207)	589 ± 28 (250)	579 ± 25 (236)	362 ± 60 (267)
n	93	82	90	20
4-months CA**				
Mean ± SEM (SD)	590 ± 22 (221)	600 ± 24 (229)	610 ± 25 (244)	390 ± 52 (256)
n	100	91	93	24
5-months CA**				
Mean ± SEM (SD)	590 ± 22 (210)	616 ± 26 (245)	626 ± 26 (230)	399 ± 47 (245)
n	90	89	77	27
6-months CA**				
Mean ± SEM (SD)	593 ± 23 (225)	619 ± 26 (290)	632 ± 25 (236)	391 ± 47 (250)
n	95	88	90	28
9-months CA**				
Mean ± SEM (SD)	597± 26 (249)	588 ± 28 (250)	608 ± 29 (260)	447 ± 41 (231)
n	89	80	81	32

000273

Table VII-37
Mean Reported Energy Intake from Study Formula
Evaluable Analysis*

	Control	AA + DHA (Fungal/Fish)	AA + DHA (Egg-DTG/Fish)	Exclusive Human Milk †
12-months CA**				
Mean ± SEM (SD)	576 ± 27 (230)	529 ± 29 (243)	555 ± 27 (230)	387 ± 41 (233)
N	73	71	73	32

* Differences among study formula groups were evaluated using ANOVA controlled for a possible site effect. No statistically significant differences were found.

† From in hospital flow sheets. Energy intake from all sources was included (eg, parenteral nutrition, formula, supplements, human milk) if the information was provided in the medical records. The volume of human milk was not included if the infant was fed at its mother's breast.

‡ From 3-day diet diaries collected immediately prior to the term-CA clinic visit. The following subjects were still in hospital at term CA and hence in hospital records were used (>80% of energy from human milk at term).

** Differences across study variables were determined using PROC MIXED for repeated measures analysis controlling for site, visit, gender, birth weight stratum, feeding*visit, feeding*gender, and feeding*birth weight stratum. No statistically significant feeding, feeding*visit, feeding*gender, or feeding*birth weight stratum effects were found.

† The Exclusive Human Milk group includes infants who consumed > 80% of their feedings from human milk from SDAY 1 to the term CA visit. After the term CA visit, infants in this group many have consumed a study formula (each infant was assigned a study formula at randomization) and/or human milk. Infants in the Exclusive Human Milk group served as a reference only; the data from these infants were not included in any of the statistical analyses.

000274

Table VII-38 Stool Characteristics of Infants				
	Control	**AA + DHA (fungal/fish)**	**AA + DHA (egg-DTG/fish)**	**EHM†**
SSC Phase: In-Hospital				
Watery stools, % of infants experiencing (n) [a]	56.5 (138)	55.6 (133)	59.6 (136)	NA
Hard stools, % of infants experiencing (n) [a]	2.9 (139)	3.8 (133)	7.4 (136)	NA
SSC Phase: Term CA				
Mean number of stools, n/day [b]	2.2 ± 0.2 (104)	1.8 ± 0.2 (90)	1.7 ± 0.1 (96)	4.9 ± 0.3 (39)
Mean Rank Stool Consistency [b, c]	2.7 ± 0.1 (140)	2.6 ± 0.1 (87)	2.6 ± 0.1 (95)	2.0 ± 0.1 (39)
Predominant Stool Consistency, % [c]				
Watery	2	8	11	15
Loose/mushy	38	35	34	62
Soft	47	49	46	23
Formed	8	8	7	0
Hard	4	0	1	0
Predominant Stool Color, % [a]				
Yellow	23	20	20	87
Green	49	52	51	5
Brown	26	27	27	8
Black	2	1	2	0
Similac NeoSure® Phase				
Mean number of stools, n/day (n)[d]				
2-months CA	1.3 ± 0.1 (103)	1.1 ± 0.1 (97)	1.3 ± 0.1 (99)	2.1 ± 0.3 (39)
4-months CA	1.3 ± 0.1 (103)	1.4 ± 0.1 (94)	1.3 ± 0.1 (95)	1.4 ± 0.1 (39)
Mean Rank Stool Consistency [e, f]				
2-months CA	2.5 ± 0.1 (99)	2.6 ± 0.1 (94)	2.6 ± 0.1 (95)	2.1 ± 0.1 (37)
4-months CA	2.9 ± 0.1 (97)	3.0 ± 0.1 (94)	2.9 ± 0.1 (90)	2.6 ± 0.1 (36)

000275

Table VII-38 Stool Characteristics of Infants				
	Control	AA + DHA (fungal/fish)	AA + DHA (egg-DTG/fish)	EHM[†]

[†] The Exclusive Human Milk group includes infants who consumed > 80% of their feedings from human milk from SDAY 1 to the term CA visit. After the term CA visit, infants in this group many have consumed a study formula (each infant was assigned a study formula at randomization) and/or human milk. Infants in the Exclusive Human Milk group served as a reference only; the data from these infants were not included in any of the statistical analyses.

[a] Differences among study feeding groups were evaluated using Chi-square analyses. No statistically significant differences were found.

[b] Scores are based on a 5-point scale: 1 = watery; 5 = hard.

[c] Differences among study feeding groups were evaluated using ANOVA statistics controlling for a possible site effect. No statistically significant differences were found.

[d] A statistically significant interaction for the feeding*birth weight stratum was found in mean stool number per day at 2-mo CA ($p = 0.0132$) and at 4-mo CA ($p = 0.0299$). Pairwise comparison demonstrated that at 2-mo CA, infants with birth weights > 1250 g fed AA + DHA (egg-DTG/fish) had a greater number of stools than did those infants fed AA + DHA (fungal/fish) ($p = 0.0042$).

[e] Scores are based on a 5-point scale: 1 = watery; 5 = hard.

[f] A statistically significant interaction for the feeding*birth weight stratum was found in mean rank stool consistency at 2-mo CA ($p = 0.0315$).

000276

Table VII-39
Safety and Monitoring Measures of
Preterm Infants Fed Human Milk and/or a Preterm Formula
With or Without AA and DHA or Exclusively Human Milk*
Feeding Protocol Followers

	Control	Fungal/Fish	Egg-DTG/Fish	EHM†
Feedings withheld due to gastric residuals‡				
% of infants	20	19	17	13
Number of infants	138	133	136	54
Days to full enteral feeds (100 kcal/kg/day)§				
Mean ± SEM	12.4 ± 1.2	12.6 ± 1.1	12.2 ± 1.2	14.7 ± 3.1
Number of infants	126	120	128	35
NPO for at least 1 day‡				
% of infants	28	28	29	32
Number of infants	138	133	136	54
Days from SD1 to Hospital Discharge, n¶				
Mean ± SEM	38.0 ± 1.8	37.1 ± 1.9	39.0 ± 1.8	37.9 ± 2.8
Number of infants	138	133	136	54
Suspected NEC‡ **				
% of infants	25	20	25	20
Number of infants	138	133	136	54
Confirmed NEC‡ **				
% of infants	3	2	2	0
Number of infants	138	133	136	54
Suspected systemic infection‡ ††				
% of infants	36	33	39	35
Number of infants	138	133	136	54
Confirmed systemic infection‡				
% of infants	16	12	15	9
Number of infants	138	133	136	54
Cases of lung disease‡				
% of infants	24	25	22	30
Number of infants	136	133	15	54

* From the time of randomization (Study Day 1) until hospital discharge, except where indicated.
† EHM = Exclusive Human Milk infants are defined as those infants receiving exclusively human milk at the time of these assessments.
‡ Differences across study formula groups were evaluated Cochran-Mantel-Haenszel statistics controlling for site. No statistically different differences were found.
§ Differences across study formula groups were evaluated by survival analysis. No statistically significant difference was found.
¶ Differences across study formula groups where determined by ANOVA controlled for site, gender, and birth weight strata.
** Necrotizing enterocolitis was confirmed by one of the following: x-ray evidence of pneumatosis, intestinal free air or gas in the portal tract, or perforation (ii), surgery (iii), or autopsy.
†† Systemic infection was confirmed by blood culture or, in the case of meningitis and pneumonia, a positive culture in the cerebral spinal fluid and positive x-ray, respectively.

000277

Table VII-40 Respiratory Assistance, Corticosteroid Therapy, Antibiotic Use and Blood Transfusions of Preterm Infants Fed Human Milk and/or a Preterm Formula With or Without AA and DHA or Exclusively Human Milk				
	Control	Fungal/Fish	Egg-DTG/Fish	EHM†
Prior to Study Day 1				
Ventilatory support, days				
Mean ± SEM	4.1 ± 0.4	3.8 ± 0.3	3.4 ± 0.3	4.5 ± 0.5
n	137	133	135	54
% Subjects	82	84	78	93
Highest inspired O_2 > 40%, days				
Mean ± SEM	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.2	1.8 ± 0.3
n	143	139	140	39
% Subjects	62	65	60	69
Apnea/bradycardia episodes, days				
Mean ± SEM	1.5 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	2.5 ± 0.3
n	143	139	140	39
% Subjects	58	60	61	70
Surfactant therapy, days				
Mean ± SEM	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
n	143	139	140	39
% Subjects	50	50	50	59
Corticosteroid therapy, days				
Mean ± SEM	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.3
n	143	139	140	39
% Subjects	9	7	7	17
Average # antibiotics, days/infant				
Mean ± SEM	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
n	143	139	140	39
% Subjects	82	92	90	93
Transfusions, # (days)				
Mean ± SEM	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
n	144	139	141	39
% Subjects	23	20	18	28
Study Day 1 to Hospital Discharge				
Ventilatory support, days				
Mean ± SEM	15.0 ± 2.0	15.1 ± 2.0	16.0 ± 2.1	14.5 ± 2.8
n	144	139	141	39
% Subjects	57	54	65	59
Highest inspired O_2 > 40%, days				
Mean ± SEM	6.1 ± 1.3	6.1 ± 1.2	5.7 ± 1.2	3.1 ± 1.8
n	137	133	135	54
% Subjects	35	36	40	43
Apnea/bradycardia episodes, days				
Mean ± SEM	14.4 ± 1.3	15.2 ± 1.4	15.5 ± 1.4	18.0 ± 2.2
n	144	139	141	39
% Subjects	86	81	85	91
Surfactant therapy, days				
Mean ± SEM	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
n	144	139	141	39
% Subjects	0	0	0	0

000278

Table VII-40
Respiratory Assistance, Corticosteroid Therapy, Antibiotic Use and Blood Transfusions of Preterm Infants Fed Human Milk and/or a Preterm Formula With or Without AA and DHA or Exclusively Human Milk

	Control	Fungal/Fish	Egg-DTG/Fish	EHM†
Corticosteroid therapy, days				
Mean ± SEM	3.4 ± 0.8	2.9 ± 0.8	2.3 ± 0.5	2.9 ± 1.3
n	144	139	141	39
% Subjects	21	16	18	28
Average # antibiotics, days/infant				
Mean ± SEM	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
n	144	139	141	39
% Subjects	58	68	63	57
Transfusions, # (days)				
Mean ± SEM	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.0 ± 0.3
n	144	139	141	39
% Subjects	49	43	43	44

† EHM = Exclusive Human Milk infants are defined as those infants receiving exclusively human milk at the time of these assessments.

The incidence rate (% subjects) of all the listed events were analyzed using Chi-square or Fisher's exact tests. Average days of antibiotics use per infant was also analyzed by ANOVA. No statistically significant differences were found.

000279

C. Clinical Trial of Growth, Development, and Visual Function of Term Infants Fed Formula Containing SUNTGA25 AA-Rich Oil and DHA-Rich Tuna Oil

Between December 1995, and November 1997, Ross Products Division conducted a prospective, double-masked, randomized, parallel feeding study to evaluate the addition of AA and DHA to formulas for term infants (Ross Study AF92, Ross Products Division, 2000). Multiple indices of growth and development were measured to evaluate the suitability of two AA and DHA sources and to determine whether adding AA + DHA to term formula enhances development. Infants were fed the formulas from 8 days to 12 months of age. Reference groups of breastfed infants weaned to formulas with and without AA + DHA also were studied. Infants in the formula groups were randomized to a control formula with no AA or DHA or one of two otherwise identical formulas containing AA + DHA from either AA-rich fungal oil and DHA-rich fish oil (fungal/fish), or egg-derived triglyceride (egg-DTG) at approximately 0.46% and 0.13% respectively, levels similar to the average in human milk samples. Infants in the breast-fed groups were breastfed exclusively for at least 3 months and when formula supplementation was introduced, were fed the egg-DTG or control formulas as randomized at enrollment.

1. Materials and Methods

Study Participants

Cohorts of formula-fed and breast-fed infants were recruited for the study. A total of 404 infants were randomized into the study. Infants were recruited from three hospitals and five pediatric practices in the United States and through referrals from the local community (Kansas City, MO; Little Rock, AK; Pittsburgh, PA; Tucson, AZ). Study visits were conducted at hospitals or private pediatric offices. The study was approved by the IRB of each study site.

Infants born at term who met the following inclusion criteria were eligible for study participation: in good health, full term (gestational age 37-42 weeks), less than 9 days of age (formula groups), less than 11 days and currently breastfed (breastfed group), birth weight of 2500 g or greater, able to tolerate a milk-based formula, 5-minute Apgar score of 7 or greater, parent or guardian agreed to feed the assigned study formula *ad libitum* according to the study design, and parent or guardian voluntarily signed the informed consent form. Infants were not enrolled if they met any of the following exclusion criteria: evidence of significant cardiac, respiratory, ophthalmological, gastrointestinal, hematological or metabolic disease, or a milk protein allergy, or a maternal medical history known to have provided adverse effects on the fetus, such as diabetes (gestational diabetes was acceptable if the infant's birth weight was less than 4150 g), tuberculosis, HIV infection, perinatal infections, or substance abuse. A computer-generated randomization schedule was prepared for each study center. Randomization was blocked by gender (50% male, 50% female). Twins were assigned to the same feeding group.

Study Design

Infants in the formula groups were randomized before 9 days of age and fed the control formula or one of the formulas containing AA and DHA from enrollment to 12 months of age. Infants in the breast fed groups were enrolled before 11 days of age and breast fed exclusively for at least the first 3 months of age. At enrollment, breastfed infants were randomized to the control or the egg-DTG formula, and were not to begin formula feeding before 3 months of age.

Solid foods were permitted for all infants after 4 months of age. Infants in the breast-fed group were permitted supplemental formula before 3 months, but this was limited to less than 16 oz (480 mL) per week. If the study formulas were not readily available when parents of the breast-fed infants chose to begin supplementing with formula, infants were permitted any commercially available formula for up to 5 days. Supplemental multivitamins and fluoride were permitted, as long as the majority of the infant's feeding was human milk. Water was permitted *ad libitum* for all infants. Alternate formulas were permitted for all infants for up to 5 days if recommended by their primary physician or by the investigator.

Study visits after enrollment took place at 1-, 2-, 4-, 6-, 9- and 12-months; a mail-in questionnaire was completed at 14-months. A window of ± 5 days was permitted for all visits through the 9-month visit; the 12- and 14-month visits had a window of ± 7 days. Infant and family demographic information was obtained at the enrollment visit. The Study Exit form was completed at the 12-month study visit or at the visit after the last known study feeding (formula and/or breastmilk) for infants who exited early.

Formulas

At enrollment, formula-fed infants were randomized to a control formula or one of two formulas supplemented with AA and DHA: 1) AA-rich fungal oil (Suntory Ltd, Japan; fungal/fish) and DHA-rich fish oil (Mochida International Co, Ltd, Japan), or 2) egg-derived triglyceride (egg-DTG).

The fungal/fish supplemented formula contained AA and DHA at 0.46% and 0.13% fatty acids, respectively; the egg-DTG contained AA and DHA at 0.45% and 0.14% fatty acids, respectively. Eicosapentaenoic acid (EPA; 20:5n-3) was 0.04% in the formula with fish oil (fungal/fish) and was not detectable in the egg-DTG formula. All formulas provided the dietary essential fatty acids linoleic acid and alpha-linolenic acid (21.0 – 22.4% and 2.4 – 2.6%, respectively).

All formulas provided 20 kcal/fl oz and were packaged in clinically labeled (coded) 32-fl oz ready-to-feed cans. The control formula was manufactured essentially as commercial *Improved SWI* and coded as a clinical study product.

Study Outcomes Measured

Table VII-41 presents an overview of the study variables that were collected and the time at which they were collected. Descriptions of the study outcomes are shown below. In this summary, outcomes have been classified as one of three types of safety assessments: growth, development, and monitoring of parameters including blood biochemistries, tolerance, morbidity, and adverse events.

Demographics

Infant and family demographic information was obtained at the enrollment visit. Information about the infants included: race, gender, 5-minute Apgar score, multiple birth status, birth weight, length and head circumference. Family demographic information collected included biological mother's history of smoking, age, and parental height, education, marital status, and family size.

Table VII-41
Study Variables and Data Collection: Term Infant Study

Study Time Points, months	Enroll (E)	1	2	3	4	6	9	12	Daily E to12	14
Formula Groups (enrolled at ≤8 days of age)	**Control, AA + DHA (Fungal/Fish), AA + DHA (Egg-DTG)**									
Breastfed Groups (enrolled at ≤10 days of age)	**Breastmilk exclusively**			**Breastmilk and/or assigned study formula:** Control or AA + DHA (Egg-DTG)						
Assessment Periods:										
Informed Consent										
Randomization	X									
Demographics	X									
Growth: Weight, Length, Head Circumference	X	X	X		X	X	X	X		
Development: Visual										
Visual acuity			X		X	X		X		
Development: Information Processing										
Fagan Test of Infant Intelligence						X*	X*			
Development: General										
Bayley Scales of Infant Development (PDI, MDI)						X		X*		
Development: Language										
MacArthur Communicative Development Inventories							X			X
Development: Temperament										
Infant Behavior Questionnaire (Rothbart)						X		X		
Bayley Scales of Infant Development (BRS)						X		X*		
Monitoring: Blood biochemistries (CBC, antioxidant status, fatty acids,)					X*			X*		
Monitoring: Human Milk Fatty Acids					X*					
Monitoring: Tolerance to formulas										
Formula intake and spit up records‡		X	X		X	X	X	X		
Stool characteristic records§		X	X		X					
Ross feeding and stool questionnaire		X			X					
Ross formula satisfaction questionnaire		X			X					
Monitoring: Morbidity†									X	
Monitoring: Adverse events (hospitalizations)									X	

* Assessed with a subset of infants; not all sites participated.
† Otitis media, diarrhea, antibiotic use, candida, thrush, antifungal use by parent diaries and/or physician records.
‡ 3-day parent diaries for 3 days preceding the study visit, except for in the first month (daily diaries from 9 to 27 days of age).
§ Infant stool records maintained between 9 and 27 days of age, and for 3 d before the 2- and 4-month study visits.

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Growth

Anthropometric measurements (weight, length, and head circumference) were collected according to standard procedures. Growth measures were collected at enrollment and at the study visits at 1-, 2-, 4-, 6-, 9-, and 12-months.

Development

Visual Development

Visual acuity is the ability to resolve fine spatial detail in the visual image. Visual acuity was assessed using the Teller Acuity Card procedure at 2-, 4-, 6-, and 12-months. Infants were shown a series of 25.5 x 51 cm cards with black and white stripes varying in spatial frequency (stripe width) by half-octave steps, where one octave represents a halving or doubling of spatial frequency. The finest grating (stripe width) to which the infant showed a consistent fixation response is the visual acuity threshold in cyc/deg with variance in octaves.

Information Processing

The Fagan Test of Infant Intelligence, which determines preference for novelty by infant looking behaviors, was administered to assess infant information processing at the 6- and 9-month visits. During a familiarization period, a face stimulus was shown until the infant accumulated a predetermined amount of looking time; during the subsequent test period, the familiar face stimulus was shown concurrently with a novel face stimulus. The amount of looking time spent on each stimulus was recorded, and "novelty preference" (percent of total looking time spent looking at the novel stimulus during the test phase averaged across 10 tests) was computed. In addition, mean duration of looking time, construed as a measure of efficacy of information processing, was computed for both the familiarization and test periods by dividing the total looking time by the number of looks averaged across 10 tests.

General Development

The Psychomotor Developmental Index (PDI) and the Mental Developmental Index (MDI) of the Bayley Scales of Infant Development were administered at 6- and 12-months to assess motor and cognitive development, respectively. The PDI and MDI involve a series of tasks that are presented to the infant while the parent/guardian is present, beginning with a set of items corresponding to the infant's chronological age. The standardized tests are designed to assess development of infants from 1 to 42 months of age.

Language Development

The infant version of the MacArthur Communicative Development Inventories was completed at 9- and 14-months. The standardized parent-report instrument consists of a checklist of words, phrases, gestures, and actions and provides information about comprehension vocabulary (words and phrases the child understands) and expressive vocabulary (words and phrases the child says). Percentile scores were computed from gender-specific norms and transformed to standard scores by convention.

Temperament

The Infant Behavior Questionnaire (Rothbart) was completed by the parent(s) at 6- and 12-months. The questionnaire is a standardized 94-item instrument that measures activity level,

distress to novel stimuli, distress to limitations, soothability, smiling and laughter, and duration of orientating. Infant temperament was also assessed using the Behavior Rating Scale of the Bayley Scales, which provides an index of the examiner's overall impression of the child's behavior. The BRS is completed by the tester after the Bayley Scales test session, and involves ratings of the infant's behavior during the session. Based on these ratings, scores on three subscales (orientation/engagement, emotional regulation, motor quality) were calculated.

Monitoring

Blood biochemistries

At the 4- and 12-month visits, blood was drawn from a subset of infants (~37 per feeding group) whose parents agreed to the procedure at the Kansas City, Pittsburgh, and Little Rock locations. Requests for blood samples were discontinued after ~37 samples per feeding group had been obtained. Several analyses were completed on the samples.

Complete Blood Counts

An aliquot of the blood samples collected for the blood biochemistries at the 4-and 12-month visits was analyzed to determine hemoglobin, MCV, MCH, MCHC.

Plasma Antioxidants

The concentrations of retinol and vitamin E (α-tocopherol) were measured in the samples. The total antioxidant potential (TRAP) in plasma also was quantified and lipid peroxide levels in plasma were measured.

Plasma and Red Blood Cell Fatty Acids

Fatty acid levels, including AA and DHA, were measured in plasma phospholipids, cholesterol esters, and triglycerides and reported as concentrations (microgram/liter) and as g/100 g of the total fatty acids (wt %). Additionally, fatty acid levels were measured in red blood cell membrane phosphatidylcholine and phosphatidylethanolamine (wt % of fatty acids).

Human Milk Fatty Acid Composition

Breast milk samples were obtained at the 4-month visit from a subset of the breastfed group at the Kansas City, Pittsburgh, and Little Rock study sites. Only mothers of infants who had blood samples drawn were asked for breast milk samples. Fatty acid analyses were completed on the samples.

Tolerance

Formula Intake and Spit Up

Parents or guardians were given forms (diaries) to record the volume of formula consumed at each feeding, the daily number of human milk feedings (breastfed groups), when the infant spit up or vomited, and when foods other than the study formula were consumed. Parent(s) or guardian(s) of breastfed infants also recorded the date that they started supplementing with formula and the last date of breastfeeding.

Stool Characteristics

The parents/guardians also recorded information about their infant's stool characteristics, including the color and consistency of each stool, whether their infant strained during stooling, and the frequency of gas at designated intervals before their infants reached 4 months of age.

Infant Feeding & Stool Pattern and Formula Satisfaction Questionnaires

Parents completed two questionnaires, both developed by Ross, at the 1- and 4-month study visits. The Infant Feeding and Stool Patterns Questionnaire is designed to assess parental perceptions about their infant's reactions to the study feedings, including ratings of stool characteristics, incidence of spitting up and vomiting, frequency of burping, gassiness, and acceptance of the feedings. Parents/guardians of infants in both the human milk and formula groups completed this questionnaire, with substitution of the word "formula" with "human milk" or "feeding" for the infants in the human milk group. The Formula Satisfaction Questionnaire assessed parents' overall satisfaction with the formula, what they liked most and least about the formula, and ratings of the organoleptic characteristics of the formula. Only parents/guardians of infants in the formula groups completed this questionnaire.

Morbidity and Adverse Events

Morbidity data (diarrhea; otitis media; candida; thrush; medical treatments; medications; visits to physician's office, clinic, or emergency room; hospitalizations) were collected by parents and physicians. Parents recorded on calendars (daily diaries) the occurrence of diarrhea, prescription medications used, and treatments administered to their infants. The physician medical records for each child were reviewed and episodes of otitis media, diarrhea, thrush or candida infections were recorded. Additionally, antibiotic prescriptions, as well as other medications and treatments were recorded. The day of and reason for physician office visits, clinic visits, emergency room visits and hospitalizations were also recorded.

Data Quality Assurance

Testing and scoring for all developmental measures and clinical procedures were standardized across all sites, and adherence to testing and scoring procedures was monitored throughout the study to assure reliability. Administrators and coders for the developmental testing were trained in proper procedures, had extensive experience in child development, and met certification requirements. All testers were masked to the infant feeding groups.

Determination of Sample Size

Enrollment of 380 infants was planned, allowing for 76 infants in each of five treatment groups. This sample size reflects over-sampling to ensure adequate sample size (20% attrition). Evaluations of infant growth and assessment of language (vocabulary) scores at 14 months of age were used to calculated sample size for the three formula group comparisons. Fifty-four subjects (27 male, 27 female) in three randomized formula groups were needed to detect a one standard deviation difference in growth for each gender with (probability of type II error) of 0.10 (90% power). Assuming 20% attrition, a minimum of 68 subjects per group were to be enrolled. An additional sample size estimate was based on the results of a study conducted by Ross (Scott et al., 1998); the power analysis estimated that 47 infants per group were needed to detect a 0.75 standard deviation difference in vocabulary scores with 90% power. Assuming 20% attrition, a minimum of 60 subjects per group would be enrolled based on this estimate.

Blood samples were to be drawn from a subset of infant at 4 and 12 months of age. Twenty-seven subjects were required to detect a one standard deviation difference with (probability of type II error) of 0.10 (90% power). Assuming 20% attrition and over sampling, 37 blood samples per feeding group were planned.

Statistical Analyses

The primary analyses were conducted using all available data and the secondary analyses using preplanned covariates for many of the assessments. All statistical tests were two-sided, using $\alpha = 0.05$ to determine statistical significance. All analyses controlled for site (i.e., site was used as a covariate) to minimize the potential confounding effect of intersite variability. Comparisons among the three formula groups and between the two breastfed groups were done twice, once with no additional covariates and once with additional covariates selected *a priori*. The covariates for each primary variable group (growth and developmental outcomes) were defined using published reports, previous study findings, and expert opinion. Categorical variables were analyzed using Chi-square or Cochran-Mantel-Haenszel tests and continuous variables by analysis of variance (ANOVA) and/or analysis of covariance (ANCOVA). Repeated measures analyses were used for data collected at more than one time period. *Post-hoc* comparisons of formula- and breastfed infants were done using the breastfed and corresponding formula groups to provide the greatest power to detect differences between these cohorts. Missing data were not imputed.

2. Results

Study Population

A total of 404 infants were randomized into the study: 239 infants in the formula fed groups, and 165 in the breastfed groups. A total of 294 infants completed the study. Some infants were randomized to the study prior to the day on which the study feeding was to begin; this resulted in a total of seven infants withdrawing from the study on or prior to the first study day and consequently before receiving a study formula. Select baseline demographic and exit data were available for two of the infants, thus these infants were included in some analyses.

Table VII-42 presents a summary of the number of infants participating at each phase of the study. Sixty-seven of the 110 withdrawals occurred within the first 2-months of the study. The primary reason for early exit for the formula-fed infants was "intolerance" and the primary reason for early exit for the breastfed groups was "noncompliance". There were no statistically significant differences among the feeding groups for disposition of subjects, number of days in study, or primary reason for exit. Sixteen of the 404 infants enrolled did not meet all of the inclusion criteria and two infants met at least one of the exclusion criteria. These 18 infants were represented across the study groups.

Reported intake of formula supplements or non-study formula occurred in 5% or fewer of the infants. Similarly, reported intake of non-study formula occurred in 12 (6%) of infants in the formula groups in the first month and, subsequently, in 2% or fewer of infants (data not shown).

Solid foods were not permitted before the 4-month visit. The reported intake of solid foods in the study was variable (13% to 39% of infants at the 1, 2, and 4-month visits), with the greatest number of deviations at 4 months (data not shown). Small amounts of cereal accounted for many of these deviations, and the majority of the caloric and fat intake was thus from formula and/or breastmilk. The potential impact of these protocol deviations on the study outcome is considered very small.

Demographics

Summaries of the infant and family demographic characteristics of the study population are presented in Table VII-43 and Table VII-44, respectively. The demographics did not differ among the three formula groups. Most (87%) infants were appropriate for gestational age, with 11% large for gestational age and < 2% small for gestational age. The ethnic distribution was 85% Caucasian, 5% Hispanic, 3% African-American, with the remaining 7% from other ethnic origins. English was the predominant language in all but two homes (i.e., 99.5% of homes). There were no differences between the two breastfed reference groups with the exception of a statistically significant difference in gestational age (control, 39.2 weeks; egg-DTG, 39.6 weeks) and maternal age (control, 29.1 years; egg-DTG, 30.8 years). There was an equal distribution of males and females.

Growth

Indices of growth, including weight, length, and head circumference were measured at enrollment and 1-, 2-, 4-, 6-, 9-, and 12-months. There were no overall or gender specific differences for increases in weight, length, or head circumference among groups during the 12-month study (Figure VII-3).

Weight gain from enrollment to 12-months was 17.7 ± 2.6, 17.8 ± 2.4, 17.2 ± 2.6 g/day for infants fed the control, AA + DHA (fungal/fish), and AA + DHA (egg-DTG) formulas and 17.4 ± 2.6 and 18.0 ± 3.1 g/day for infants in the breastfed-control and breastfed-AA + DHA groups, respectively (Table VII-45). There were no differences in length or head circumference between 0 and 4 months and 0 and 12 months. Gains in length and head circumference between 0 and 4 months and 0 and 12 months were not different between the BF/control and BF/AA + DHA groups. Weight gain for male infants between enrollment and 4 months, but not between enrollment and 12 months, was greater for the AA + DHA (fungal/fish) group than the control group (31.4 ± 4.6 and 27.8 ± 4.2 g/day, respectively) (Table VII-46). Weight gain for infants fed AA + DHA from egg-DTG was not greater than the control group over the same period. Gains in weight between 0 and 4 months and 0 and 12 months were not different between the BF/control and BF/AA + DHA groups. There were no differences in weight gain for female infants (Table VII-47).

Development

Visual Development

Visual acuity was not different in infants fed the AA + DHA formulas than infants in the control group between 2 and 12 months of age, regardless of whether they were exclusively formula fed or breastfed for at least three months (Figure VII-4, Table VII-48).

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Information Processing

The Fagan Test of Infant Intelligence was administered at 6 and 9 months of age. Scores did not differ between infants in the formula control and AA + DHA groups or between BF/control and BF/AA + DHA groups at either time period (Table VII-49). Look duration during the familiarization and testing periods also did not differ in relation to adding AA and DHA to the formulas.

General Development

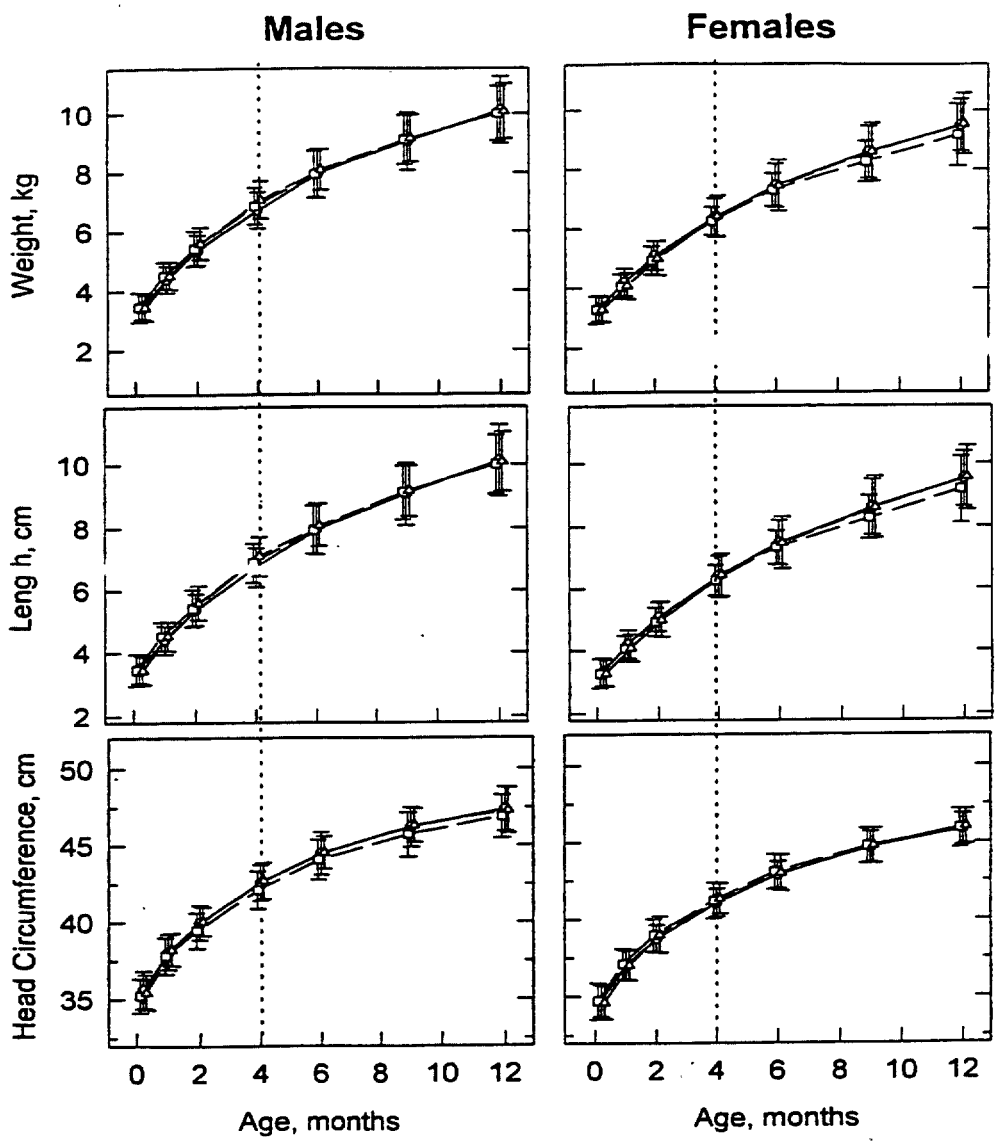
The mental and motor index test scores (MDI and PDI) of the Bayley Scales of Infant Development test were not different among the control and the AA + DHA formula groups at 6 and 12 months (Table VII-50). No differences were found in the percentages of infants who scored < 70, which may suggest significant delayed performance, or between 71 and 84, which may suggest mildly delayed performance (data not shown).

Language Development

Language development was assessed using the MacArthur Communicative Development Inventories. Vocabulary comprehension was not different in relation to AA + DHA supplementation among the three formula groups and between the two breastfed groups (Table VII-51). No differences were found in scores of vocabulary production at the 9-month visit, but at 14 months, infants in the AA + DHA (fungal/fish) formula group had a significantly higher score as compared to infants fed the AA + DHA (egg-DTG) formula (104 ± 13 and 96 ± 18, respectively) ($p<0.05$ by ANOVA and ANCOVA). Neither AA + DHA group was significantly different from the control formula group, and there were no differences between the breastfed and corresponding formula groups.

No differences in relation to AA + DHA supplementation among the three formula groups were found with regard to number of phrases or gestures (early, later, and total) understood (Table VII-52). A small but statistically significant difference between the breastfed control and breastfed AA + DHA (egg-DTG) groups for late gestures and total gestures was found; scores were higher in the control group.

FIGURE VII-3
Growth Curves of Male and Female Infants in Clinical Trial AF92



Growth (weight, length, and head circumference) of infants fed formulas with or without AA + DHA. The vertical dotted line at 4-months indicates when infants could begin eating solid foods. Left Panels, male infants; Right Panels, female infants. Circles, control formula groups; Squares, AA + DHA [egg-DTG] groups; Triangles, AA + DHA [fungal/fish oil] group. Values are mean ± SD. There were no statistically significant differences among the groups. (Auestad et al. 2001)

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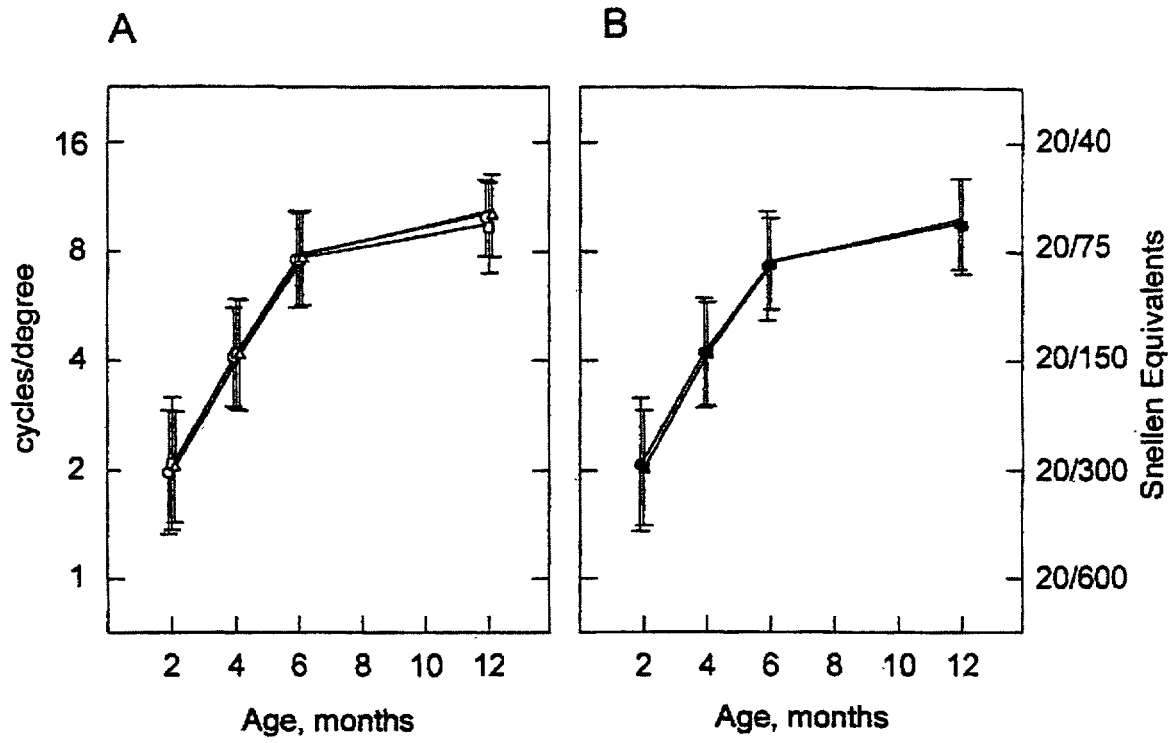
FIGURE VII-4
Visual Acuity of Infants in Clinical Trial AF92



Visual acuity measured by the acuity card procedure for infants fed formulas with or without AA + DHA or breastfed for at least the first 3 months after which formula with or without AA + DHA may have been fed as a supplement or weaning formula. Panel A. Formula groups: *Open circles*, control formula; *Open squares*, AA + DHA (egg-DTG) supplemented formula. *Open triangles*, AA + DHA (fungal/fish) supplemented formula. Panel B. Breastfed groups: *Closed circles*, BF/control formula; Closed *squares*, BF/AA + DHA (egg-DTG) supplemented formula. Data are shown on the left axis as mean (cycles/degree; cy/deg) ± SD (octaves). No differences in visual acuity among groups by repeated-measures analysis were found. (Auestad et al. 2001)

Temperament

Infant behavior and temperament were assessed using the Infant Behavior Questionnaire (Rothbart) and the Behavior Rating Scales (BRS) of the Bayley Scales. No differences among feeding groups were found for five of the six dimensions of the Rothbart parent questionnaire (activity level, distress to novel stimuli, distress to limitations, soothability, and duration of orienting) (Table VII-53). Scores on the sixth dimension, smiling and laughter, were statistically higher for the control formula group as compared to the egg-DTG group; no differences were found between the control and AA + DHA (fungal/fish) group. The percentages of infants with questionable or no optimal BRS facet scores (motor quality, orientation/engagement, emotional regulation) on the Bayley Scales did not differ among any groups in relation to AA + DHA supplementation (Table VII-54). No differences emerged between the BF/control and the

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BF/AA + DHA groups on the dimensions of Infant Behavior Questionnaire or on the facet scores of the Bayley Scales.

Monitoring

Blood Biochemistries

Complete Blood Counts

With few exceptions, plasma levels of hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, and mean cell volume measured in the 4-and 12-month samples were within the normal ranges for age matched cohorts (Table VII-55). There was a small but statistically significant difference in plasma hemoglobin between the AA + DHA (egg-DTG) and AA + DHA (fungal/fish) groups ($p<0.05$ by repeated measures ANOVA); values in the fungal/fish group were greater than values in the egg-DTG group. There were no differences between the control and AA + DHA (fungal/fish) groups.

Plasma Antioxidants

The potential for increased oxidation of the dietary fatty acids was assessed by measuring plasma levels of lipid peroxides, and a measure of the total antioxidant potential in plasma (TRAP), and α-tocopherol (vitamin E) in a subset of infants (Table VII-56). Lipid peroxide levels in plasma were below detection limits for greater than 90% of the samples, and therefore were not analyzed statistically. The values for TRAP were not different in regard to AA + DHA supplementation.

A small, but statistically significant difference in plasma α-tocopherol levels between the control formula group and the egg-DTG groups was found by repeated measures of ANOVA ($p < 0.05$), where means levels in the egg-DTG group were greater. A similar statistically significant difference between these groups was also found for plasma vitamin A (retinol) levels. No differences were found between the control group and the fungal/fish group, or among the breastfed groups.

Plasma and Red Blood Cell Fatty Acids

Infants fed the AA + DHA formulas had approximately a two-fold increase in AA and DHA in plasma phospholipids, whether expressed as a relative percent of total fatty acids or as concentrations in plasma (Tables VII-57 and VII-58). Similar two-fold increases in DHA were found in red cell phospholipids (Tables VII-59 and VII-60). The levels of AA and DHA in cholesterol esters and triglycerides were near the detection limit (Tables VII-61 and VII-62); the coefficient of variation was large, thus, the sensitivity poor. The levels of AA and DHA in plasma and red cells of the AA + DHA supplemented formula groups at 4 months are within a similar range for those of the breastfed groups, at which time about half of the breastfeeding mothers had begun weaning.

Human Milk Fatty Acid Levels

The average levels of AA and DHA in the supplemented formulas were 0.46% and 0.13%, respectively, very similar to the average levels in the subset of breastfeeding women in this study. The average levels of AA, DHA and EPA were 0.51%, 0.12% and 0.05 (g/100g total fatty acids), respectively, in breastmilk samples obtained from a subset of 43 mothers from

Kansas City, Pittsburgh, or Little Rock, who were still exclusively breastfeeding at the 4-month study visit (data not shown).

Tolerance

Formula Intake

Formula intake (average daily caloric intake of formula over the data collection period) was not different among the formula groups (Table VII-63) or between the two breastfed groups (data not shown). The frequency of spitting up (percent of feedings with spit up and or vomiting during data collection period) was analyzed only for the 1- and 2-month data collection periods after which spit up was infrequent for most infants. There were no differences among the formula groups for the frequency of spitting up and/or vomiting in the first two months. Breastfed infants spit up infrequently, thus the frequency of spit up was not analyzed statistically.

Stool Characteristics

Parents were asked to describe the consistency of each stool as watery, loose/mushy, soft, formed, or hard. Stool consistency was evaluated at the 1-, 2-, and 3-month study visits and was summarized as: i) predominant stool consistency; ii) mean rank stool consistency, and; iii) the percent of stools with a specific consistency (e.g., watery). The latter was not analyzed statistically. The predominant stool consistency (the most frequently reported stool consistency over the 19- and the 3-day data collection periods) was not different among the three formula groups or between the two breastfed groups (data not shown). The consistency of each stool was also ranked numerically from 1 (watery) to 5 (hard) and the average rank (mean rank) over each data collection was calculated and analyzed. There were no differences among groups (Table VII-64). The percent of stools that were watery was not evaluated statistically; 12% of stools were watery for infants fed the control formula, 6% for those in the egg-DTG group, and 9% for those fed the fungal/fish oil formula. Stool color was not different among groups (data not shown).

Infant Feeding and Stool Patterns and Formula Satisfaction Questionnaires

Parents' perceptions about formula tolerance and the acceptability of the formula were assessed using questionnaires at the end of the first month and the end of the exclusive formula feeding period (4 months). Responses to questions about infants' acceptance of the formula and stool patterns and characteristics were not different when either the control or the AA + DHA formulas were fed (data not shown).

Morbidity

Approximately half of the infants had at least one reported incidence of otitis media. The percentage of infants who were diagnosed at least once with otitis media (40% to 61% of infants) or who had two or more episodes (23% to 37% of infants) did not differ in relation to formula LCP supplementation for either the formula or breastfed cohorts (Table VII-65). Similarly, about half of the infants were given a prescription for antibiotics at least once during the first year (45% to 64% of infants) or were given two or more prescriptions (27% to 42% of infants) (Table VII-65). There were no differences among groups in relation to AA + DHA supplementation in the two cohorts.

Approximately half of the parents reported at least one episode of diarrhea and approximately 25% of infants had a physician diagnosis of diarrhea at least once in the first year of life (Table VII-66). The percentage of infants with one episode of diarrhea, who were given electrolyte solutions for the treatment of diarrhea and/or whose physician diagnosed at least one episode of diarrhea was not different among the three formula groups or between the two breastfed groups.

The number of infants whose physician diagnosed at least one episode of thrush or Candida or prescribed antifungal medication at least once was too few (2 to 21/group) for a valid statistical analysis, though the results do not suggest differences among the formula groups (Table VII-67).

Adverse Events

Serious adverse events, defined as any experience that was fatal, life-threatening, disabling, requiring hospital admission, or intervention to prevent permanent impairment, were reported for 30 infants (formula control group, 10/77; AA + DHA/egg-DTG, 6/80; AA + DHA/fungal-fish, 4/82; breastfed control group, 3/82; breastfed AA + DHA/egg-DTG groups, 5/83). There was no preplanned presentation or analysis of adverse events by specific events. Review of the data indicates that the reported serious adverse events were primarily hospitalizations for treatment of chronic otitis media and infectious diseases. There were no differences among groups within each cohort for hospitalizations. The data collection methods did not permit reliable analysis of visits to physician offices or emergency rooms, thus these data are not reported. There were no deaths or other serious or significant adverse events.

3. Discussion

This clinical trial currently represents the largest study of the effects of AA + DHA supplemented formulas on the development of term infants in the published literature. A total of 404 term infants were enrolled this prospective, masked, randomized, multicenter parallel feeding study to evaluate the effects of formulas with or without different sources of AA and DHA, on the growth and multiple indices of development of term infants over the first 14 months after birth. Approximately 20% of the infants in this study consumed SWI that contained 0.46% AA from AA-rich oil and 0.13% DHA from DHA-rich oil. Study subjects were recruited from homes generally comparable to the U.S. population and are therefore representative of healthy term infants in the U.S.

Multiple measures of safety of the supplemented formulas were assessed throughout the first 14 months of the infants' lives. All assessments were conducted with masked clinical tests and by parent report instruments. Results of multiple assessments throughout the study, including measures of growth, measures of development, and systematic monitoring of effects on blood chemistries, tolerance, morbidity, and incidence of adverse events revealed that infants fed the formula supplemented with AA- and DHA-rich oils were not different as compared to infants fed a standard infant formula. The comparisons in the trial were adequately powered to detect differences among the formula groups.

Growth rates, as assessed by gains in weight, length, and head circumference were not lower in either of the supplemented groups as compared to the control groups. Addition of AA-

and DHA-rich oil from single-cell and fish sources, respectively, to term infant formula also did not adversely affect visual development, information processing, general development, language development, or temperament as assessed by trained individuals. The variance in the visual and developmental tests was as expected, and was not increased with multiple testers at multiple sites. This provides evidence that the sensitivity of the tests was not compromised in the multicenter trial.

Addition of AA-rich oil derived from a singe-cell source and DHA-rich oil from tuna oil to term infant formula also did not affect antioxidant status as measured by vitamin E, vitamin A, and TRAP. Iron status, as assessed by hemoglobin, MCH, MCHC, and MCV also was unaffected by addition of the fungal/fish oils to formula. Given the absence of any differences in these hematology parameters, combined with no observed differences in incidence of infections, the data suggest that immune function was not affected by consumption of the fungal/fish supplemented formula.

As anticipated, the addition of AA and DHA did increase the levels of AA and DHA fatty acids in plasma and RBC phospholipids, as well as plasma triglycerides and cholesterol-ester. The levels of AA and DHA in plasma and red blood cells of the AA + DHA supplemented formula groups at 4 months are within a similar range for those of the breastfed groups.

Tolerance and morbidity of the infants who consumed the formula with the AA- and DHA-rich oil sources were comparable to that of infants fed the control formula. The average daily caloric intake of formula, the frequency of spitting up and/or vomiting, the frequency and consistency of stools, and the extent to which infants were gassy were used to evaluate how well infants tolerated the formula feeding. Parents completed diaries of formula intake, reports of spit up/vomit, stool characteristics, and questionnaires on feeding and stool patterns and formula satisfaction. No differences among the formula groups were found. Similarly, the supplemented formulas were not associated with increased morbidity as determined by comparison among formula groups of physicians' reports of diagnosed otitis media, incidence of diarrhea, use of electrolyte replacements and antibiotics, oral thrush and candida diagnoses, and use of antifungals.

In conclusion, the addition of 0.46% AA from an AA-rich oil and 0.13% DHA from a DHA-rich oil source to SWI had no adverse effects on growth, development, tolerance, and morbidity as consumed by term infants under the conditions of this clinical trial. The observed increases in plasma and RBC concentrations of AA and DHA were anticipated and the resulting levels were within a similar range for those of breastfed infants.

Table VII-42 Disposition of Study Infants Subjects Randomized to the Feeding Protocol (n = 404)					
	Formula Groups			Breastfed Groups	
	Control	AA + DHA (fungal/fish)	AA + DHA (egg-DTG)	Control	AA + DHA (egg-DTG)
Randomized to study	239			165	
	77	82	80	82	83
Withdrew from study: No. (%)	30 (39)	23 (28)	20 (25)	21 (26)	16 (19)
Completed study: No, (%)	47 (61)	59 (72)	60 (75)	61 (74)	67 (81)
Primary reasons for early exit: No. of infants					
Non-compliance	9	7	4	14	14
Intolerance	16	14	13	5	1
Illness	2	1	2	1	0
Other	3	1	1	1	1
Days in study (median)	359	360	359	357	357

000295

Table VII-43
Infant Demographic Characteristics

	Formula Groups				Breastfed Groups		
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	ANOVA	Control	AA + DHA Egg-DTG	ANOVA
Number of Subjects	77	80	82		82	83	
Birth Weight, kg	3.45 ± 0.44	3.39 ± 0.47	3.41 ± 0.41	NS	3.49 ± 0.48	3.54 ± 0.41	NS
Birth Length, cm	50.8 ± 2.5	50.3 ± 2.6	50.6 ± 2.1	NS	50.9 ± 2.4	51.1 ± 2.6	NS
Gestational age, (n)	39.4 ± 1.2	39.0 ± 1.3	39.3 ± 1.2	NS	39.2 ± 1.2	39.6 ± 1.3	$p < 0.05$
Gestational approp, n (%)							
AGA	66 (86.8)	69 (86.3)	71 (89.9)	NS	72 (88.9)	69 (83.1)	NS
SGA	2 (2.6)	2 (2.5)	0 (0.0)		1 (1.2)	1 (1.2)	
LGA	8 (10.5)	9 (11.3)	8 (10.1)		8 (9.9)	13 (15.7)	
unknown (missing)*	1	0	3		1	0	
Apgar, 5 min	9.1 ± 0.4	9.1 ± 0.4	9.1 ± 0.5	NS	9.1 ± 0.4	9.1 ± 0.7	NS
Males, n (%)	37 (48.1)	39 (48.7)	40 (48.8)	NS	42 (51.2)	43 (51.8)	NS
Births, n (%)							
Singleton	75 (97.4)	74 (92.5)	80 (97.6)	NS	80 (97.6)	83 (100.0)	NS
Twin	2 (2.6)	6 (7.5)	2 (2.4)		2 (2.4)	0 (0.0)	
Birth order, n (%)							
1	30 (39)	22 (28)	29 (36)	NS	32 (39)	32 (39)	NS
2	25 (32)	36 (45)	38 (47)		27 (33)	30 (36)	
≥3	22 (29)	22 (28)	14 (17)		23 (28)	21 (25)	
Ethnicity, n (%)							
Caucasian	61 (79.2)	70 (87.5)	72 (87.8)	NS	67 (81.7)	74 (90.2)	NS
African-American	3 (3.9)	2 (2.5)	3 (3.7)		4 (4.9)	0 (0.0)	
Hispanic	2 (2.6)	4 (5.0)	4 (4.8)		6 (7.3)	3 (3.7)	
Asian	1 (1.3)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Other	10 (13.0)	4 (5.0)	3 (3.7)		5 (6.1)	5 (6.1)	
Unknown/missing	--	--	--		1	--	

Values are mean ± SD unless otherwise noted.
* Unknown (missing) values are not included in total percentages
NS, not statistically significantly different

000296

Table VII-44
Family Demographic Characteristics

VARIABLE	FORMULA GROUPS				BREASTFED GROUPS		
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	ANOVA	Control	AA + DHA Egg-DTG	ANOVA
Number of Subjects Enrolled*	77	80	82		82	83	
Maternal Age, (yrs)	28.1 ± 5.9	29.0 ± 5.1	29.2 ± 5.0	NS	29.1 ± 5.1	30.8 ± 4.6	$p < 0.05$
Maternal Height, in	64.5 ± 3.1	64.8 ± 2.6	64.6 ± 2.7	NS	64.3 ± 3.0	65.4 ± 2.8	NS
Paternal Height, in	70.4 ± 3.4	70.8 ± 2.7	70.9 ± 2.6	NS	70.7 ± 2.8	71.1 ± 2.9	NS
Smoking, n (%)							
Mother during pregnancy	12 (15.6)	16 (20.0)	15 (18.5)	NS	7 (8.5)	6 (7.2)	NS
Any smoking in household	16 (20.8)	26 (32.5)	14 (17.3)		11 (13.4)	7 (8.4)	
Parental Education, yrs							
Mother	13.7 ± 2.1	14.2 ± 1.9	14.1 ± 2.5	NS	15.1 ± 2.3	15.3 ± 2.4	NS
Father (range)	13.8 ± 2.0 (10-18)	14.0 ± 2.1 (9 - 19)	14.6 ± 2.1 (11 - 20)	NS	15.0 ± 2.4 (10 - 21)	15.3 ± 2.6 (9 - 24)	NS
Highest Degree Attained, n (%)							
Mother							
none	5 (6.5)	3 (3.8)	6 (7.4)	NA	3 (3.7)	1 (1.2)	NA
high school	37 (48.0)	38 (47.5)	32 (39.5)		22 (26.8)	26 (31.3)	
associate	12 (15.6)	13 (16.3)	6 (7.4)		12 (14.6)	6 (7.2)	
vocational	1 (1.3)	2 (2.5)	10 (12.4)		0 (0.0)	3 (3.6)	
bachelor	21 (27.3)	19 (23.8)	18 (22.2)		34 (41.5)	34 (41.0)	
masters	1 (1.3)	4 (5.0)	9 (11.1)		8 (9.8)	10 (12.1)	
PhD, JD, MD	0 (0.0)	1 (1.3)	0 (0.0)		3 (3.7)	3 (3.6)	
unknown	0 (0.0)	0 (0.0)	1		0 (0.0)	0 (0.0)	
Father				NA			NA
none	3 (4.0)	3 (3.8)	5 (6.3)		2 (2.4)	2 (2.4)	
high school	43 (56.6)	44 (55.7)	29 (36.7)		29 (35.4)	23 (27.7)	
associate	5 (6.6)	6 (7.6)	6 (7.6)		6 (7.3)	4 (4.8)	
vocational	0 (0.0)	3 (3.8)	2 (2.5)		5 (6.1)	7 (8.4)	
bachelor	23 (30.3)	17 (21.5)	32 (40.5)		30 (36.6)	32 (38.6)	
masters	2 (2.6)	4 (5.0)	4 (5.0)		8 (9.8)	11 (13.2)	
PhD, JD, MD	0 (0.0)	2 (2.5)	1 (1.3)		2 (2.4)	4 (4.8)	
unknown	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	

000297

Table VII-44
Family Demographic Characteristics

VARIABLE	FORMULA GROUPS				BREASTFED GROUPS		
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	ANOVA	Control	AA + DHA Egg-DTG	ANOVA
Maternal Marital Status, n (%)				NA			NA
married	60 (77.9)	63 (78.8)	68 (82.9)		70 (85.4)	80 (96.4)	
single	14 (18.2)	15 (18.8)	14 (17.1)		10 (12.2)	3 (3.6)	
separated/divorced	3 (3.9)	2 (2.5)	0 (0.0)		2 (2.4)	0 (0.0)	
Siblings Living in Home n (%)				NA			NA
0	27 (35.1)	20 (25.0)	27 (33.3)		30 (36.6)	29 (35.0)	
1	27 (35.1)	34 (42.5)	38 (47.0)		29 (35.4)	31 (37.4)	
2	19 (24.7)	19 (23.8)	10 (12.4)		14 (17.1)	16 (19.3)	
3	3 (3.9)	5 (6.3)	4 (5.0)		6 (7.3)	4 (4.8)	
4 or more	1 (1.3)	2 (2.5)	2 (2.5)		3 (3.7)	3 (3.6)	
Household Density, no. of People/room, median [IQR]	0.57 [0.50 - 0.80]	0.60 [0.50 - 0.83]	0.57 [0.50 - 0.75]	NS	0.60 [0.44 - 0.75]	0.57 [0.43 - 0.71]	NS
Occupation							
Mother				NA			NA
managerial/professional	14 (18.4)	21 (26.3)	18 (22.8)		24 (29.3)	27 (33.0)	
technical/sales	34 (44.7)	30 (37.5)	28 (35.4)		27 (33.0)	26 (31.7)	
service occupation	6 (7.9)	7 (8.8)	10 (12.7)		4 (4.9)	7 (8.5)	
farming/forestry	0 (0.0)	0 (0.0)	1 (1.3)		0 (0.0)	0 (0.0)	
production	0 (0.0)	0 (0.0)	0 (0.0)		1 (1.2)	0 (0.0)	
operator/fabricator	1 (1.3)	0 (0.0)	0 (0.0)		2 (2.4)	1 (1.2)	
experienced unemployed	20 (26.3)	21 (26.3)	19 (24.1)		24 (29.3)	19 (23.2)	
other	1 (1.3)	1 (1.3)	3 (3.8)		0 (0.0)	2 (2.4)	
missing	1 (1.3)	0 (0.0)	3 (3.8)		0 (0.0)	1 (1.2)	
Father				NA			NA
managerial/professional	28 (37.8)	20 (25.3)	19 (24.7)		33 (40.2)	34 (41.0)	
technical/sales	18 (24.3)	19 (24.1)	23 (29.9)		24 (29.3)	24 (28.9)	
service occupation	7 (9.5)	5 (6.3)	5 (6.5)		4 (4.9)	5 (6.0)	
farming/forestry	3 (4.1)	1 (1.3)	1 (1.3)		0 (0.0)	1 (1.2)	
production	9 (12.2)	16 (20.3)	15 (19.5)		14 (17.1)	9 (10.8)	
operator/fabricator	8 (10.8)	13 (16.5)	8 (10.4)		5 (6.1)	6 (7.2)	
military	0 (0.0)	2 (2.5)	1 (1.3)		0 (0.0)	0 (0.0)	
experienced unemployed	0 (0.0)	1 (1.3)	1 (1.3)		0 (0.0)	1 (1.2)	
other	1 (1.4)	2 (2.5)	3 (3.9)		2 (2.4)	3 (3.6)	
missing	3 (4.1)	1 (1.3)	5 (6.5)		0 (0.0)	0 (0.0)	

000298

Table VII-44 Family Demographic Characteristics							
VARIABLE	FORMULA GROUPS				BREASTFED GROUPS		
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	ANOVA	Control	AA + DHA Egg-DTG	ANOVA

Values are mean ± SD unless otherwise noted.
* For some variables, the numbers were reduced by one or two subjects due to missing data.
IQR, interquartile range; NA, not analyzed statistically, presented as descriptive; NS, not statistically significantly different.

000299

Table VII-45
Growth: All Infants

Variable	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg- DTG	AA + DHA Fish/ Fungal	Control	AA + DHA Egg- DTG
Weight, kg					
Enrollment	3.42 ± 0.46 (77)*	3.36 ± 0.48 (80)	3.37 ± 0.43 (82)	3.47 ± 0.49 (82)	3.47 ± 0.42 (83)
4 months	6.54 ± 0.66 (54)	6.54 ± 0.63 (63)	6.78 ± 0.74 (60)	6.80 ± 0.84 (67)	6.87 ± 0.85 (72)
12 months	9.78 ± 1.04 (48)	9.52 ± 1.06 (61)	9.83 ± 1.00 (59)	9.71 ± 1.11 (60)	9.98 ± 1.11 (68)
Weight Gain, g/d					
Enroll to 4 months	27.5 ± 4.7 (54)	27.8 ± 4.8 (63)	29.1 ± 4.9 (60)	29.1 ± 5.7 (67)	29.2 ± 7.1 (72)
Enroll to 12 months	17.7 ± 2.6 (48)	17.2 ± 2.6 (61)	17.8 ± 2.4 (59)	17.4 ± 2.6 (60)	18.0 ± 3.1 (68)
Length, cm					
Enrollment	50.6 ± 2.1	50.4 ± 2.2	50.7 ± 1.9	50.8 ± 2.1	51.1 ± 2.2
4 months	63.0 ± 2.3	62.6 ± 2.2	63.1 ± 1.9	62.9 ± 2.5	63.2 ± 2.1
12 months	75.4 ± 2.7	74.8 ± 2.5	75.6 ± 2.4	74.6 ± 2.8	75.1 ± 2.6
Length Gain, cm/wk					
Enrollment to 4 months	0.76 ± 0.09	0.74 ± 0.09	0.74 ± 0.11	0.74 ± 0.11	0.72 ± 0.10
Enrollment to 12 months	0.48 ± 0.04	0.48 ± 0.04	0.48 ± 0.05	0.46 ± 0.05	0.47 ± 0.05
Head Circumference, cm					
Enrollment	35.2 ± 1.2	35.0 ± 1.2	35.0 ± 1.2	35.3 ± 1.4	35.3 ± 1.4
4 months	41.8 ± 1.3	41.6 ± 1.3	42.1 ± 1.3	41.9 ± 1.3	41.9 ± 1.2
12 months	46.5 ± 1.5	46.4 ± 1.4	46.7 ± 1.5	46.6 ± 1.4	46.7 ± 1.3
Head Circumference Gain, cm/wk					
Enrollment to 4 months	0.40 ± 0.05	0.41 ± 0.05	0.41 ± 0.05	0.39 ± 0.07	0.40 ± 0.07
Enrollment to 12 months	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.03

* Values are mean ± SD (number of infants)

000300

Table VII-46
Growth: Males

Variable	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg- DTG	AA + DHA Fish/ Fungal	Control	AA + DHA Egg- DTG
Weight, kg					
Enrollment	3.55 ± 0.43 (37)*	3.48 ± 0.50 (39)	3.45 ± 0.43 (40)	3.52 ± 0.53 (42)	3.50 ± 0.41 (43)
4 months	6.73 ± 0.61 (26)	6.87 ± 0.63 (30)	7.08 ± 0.64 (32)	7.08 ± 0.82	7.05 ± 0.78 (38)
12 months	10.11 ± 1.13 (22)	9.92 ± 0.93 (29)	10.08 ± 0.94 (32)	10.07 ± 1.03	10.30 ± 1.02 (35)
Weight Gain, g/d					
Enrollment to 4 months	27.8 ± 4.2 (26)	29.1 ± 5.1 (30)	31.4 ± 4.6 (32)	31.3 ± 4.8 (35)	30.7 ± 6.5 (38)
Enrollment to 12 months	18.3 ± 2.8 (22)	17.9 ± 2.3 (29)	18.3 ± 2.5 (32)	18.3 ± 2.2 (32)	19.0 ± 2.9 (35)
Length, cm					
Enrollment	51.0 ± 2.7	51.0 ± 2.5	51.2 ± 2.0	51.3 ± 2.5	51.6 ± 2.7
4 months	63.7 ± 2.1	63.7 ± 1.9	63.9 ± 1.6	63.5 ± 2.6	63.9 ± 2.0
12 months	76.0 ± 2.5	75.7 ± 2.6	76.4 ± 2.5	75.3 ± 2.8	75.9 ± 2.5
Length Gain, cm/wk					
Enrollment to 4 months	0.77 ± 0.07	0.75 ± 0.09	0.78 ± 0.10	0.77 ± 0.08	0.74 ± 0.10
Enrollment to 12 months	0.48 ± 0.04	0.48 ± 0.04	0.49 ± 0.05	0.47 ± 0.04	0.47 ± 0.04
Head Circumference, cm					
Enrollment	35.6 ± 1.2	35.3 ± 1.1	35.5 ± 1.1	35.5 ± 1.6	35.6 ± 1.4
4 months	42.6 ± 1.2	42.1 ± 1.3	42.7 ± 1.2	42.3 ± 1.2	42.3 ± 1.2
12 months	47.4 ± 1.4	46.9 ± 1.4	47.3 ± 1.5	47.1 ± 1.3	47.3 ± 1.2
Head Circumference Gain, cm/wk					
Enrollment to 4 months	0.41 ± 0.04	0.41 ± 0.05	0.43 ± 0.05	0.41 ± 0.07	0.40 ± 0.06
Enrollment to 12 months	0.23 ± 0.02	0.23 ± 0.02	0.23 ± 0.03	0.23 ± 0.03	0.23 ± 0.02

* Values are mean ± SD (number of infants).

000301

Table VII-47 Growth: Females					
Variable	**Formula Groups**			**Breastfed Groups**	
	Control	**AA + DHA Egg- DTG**	**AA + DHA Fish/ Fungal**	**Control**	**AA + DHA Egg- DTG**
Weight, kg					
Enrollment	3.33 ± 0.44 (40)*	3.28 ± 0.45 (41)	3.30 ± 0.38 (42)	3.46 ± 0.41 (39)	3.50 ± 0.4 (40)
4 months	6.37 ± 0.66 (28)	6.25 ± 0.48 (33)	6.42 ± 0.69 (28)	6.50 ± 0.78 (32)	6.68 ± 0.89 (34)
12 months	9.49 ± 0.87 (26)	9.16 ± 1.05 (32)	9.54 ± 1.02 (27)	9.30 ± 1.06 (28)	9.63 ± 1.11 (33)
Weight Gain, g/d					
Enrollment to 4 months	27.1 ± 5.3 (28)	26.7 ± 4.2 (33)	26.5 ± 3.9 (28)	26.6 ± 5.6 (32)	27.5 ± 7.4 (34)
Enrollment to 12 months	17.2 ± 2.4 (26)	16.7 ± 2.8 (32)	17.2 ± 2.3 (27)	16.4 ± 2.7 (28)	17.0 ± 3.0 (33)
Length, cm					
Enrollment	50.8 ± 2.2	49.7 ± 2.5	50.1 ± 2.1	50.6 ± 2.3	50.5 ± 2.2
4 months	62.4 ± 2.3	61.6 ± 2.0	62.2 ± 1.9	62.2 ± 2.1	62.4 ± 2.1
12 months	74.9 ± 2.8	74.0 ± 2.1	74.7 ± 1.9	73.8 ± 2.5	74.3 ± 2.6
Length Gain, cm/wk					
Enrollment to 4 months	0.75 ± 0.10	0.73 ± 0.09	0.70 ± 0.10	0.70 ± 0.13	0.70 ± 0.09
Enrollment to 12 months	0.48 ± 0.05	0.48 ± 0.04	0.47 ± 0.04	0.45 ± 0.05	0.46 ± 0.05
Head Circumference, cm					
Enrollment	34.7 ± 1.0	34.7 ± 1.2	34.6 ± 1.1	35.2 ± 1.2	34.9 ± 1.4
4 months	41.1 ± 0.9	41.2 ± 1.2	41.3 ± 1.0	41.3 ± 1.1	41.5 ± 1.0
12 months	45.8 ± 1.0	45.9 ± 1.2	46.0 ± 1.1	46.1 ± 1.3	46.1 ± 1.1
Head Circumference Gain, cm/wk					
Enrollment to 4 months	0.40 ± 0.06	0.41 ± 0.05	0.39 ± 0.04	0.70 ± 0.13	0.70 ± 0.09
Enrollment to 12 months	0.22 ± 0.02	0.22 ± 0.03	0.22 ± 0.02	0.45 ± 0.05	0.46 ± 0.05

* Values are mean ± SD (number of infants)

000302

Table VII-48
Visual Development: Acuity Card Procedure

	Formula Groups			Breastfed Groups	
	Control	**AA + DHA Egg-DTG**	**AA + DHA Fungal/fish**	**Control**	**AA + DHA Egg-DTG**
2 months	1.97 ± 0.57 (54)	2.08 ± 0.61 (66)	2.04 ± 0.51 (63)	2.03 ± 0.53 (74)	2.07 ± 0.61 (73)
4 months	4.08 ± 0.45 (53)	4.17 ± 0.50 (63)	4.14 ± 0.50 (58)	4.17 ± 0.47 (66)	4.20 ± 0.50 (71)
6 months	7.57 ± 0.44 (50)	7.52 ± 0.43 (63)	7.62 ± 0.43 (57)	7.36 ± 0.42 (65)	7.26 ± 0.50 (70)
12 months	9.92 ± 0.35 (48)	9.32 ± 0.42 (59)	10.06 ± 0.38 (59)	9.45 ± 0.44 (61)	9.58 ± 0.42 (66)

Values are mean [cycles/degree] ± SD [octaves], (number of infants)

000303

Table VII-49
Information Processing: Fagan Test of Infant Intelligence

	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
No of infants, (n)					
6 months	34	43	36	47	48
9 months	32	42	37	44	45
% Novelty Preference					
6 months	59.0 ± 6.1	58.2 ± 6.5	59.7 ± 6.1	58.6 ± 6.0	59.5 ± 6.2
9 months	57.1 ± 5.3	58.1 ± 7.4	57.4 ± 5.9	57.7 ± 5.0	57.1 ± 6.6
6 + 9 months average	57.6	57.8	58.2	58.4	58.2
Fixation during familiarization, sec					
6 months	1.96 ± 0.68	2.04 ± 0.95	1.91 ± 0.62	2.15 ± 0.84	1.89 ± 0.65
9 months	1.51 ± 0.45	1.48 ± 0.46	1.46 ± 0.43	1.45 ± 0.33	1.59 ± 0.52
Fixation during testing phase, sec					
6 months	1.60 ± 0.58	1.62 ± 0.49	1.72 ± 0.61	1.87 ± 0.62	1.66 ± 0.64
9 months	1.17 ± 0.28	1.22 ± 0.32	1.05 ± 0.28	1.16 ± 0.27	1.22 ± 0.38
Ratio left/right looks					
6 months	2.61 (0.78 - 4.04) *	2.37 (1.81 - 4.00)	2.76 (1.71 - 4.22)	2.09 (0.91 - 4.47)	2.71 (1.54 - 4.76)
9 months	1.12 (0.55 - 2.57)	1.60 (0.69 - 2.50)	1.33 (0.50 - 2.58)	1.04 (0.68 - 1.76)	1.23 (0.69 - 2.01)

Values are mean ± SD unless otherwise noted.
* Median (Interquartile range)

000304

Table VII-50
General Development: Mental Development Index and Psychomotor Developmental Index Scores of the Bayley Scales of Infant Development

	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
Number of infants					
6 months	49	62	55	65	69
12 months	48	60	57	61	65
Mental Development Index*					
6 months	100.4 ± 5.0	99.5 ± 6.6	99.8 ± 5.6	101.1 ± 5.3	100.4 ± 5.4
12 months	97.8 ± 8.3	95.7 ± 10.0	97.8 ± 8.5	101.3 ± 7.8	98.6 ± 9.7
Psychomotor Developmental Index*					
6 months	99.1 ± 12.3	98.5 ± 11.1	97.1 ± 11.3	100.2 ± 10.2	100.2 ± 10.5
12 months	94.6 ± 12.5	96.0 ± 13.8	91.9 ± 12.7	96.1 ± 11.4	97.1 ± 12.9

Values are mean ± SD unless otherwise noted.
* Standard scores

000305

Table VII-51
Language Development: Vocabulary Comprehension and Production, MacArthur Communicative Developmental Inventories

	Raw Scores		Standard Scores				
			Formula Groups			Breastfed Groups	
	Reference Population Range*	Study Sample Range	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
No. of infants							
9 months	100	286	47	58	58	59	63
14 month		251	39	49	51	53	59
Vocabulary Comprehension	*no. of words*						
9 months	2 - 201	0 - 378	101 ± 17	103 ± 19	98 ± 17	99 ± 16	98 ± 21
14 months	31 - 343	17 - 396	100 ± 11	100 ± 13	103 ± 17	103 ± 15	101 ± 14
Vocabulary Production	*no. of words*						
14 months	0-193	0-134	99 ± 12[a, b]	96 ± 18[a]	104 ± 13[b]	98 ± 15	96 ± 19

Values are mean ± SD unless otherwise noted.
* From MacArthur's Communicative Developmental Inventories (1991).
Values with different superscripts within a feeding cohort (formula, breastfed) are significantly different ($p<0.05$ by ANOVA and ANCOVA).

000306

Table VII-52
Language Development: Phrases Understood and Early, Late, and Total Gestures, MacArthur Communicative Developmental Inventories

	Raw Scores		Standard Scores				
			Formula Groups			Breastfed Groups	
	Reference Population Range*	Study Sample Range	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
No. of infants							
9 months	100	286	47	58	58	59	63
14 months		251	39	49	51	53	59
Phrases Understood	*no. of phrases*						
9 months	0 - 26	0 - 25	97 ± 18	100 ± 21	98 ± 17	99 ± 13	99 ± 17
14 months	10 - 28	5 - 28	103 ± 13	105 ± 16	107 ± 17	110 ± 16	106 ± 17
Early Gestures	*no. of gestures*						
9 months	0 - 15	0 - 17	104 ± 16	100 ± 19	102 ± 15	102 ± 16	98 ± 16
14 months	6 - 18	7 - 18	111 ± 13	111 ± 15	111 ± 15	110 ± 16	106 ± 14
Later Gestures†	*no. of gestures*						
9 months	0 - 27	0 - 27	96 ± 15	97 ± 21	98 ± 15	99 ± 18	96 ± 18
14 months	8 - 42	5 - 43	105 ± 12	102 ± 15	103 ± 15	110 ± 13	103 ± 13
Total Gestures†	*no. of gestures*						
9 months	NA	0 - 39	100 ± 15	100 ± 18	100 ± 14	101 ± 14	97 ± 16
14 months	NA	13 - 60	105 ± 13	103 ± 14	105 ± 14	110 ± 14	102 ± 13

Values are mean ± SD unless otherwise noted.
* From MacArthur's Communicative Developmental Inventories (1991).
† By repeated measures ANOVA and ANCOVA, the breastfed-control and breastfed-egg-DTG groups are statistically significantly different ($p<0.05$).
NA = not available

0003307

Table VII-53
Temperament: Infant Behavior Questionnaire (Rothbart 1981)

	Formula Groups				Breastfed Groups		
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Overall *p*-values	Control	AA + DHA Egg-DTG	Overall *p*-values
Number of infants							
6 months	50	63	59		65	70	
12 months	46	59	56		61	68	
Activity Level[1]				0.76			0.18
6 months	4.39 ± 0.85	4.41 ± 0.72	4.28 ± 0.84		4.46 ± 0.94	4.24 ± 0.82	
12 months	4.40 ± 0.89	4.51 ± 0.88	4.56 ± 0.82		4.44 ± 0.94	4.30 ± 0.81	
Distress to Novel Stimuli[2]				0.46			0.20
6 months	2.55 ± 0.85	2.60 ± 0.81	2.57 ± 0.85		2.52 ± 0.76	2.40 ± 0.78	
12 months	2.84 ± 0.72	3.09 ± 0.78	2.88 ± 0.73		3.04 ± 0.79	2.87 ± 0.69	
Soothability[3]				0.50			0.98
6 months	5.50 ± 0.91	5.41 ± 0.84	5.20 ± 0.81		5.23 ± 0.81	5.29 ± 0.64	
12 months	5.27 ± 0.90	5.15 ± 0.78	5.25 ± 0.90		5.34 ± 0.86	5.24 ± 0.88	
Distress at Limitations[4]				0.56			0.25
6 months	2.89 ± 0.73	3.13 ± 0.74	3.05 ± 0.73		3.21 ± 0.86	3.18 ± 0.76	
12 months	3.49 ± 0.77	3.50 ± 0.72	3.58 ± 0.78		3.71 ± 0.87	3.49 ± 0.74	
Smiling and Laughter †				0.05			0.92
6 months	5.58 ± 0.73	5.39 ± 0.79	5.40 ± 0.88		5.45 ± 0.78	5.39 ± 0.79	
12 months	5.66 ± 0.60	5.29 ± 0.71	5.44 ± 0.79		5.35 ± 0.73	5.37 ± 0.73	
Duration of Orienting[5]				0.70			0.54
6 months	4.29 ± 1.22	4.13 ± 1.17	4.32 ± 1.00		4.13 ± 1.08	4.23 ± 1.01	
12 months	3.70 ± 1.03	3.58 ± 1.06	3.62 ± 1.14		3.45 ± 1.02	3.59 ± 0.87	

Values are mean ± SD unless otherwise noted; a 7-point scale was used; 1 = never, 2 = very rarely, 3 = less than half the time, 4 = about half the time, 5 = more than half the time, 6 = almost always, 7 = always.

[1] Gross motor and locomotor activity

[2] Distress and extended latency to approach intense or novel stimuli

[3] Reduction in fussiness when soothing techniques are used

[4] Crying or fussiness while waiting for food or when prevented access to a toy

[5] Vocalizing, playing with, or looking at an object for extended time period

† Repeated measures analysis of variance (ANOVA, ANCOVA) found no differences among groups within this cohort, with one exception: Bonferroni adjusted pairwise comparisons found a significant difference between the control and Egg-DTG formula groups, p=0.05.

000308

Table VII-54
Temperament: Behavior Rating Scale Scores of The Bayley Scales of Infant Development

	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
Number of infants					
6 months	49	62	55	65	69
12 months	48	60	57	61	65
Behavior Rating Scale of infants[†], n (%)					
6 months					
Questionable	6 (12)	10 (16)	12 (22)	10 (15)	10 (14)
Nonoptimal	2 (4)	2 (3)	3 (5)	2 (3)	2 (3)
12 months					
Questionable	7 (15)	10 (17)	10 (18)	10 (16)	11 (17)
Nonoptimal	3 (6)	3 (5)	4 (7)	1 (2)	2 (3)

[†] Number (%) of infants with scores ≤ 10th percentile (nonoptimal) or 11th - 25th percentile (questionable) in comparison to the reference population described in the manual.

000309

Table VII-55
Complete Blood Count: Hemoglobin, MCH, MCHC, MCV

	Reference Ranges* 2 mos - 2 yrs	Formula Groups			Breastfed Groups	
		Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
Number of Infants						
4 months		22	25	25	30	27
12 months		21	27	32	36	34
Hemoglobin, g/dL †	9.4 - 14.1					
4 months		12.0 (10.5 - 13.2)	11.8 (10.3 - 13.1)	12.4 (10.1 - 14.0)	11.9 (6.2 - 34.8) ‡	11.6 (10.3 - 13.4)
12 months		12.1 (10.2 - 13.6)	12.0 (10.6 -13.7)	12.2 (10.9 - 14.5)	11.9 (10.6 - 13.4)	11.7 (10.4 - 13.3)
MCH§**, pg/cell**	24 - 34					
4 months		29.2 (27.0 - 30.5)	29.2 (26.6 - 30.7)	28.9 (26.3 - 30.6)	27.9 (25.4 - 30.6)	27.6 (23.6 - 30.6)
12 months		27.8 (26.1 - 29.5)	27.4 (24.7 - 32.7)	27.4 (24.4 - 29.9)	26.8 (24.2 - 29.4)	26.5 (23.6 - 30.5)
MCHC¶**, g Hb/dL Ercs**	28 - 37					
4 months		34.6 (33.2 - 35.9)	34.7 (32.9 - 36.1)	34.7 (32.9- 35.9)	34.7 (32.6 - 36.1)	34.4 (32.6 - 36.0)
12 months		33.7 (32.9 - 34.6)	33.7 (32.5 - 35.7)	33.6 (32.6 - 35.1)	33.8 (32.3 - 35.7)	33.8 (31.9 - 35.7)
MCV**, **fL**	68 - 106					
4 months		84.3 (78.8 - 88.7)	83.3 (79.1 - 87.2)	82.9 (76.0 - 86.4)	80.8 (74.5 - 86.1)	80.1 (69.9 - 87.6)
12 months		82.7 (77.4 - 87.7)	80.9 (73.8 - 91.8)	82.0 (71.9 - 88.2)	80.4 (71.6 - 87.0)	79.4 (69.9 - 88.2)

Values are median (range)
* From: Tietz 1990.
† The egg-DTG and fungal/fish formula groups are statistically significantly different by repeated measures of ANOVA, $p<0.05$.
‡ One value (34.8) appears to be a data entry error, most likely this value represents hematocrit. The median (range) without this value is 11.8 (6.2 - 13.3)
§ Mean cell hemoglobin
¶ Mean cell hemoglobin concentration
** Mean cell volume

000310

Table VII-56
Plasma Vitamins A and E and Antioxidant Status

	Reference Group*	Formula Groups			Breastfed Groups	
		Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
Vitamin E (α-tocopherol), mg/L †						
4 months	3 - 9	13.0 ± 3.5 (33)	14.0 ± 3.0 (31)	13.7 ± 2.5 (29)	11.0 ± 3.3 (41)	12.0 ± 3.8 (36)
12 months	(1 - 12 years)	11.0 ± 3.1 (23)	13.2 ± 2.4 (27)	12.0 ± 2.5 (32)	11.2 ± 2.9 (39)	11.6 ± 3.8 (34)
Vitamin A (retinol), μg/L †						
4 months	200 - 430	286 ± 69 (33)	309 ± 77 (31)	284 ± 46 (29)	253 ± 58 (41)	272 ± 51 (36)
12 months	(1 - 6 years)	352 ± 48 (23)	374 ± 81 (27)	381 ± 64 (32)	350 ± 72 (39)	337 ± 69 (34)
Total Radical Trapping Capacity (TRAP, Antioxidant Status) ‡						
4 months	NA	952 ± 172 (33)	965 ± 107 (30)	994 ± 132 (26)	878 ± 126 (40)	925 ± 146 (35)
12 months		1022 ± 151 (17)	1006 ± 163 (18)	1117 ± 139 (21)	1011 ± 185 (28)	1022 ± 136 (22)

Values are mean ± SD (number of samples) unless otherwise noted.
* From: Tietz, 1990; Vitamin E, p 588; Vitamin A, p 578.
† The control formula and egg-DTG formula groups are statistically significantly different by repeated measures of ANOVA, $p<0.05$.
‡ Values reported as zero were considered missing.
NA = not available

000311

Table VII-57
Arachidonic Acid (20:4n-6) and Docosahexaenoic Acid (22:6n-3) Concentration (µg/dL) in Plasma Triglycerides (TG), Phospholipids (PL) and Cholesterol Esters (CE) at 4 Months of Age

	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/Fish	Control	AA + DHA Egg-DTG
Number of Subjects	33	29	24	41	34
Arachidonic acid, µg/dL					
TG Range	6.5 ± 4.9[a] [2.3 - 29.6]	15.4 ± 8.3[b] [3.6 - 46.8]	14.8 ± 4.9[b] [7.5 - 26.5]	13.6 ± 6.8 [4.1 - 38.3]	16.9 ± 9.2 [4.5 - 38.3]
PL Range	84.8 ± 16[a] [42.8 - 112.2]	138.5 ± 34.7[b] [66.3 - 206.9]	143.7 ± 20.3[b] [106.0 - 187.3]	130.6 ± 32.4 [77.0 - 217.5]	144.5 ± 33.0 [64.4 - 238.0]
CE Range	24.2 ± 5.9[a] [12.8 - 38.6)	47.2 ± 16.0[b] [17.9 - 86.1]	47.9 ± 8.9[b] [31.8 - 64.3]	51.0 ± 20.5 [17.4 - 101.5]	56.2 ± 14.9 [31.1 - 91.6]
Docosahexaenoic acid, µg/dL					
TG Range	1.3 ± 1.6[a] [0.0 - 8.8]	4.0 ± 1.9[b] [0.6 - 8.9]	4.1 ± 1.7[b] [1.8 - 9.0]	3.3 ± 2.3 [0.4 - 14.5]	4.0 ± 2.8 [1.0 - 11.9]
PL Range	20.1 ± 5.1[a] [8.5 - 31.4)	37.8 ± 8.6[b] [17.1 - 51.7]	41.3 ± 7.5[b] [27.8 - 55.2]	37.5 ± 12.2 [16.4 - 70.1]	38.6 ± 10.8 [15.0 - 68.9]
CE Range	2.8 ± 2.5[a] [0.4 - 12.0]	3.7 ± 1.2[b] [1.5 - 5.8]	4.3 ± 1.1[a,b] [2.7 - 6.1]	4.6 ± 2.8 [1.5 - 18.6]	4.7 ± 2.4 [2.2 - 14.8]
Hrs after last feeding, median (range)	2.0 (0.3 - 4.0)	1.5 (0.3 - 4.8)	2.0 (0.3 - 4.0)	1.5 (0.3 - 4.5)	2 (0.3 - 3.0)

Values are mean ± SD unless otherwise noted. Within each feeding cohort, values in the same line with different superscripts are statistically significantly different, $p<0.05$).

000312

Table VII-58
Plasma Phospholipid Fatty Acid Levels (g/100 g total fatty acids)

	Formula Groups						Breastfed Groups			
	Control		AA + DHA Egg-DTG		AA + DHA Fungal/fish		Control		AA + DHA Egg-DTG	
	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos
Number of Subjects	33	23	24	28	29	24	41	38	34	32
Omega-6 FA										
18:2n-6[a, b,d]	30.2 ± 1.9	29.0 ± 3.3	25.5 ± 1.5	26.1 ± 1.6	25.0 ± 1.5	25.2 ± 1.9	24.8 ± 3.9	27.5 ± 3.0	24.1 ± 2.2	26.0 ± 1.9
20:4n-6 (AA)[a, b,d]	7.9 ± 1.3	8.5 ± 1.7	12.3 ± 1.1	11.8 ± 1.3	13.0 ± 1.3	12.2 ± 1.5	12.2 ± 2.5	10.0 ± 1.9	12.8 ± 1.5	11.4 ± 1.6
Total n-6[b]	41.4 ± 0.9	41.5 ± 1.5	40.8 ± 1.2	41.4 ± 0.8	40.8 ± 0.7	40.8 ± 1.1	41.0 ± 1.6	41.5 ± 1.8	41.1 ± 1.1	41.1 ± 1.2
Omega-3 FA										
20:5n-3 (EPA)[a, b,c]	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
22:6n-3 (DHA)[a, b, c, d]	1.8 ± 0.4	1.7 ± 0.5	3.4 ± 0.4	3.5 ± 0.6	3.7 ± 0.5	3.7 ± 0.5	3.5 ± 1.0	2.4 ± 1.1	3.4 ± 0.6	3.2 ± 0.6
Total n-3[a, b, c]	2.8 ± 0.5	2.9 ± 0.6	4.0 ± 0.5	4.3 ± 0.6	4.6 ± 0.5	4.8 ± 0.6	4.7 ± 1.2	3.6 ± 1.2	4.6 ± 0.8	4.3 ± 0.5
Saturated FA	41.1 ± 1.0	40.8 ± 1.2	41.9 ± 1.7	41.0 ± 1.1	41.4 ± 0.7	41.2 ± 1.1	42.1 ± 1.2	40.5 ± 1.4	42.1 ± 1.1	40.9 ± 1.2
Monounsat FA[a, b]	14.7 ± 1.1	14.5 ± 1.1	13.3 ± 0.8	13.2 ± 0.9	13.3 ± 0.9	13.0 ± 1.2	12.2 ± 1.5	14.0 ± 1.5	12.3 ± 1.2	13.5 ± 1.4

Values are mean ± SD.
Statistically significantly different among formula groups ($p < 0.01$) by repeated measures analysis
[a] $p<0.005$, control vs egg-DTG (AA + DHA)
[b] $p<0.005$, control vs fungal/fish (AA + DHA)
[c] $p<0.005$, fish fungal (AA + DHA) vs egg-DTG
Statistically significantly different between control and AA + DHA (egg-DTG) formula feedings in breastfed groups
[d] $p<0.001$, control vs egg-DTG (AA + DHA)

000313

Table VII-59
RBC Phosphatidylethanolamine Fatty Acid Levels (g/100 g total fatty acids)

	Formula Groups					Breastfed Groups				
	Control		AA + DHA Egg-DTG		AA + DHA Fungal/fish		Control		AA + DHA Egg-DTG	
	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos
Number of Subjects	32	22	33	26	30	32	40	38	41	31
Omega-6 FA										
18:2n-6[a, b,d]	9.5 ± 1.4	9.9 ± 1.3	6.9 ± 0.8	7.3 ± 0.8	6.5 ± 1.3	7.0 ± 0.8	6.7 ± 1.9	8.4 ± 1.7	6.2 ± 1.0	7.3 ± 1.0
20:4n-6 (AA)[a,b]	23.3 ± 2.1	23.2 ± 1.9	25.4 ± 1.8	25.0 ± 2.9	25.9 ± 2.1	25.7 ± 2.4	26.7 ± 3.3	25.1 ± 2.6	27.5 ± 2.3	25.1 ± 2.7
Total n-6[a, b,d]	45.6 ± 2.5	45.4 ± 1.9	45.0 ± 2.1	44.3 ± 4.2	44.1 ± 2.8	43.7 ± 3.1	45.8 ± 4.4	46.0 ± 2.6	46.7 ± 2.6	44.3 ± 3.5
Omega-3 FA										
20:5n-3 (EPA)[a,b,c]	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
22:6n-3 (DHA)[a,b]	4.3 ± 0.7	3.2 ± 0.6	6.5 ± 1.1	6.3 ± 1.2	7.0 ± 0.8	6.6 ± 0.9	6.9 ± 1.6	4.3 ± 1.4	6.7 ± 1.2	5.6 ± 1.2
Total n-3[a,b,c]	7.6 ± 0.9	6.8 ± 0.8	8.7 ± 1.3	8.6 ± 1.6	9.8 ± 1.0	9.5 ± 1.1	10.8 ± 2.1	8.0 ± 1.7	10.6 ± 1.7	8.4 ± 1.4
Saturated FA	23.5 ± 2.3	23.7 ± 1.8	23.4 ± 2.1	24.0 ± 3.7	23.5 ± 2.0	23.6 ± 2.2	23.4 ± 3.3	23.0 ± 2.2	23.0 ± 2.5	24.7 ± 3.4
Monounsat FA	23.3 ± 3.5	24.2 ± 1.3	22.8 ± 1.5	23.1 ± 2.2	22.6 ± 1.8	23.2 ± 2.9	20.4 ± 2.8	23.1 ± 2.1	19.7 ± 1.8	22.6 ± 2.0

Values are mean ± SD.
Statistically significantly different among formula groups ($p < 0.01$) by repeated measures analysis
[a] $p<0.005$, control vs egg-DTG (AA + DHA)
[b] $p<0.005$, control vs fungal/fish (AA + DHA)
[c] $p<0.005$, fish fungal (AA + DHA) vs egg-DTG
Statistically significantly different between control and AA + DHA (egg-DTG) formula feedings in breastfed groups
[d] $p<0.001$, control vs egg-DTG (AA + DHA)

000314

Table VII-60
RBC Phosphatidylcholine Fatty Acid Levels (g/100 g total fatty acids)

	Formula Groups						Breastfed Groups			
	Control		AA + DHA Egg-DTG		AA + DHA Fungal/fish		Control		AA + DHA Egg-DTG	
	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos
Number of Subjects	32	22	30	32	33	26	40	38	41	31
Omega-6 FA										
18:2n-6[a, b,d]	25.3 ± 1.2	25.4 ± 2.2	21.4 ± 1.4	22.6 ± 2.6	21.4 ± 1.6	21.9 ± 1.7	21.9 ± 2.8	23.6 ± 2.3	20.8 ± 2.3	22.3 ± 1.5
20:4n-6 (AA)[a, b]	5.0 ± 0.9	5.1 ± 1.3	7.8 ± 1.0	7.1 ± 1.4	8.0 ± 1.0	7.6 ± 1.9	7.8 ± 1.9	6.2 ± 1.5	8.3 ± 1.3	6.7 ± 1.6
Total n-6[a, b]	33.0 ± 0.8	33.6 ± 1.6	31.7 ± 2.2	32.4 ± 3.6	31.8 ± 1.6	32.1 ± 2.6	32.9 ± 2.7	32.8 ± 1.9	32.3 ± 2.6	31.7 ± 1.9
Omega-3 FA										
20:5n-3 (EPA)[a, b,c]	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:6n-3 (DHA)[a, b, d]	0.9 ± 0.2	0.8 ± 0.3	1.8 ± 0.4	1.7 ± 0.5	1.9 ± 0.3	1.9 ± 0.5	1.8 ± 0.7	1.1 ± 0.5	1.7 ± 0.4	1.5 ± 0.5
Total n-3[a, b, c]	1.5 ± 0.3	1.5 ± 0.4	2.2 ± 0.5	2.2 ± 0.6	2.4 ± 0.4	2.5 ± 0.7	2.5 ± 0.8	1.8 ± 0.6	2.5 ± 0.6	2.1 ± 0.6
Saturated FA	45.8 ± 1.0	45.0 ± 2.1	46.2 ± 1.6	45.0 ± 2.5	46.2 ± 1.3	45.1 ± 2.9	45.8 ± 2.5	44.5 ± 2.1	45.7 ± 2.4	45.9 ± 1.8
Monounsat FA	19.6 ± 1.2	19.9 ± 1.3	19.8 ± 1.5	20.4 ± 2.3	19.6 ± 1.1	20.3 ± 1.2	18.8 ± 3.0	20.8 ± 1.6	19.5 ± 1.4	20.4 ± 1.4

Values are mean ± SD.
Statistically significantly different among formula groups ($p < 0.01$) by repeated measures analysis
[a] $p<0.005$, control vs egg-DTG (AA + DHA)
[b] $p<0.005$, control vs fungal/fish (AA + DHA)
[c] $p<0.005$, fish fungal (AA + DHA) vs egg-DTG
Statistically significantly different between control and AA + DHA (egg-DTG) formula feedings in breastfed groups
[d] $p<0.001$, control vs egg-DTG (AA + DHA)

000315

Table VII-61
Plasma Triglyceride Fatty Acid Levels (g/100 g total fatty acids)

	Formula Groups						Breastfed Groups			
	Control		AA + DHA Egg-DTG		AA + DHA Fungal/fish		Control		AA + DHA Egg-DTG	
	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos
Number of Subjects	33	23	24	28	28	24	41	37	34	30
Omega-6 FA										
18:2n-6[a, b,d]	25.1 ± 1.2	22.8 ± 3.7	25.7 ± 2.3	23.8 ± 1.4	25.6 ± 1.3	23.2 ± 3.3	20.8 ± 5.3	22.0 ± 3.3	20.6 ± 4.2	21.9 ± 4.1
20:4n-6 (AA)[a, b,d]	0.6 ± 0.2	1.0 ± 0.4	1.2 ± 0.3	1.3 ± 0.4	1.3 ± 0-.3	1.3 ± 0.4	1.2 ± 0.5	1.1 ± 0.3	1.2 ± 0.3	1.2 ± 0.4
Total n-6[b]	26.6 ± 1.2	25.1 ± 3.6	28.1 ± 2.4	26.4 ± 1.5	27.9 ± 1.6	25.8 ± 3.6	23.3 ± 5.2	24.4 ± 3.4	23.0 ± 4.3	24.4 ± 4.2
Omega-3 FA										
20:5n-3 (EPA)[a, b,c]	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:6n-3 (DHA)[a, b, c]	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.4 ± 0.2
Total n-3[a, b, c]	2.2 ± 0.3	1.9 ± 0.5	2.4 ± 0.2	2.1 ± 0.3	2.7 ± 0.2	2.4 ± 0.5	2.0 ± 0.8	1.8 ± 0.4	1.9 ± 0.5	2.0 ± 0.5
Saturated FA	23.7 ± 2.3	26.7 ± 3.9	22.6 ± 2.0	25.4 ± 1.8	22.4 ± 2.0	27.3 ± 4.2	32.2 ± 4.0	28.0 ± 3.4	30.5 ± 6.0	27.9 ± 5.7
Monounsat FA[a, b]	47.5 ± 2.1	45.3 ± 3.3	46.9 ± 1.9	45.2 ± 1.5	47.1 ± 1.3	43.7 ± 3.0	42.6 ± 5.0	44.3 ± 2.7	44.7 ± 2.9	44.3 ± 3.7

Values are mean ± SD.
Statistically significantly different among formula groups ($p < 0.01$) by repeated measures analysis
[a] $p<0.005$, control vs egg-DTG (AA + DHA)
[b] $p<0.005$, control vs fungal/fish (AA + DHA)
[c] $p<0.005$, fish fungal (AA + DHA) vs egg-DTG
Statistically significantly different between control and AA + DHA (egg-DTG) formula feedings in breastfed groups
[d] $p<0.001$, control vs egg-DTG (AA + DHA)

000316

Table VII-62
Cholesterol Ester Fatty Acid Levels (g/100 g total fatty acids) in Plasma

	Formula Groups						Breastfed Groups			
	Control		AA + DHA Egg-DTG		AA + DHA Fungal/fish		Control		AA + DHA Egg-DTG	
	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos	4 mos	12 mos
Number of Subjects	33	21	24	27	29	24	41	36	34	29
Omega-6 FA										
18:2n-6[a, b,d]	50.9 ± 3.0	55.7 ± 3.4	48.6 ± 3.1	54.6 ± 2.1	48.6 ± 2.3	52.6 ± 2.5	48.2 ± 4.1	54.0 ± 4.7	47.5 ± 3.1	53.4 ± 2.4
20:4n-6 (AA)[a, b,d]	4.0 ± 0.9	5.0 ± 1.4	7.3 ± 1.1	7.7 ± 1.0	7.5 ± 1.1	8.3 ± 1.2	7.3 ± 2.1	6.3 ± 1.7	7.8 ± 1.2	7.4 ± 1.4
Total n-6[b]	56.2 ± 3.0	62.1 ± 2.5	57.1 ± 3.5	63.4 ± 2.2	57.3 ± 2.8	62.2 ± 2.5	56.8 ± 3.6	61.5 ± 4.7	56.7 ± 3.2	62.1 ± 2.3
Omega-3 FA										
20:5n-3 (EPA)[a, b,c]	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
22:6n-3 (DHA)[a, b, c,]	0.5 ± 0.4	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.3	0.4 ± 0.2	0.6 ± 0.2	0.6 ± 0.1
Total n-3[a, b, c]	1.4 ± 0.4	1.1 ± 0.2	1.4 ± 0.3	1.3 ± 0.2	1.6 ± 0.3	1.6 ± 0.2	1.4 ± 0.4	1.1 ± 0.2	1.5 ± 0.4	1.3 ± 0.2
Saturated FA	14.6 ± 1.6	13.7 ± 1.4	15.1 ± 1.9	13.9 ± 1.9	14.9 ± 1.0	14.4 ± 1.8	18.1 ± 2.4	14.4 ± 2.0	17.6 ± 2.0	14.6 ± 1.9
Monounsat FA[a, b]	27.8 ± 2.6	22.1 ± 1.8	26.5 ± 3.3	20.5 ± 1.7	26.2 ± 2.7	20.9 ± 1.5	23.8 ± 3.3	21.5 ± 3.3	24.3 ± 3.1	20.7 ± 1.8

Values are mean ± SD.
Statistically significantly different among formula groups ($p < 0.01$) by repeated measures analysis
[a] $p<0.005$, control vs egg-DTG (AA + DHA)
[b] $p<0.005$, control vs fungal/fish (AA + DHA)
[c] $p<0.005$, fish fungal (AA + DHA) vs egg-DTG
Statistically significantly different between control and AA + DHA (egg-DTG) formula feedings in breastfed groups
[d] $p<0.001$, control vs egg-DTG (AA + DHA)

000317

Table VII-63
Feeding Frequency, Caloric Intake from Formula and Frequency of Spitting-Up/Vomiting in Infants from the Formula Groups

	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish
Number of Feedings per Day, median (range)			
1 mos (19 d)*	7.4 (4.0 - 13.5)	7.5 (5.3 - 11.5)	7.2 (5.5 - 11.4)
2 mos (3 d)	6.0 (4.0 - 8.7)	6.3 (4.0 - 9.7)	6.7 (4.7 - 11.7)
4 mos (3 d)	5.5 (3.7 - 9.0)	5.7 (3.7 - 9.3)	5.8 (3.5 - 9.0)
6 mos (3 d)	5.0 (2.0 - 8.0)	5.0 (3.3 - 8.0)	5.0 (3.7 - 10.0)
9 mos (3 d)	4.8 (2.0 - 7.0)	5.0 (3.0 - 7.7)	5.0 (2.0 - 9.3)
12 mos (3 d)	4.0 (2.0 - 6.3)	4.7 (1.7 - 7.3)	4.0 (1.0 - 9.0)
Formula Intake, kcal/day †			
1 mos (19 d)	451± 79	456 ± 84	449 ± 81
2 mos (3 d)	574 ± 105	562 ± 91	570 ± 101
4 mos (3 d)	636 ± 113	639 ± 104	635 ± 109
6 mos (3 d)	598 ± 121	604 ± 158	584 ± 119
9 mos (3 d)	551 ± 132	583 ± 166	532 ± 124
12 mos (3 d)	520 ± 167	514 ± 156	469 ± 164
Percent of Feedings with Spit-Up and/or Vomiting ‡			
1 mos (19 d)	9 [3 - 17]	9 [4 -21]	12 [5 - 19]
2 mos (3 d)	9 [0 - 25]	6 [0 - 23]	9 [0 - 33]
4 mos (3 d)	6 [0 - 33]	13 [0 - 35]	11 [0 - 36]
6 mos (3 d)	0 [0 - 7]	6 [0 - 29]	0 [0 - 8]
9 mos (3 d)	0 [0 - 6]	0 [0 - 7]	0 [0 - 0]
12 mos (3 d)	0 [0 - 0]	0 [0 - 0]	0 [0 - 0]

* Month of study visit (number of days that intake and tolerance records were maintained by parent[s])
† Values are mean ± SD
‡ Values are median [interquartile range]

000318

Table VII-64 Mean Rank Stool Consistency					
Study Visit (Observation Period)	**Formula Groups**			**Breastfed Groups**	
	Control	**AA + DHA Egg-DTG**	**AA + DHA Fungal/fish**	**Control**	**AA + DHA Egg-DTG**
Mean Rank Stool Consistency					
1 month (9 - 27 days)*	2.06 ± 0.54†	2.04 ± 0.49	2.10 ± 0.51	1.99 ± 0.57	1.98 ± 0.48
2 months (56 - 60 days)	2.03 ± 0.61	2.11 ± 0.74	2.05 ± 0.59	1.88 ± 0.56	1.89 ± 0.56
4 months (117 - 120 days)	2.21 ± 0.58	2.31 ± 0.78	2.26 ± 0.77	2.00 ± 0.54	2.05 ± 0.58

Values are mean ± SD.
* Infants ages (days) during which stool records were maintained by parents (observation period).
† Parents rated the consistency of each stool using a 5-point scale (1 = watery, 2 = loose/mushy, 3 = soft, 4 = formed, 5 = hard); Values represent the average daily consistency for all stools in the observation period.

000319

Table VII-65
Physician Diagnosed Otitis Media and Prescriptions for Antibiotics*

	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
Number of Infants	77	80	82	82	83
Otitis Media					
No (%) of infants	31 (40)	44 (55)	41 (50)	41 (50)	51 (61)
Episodes/100 d, median [IQR]	0.0 [0 - 0.3]	0.3 [0 - 0.6]	0.1 [0 - 0.6]	0.3 [0 - 0.8]	0.3 [0 - 0.6]
≥ 2 episodes, no (%) of infants	18 (23)	28 (35)	28 (34)	28 (34)	31 (37)
Antibiotic Prescriptions					
No (%) of infants	35 (45)	49 (61)	47 (57)	46 (56)	57 (69)
Prescriptions/100 d, median [IQR]	0.3 [0 - 0.6]	0.3 [0 - 1.1]	0.3 [0 - 1.1]	0.3 [0 - 1.1]	0.3 [0 - 1.1]
≥ 2 episodes, no (%) of infants	21 (27)	32 (40)	30 (37)	32 (39)	35 (42)

* No statistically significant differences

000320

Table VII-66
Diarrhea and Use of Electrolyte Replacement Solutions*

	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
Number of Infants	77	80	82	82	83
Parent Reports					
Diarrhea, no (%) of infants	35 (45)	43 (54)	50 (61)	44 (54)	46 (55)
Electrolyte replacement solutions, no (%) of infants	23 (30)	34 (43)	23 (28)	22 (27)	27 (33)
Physician Reports					
Diarrhea, no (%) of infants	14 (18)	19 (24)	13 (16)	14 (17)	22 (27)

* No statistically significant differences

000321

Table VII-67
Physician Diagnosed Oral Thrush and Candida (diaper rash) and Prescriptions for Antifungal*

	Formula Groups			Breastfed Groups	
	Control	AA + DHA Egg-DTG	AA + DHA Fungal/fish	Control	AA + DHA Egg-DTG
Number of Infants	77	80	82	82	83
Thrush, no (%)	2 (3)	8 (10)	5 (6)	6 (7)	15 (18)
Candida, no (%)	6 (8)	5 (6)	12 (15)	9 (11)	10 (12)
Antifungal, no (%)	10 (13)	9 (11)	15 (18)	13 (16)	21 (25)

* The data are shown for descriptive purposes only.

000322

D. Infant Mortality Including Reports of Sudden Infant Death Syndrome (SIDS) in Clinical Studies with Formulas Containing Long-Chain Polyunsaturated Fatty Acids

A full report of the analysis of infant mortality, including Sudden Infant Death Syndrome (SIDS) cases in clinical studies of infants fed formula supplemented with the long-chain polyunsaturated fatty acids (LCP) docosahexaenoic acid (DHA) and arachidonic acid (AA) is found in Attachment 2. This section provides a summary of the analysis and conclusions reached by an expert panel convened to evaluate this issue. In particular, the review focuses on studies of preterm infants conducted by Ross Products Division, Abbott Laboratories (Ross). Other published studies and studies in term infants are also included.

This evaluation is a follow-up to an investigation that was initiated in late 1997 regarding the potential health and safety implications of the observation of deaths, initially reported as SIDS, during the conduct of three premature infant feeding studies (AG38 [O'Connor et al 2001], Carlson et al 1998 & unpublished results; Ryan et al 1999), two of which had or were nearly completed (Carlson et al 1998; Ryan et al 1999). At that time, an advisory panel of experts in the areas of SIDS, neonatology, nutrition, lipids, toxicology, and epidemiology was convened to review the available information on deaths reported as SIDS in Ross-sponsored LCP fatty acid studies with preterm infants. The critical question before the group was whether the continuation of AG38 presented any safety risks.

The panel members were unanimous in recommending continuation of the AG38 (O'Connor et al 2001) study and made several procedural recommendations for obtaining additional information about the reported deaths and for surveillance and monitoring during the conduct of this study. The panel also reviewed subsequent quarterly reports through the completion of the study. No safety concerns emerged following the panel reviews of the quarterly safety assessments. The FDA was informed of the initial observations in late 1997 and was updated throughout the course of the O'Connor et al (2001) study.

Clinical studies have been conducted by Ross and others using LCP from various sources, including high-EPA fish oil, high-DHA fish oil, egg lipid, egg phospholipid (egg lecithin), egg-derived triglycerides (egg-DTG), and microbial sources (DHASCO, ARASCO, SUNTGA25).

The current document provides an updated evaluation of the issue of mortality and SIDS in Ross-sponsored LCP fatty acid studies. This document also extends the analysis to other published studies of LCP-supplemented infant formula. Based on all of the information evaluated, there is no persuasive evidence that any specific source of LCP plays a causative role in the occurrence of SIDS, or other cause of infant mortality.

In studies of formulas supplemented with high-DHA tuna oil and a source of AA (native TG sources), no adverse effects on growth or development were reported, and there was no indication that feeding these formulas was causally related to any adverse health effects (Ross AG38 [O'Connor et al 2001]; AF92 [Auestad et al 2001]). Similarly, non-Ross studies with other native TG sources of DHA and AA have found no evidence to suggest adverse health effects related to feeding these formulas.

The inappropriateness of a formula supplemented with DHA, without a source of added AA, for preterm infants is suggested by reports of poor growth in several studies that used such a formula (Ryan et al. 1999; Carlson et al. 1992, 1993 and Carlson et al. 1996), and by higher mortality in preterm infants fed a formula supplemented with DHA only (Ryan et al. 1999). Slower growth and increased mortality have not been reported in preterm infants fed formulas with balanced supplementation with both DHA and AA (O'Connor et al. 2001; Vanderhoof et al 1999, 2000).

The available information from a single study with an egg PL source of AA and DHA leaves open a possible increase in adverse effects, but is inconclusive. The relatively small sample sizes of the Ryan et al (1999) (DHA alone) and Carlson et al 1998 (egg PL) studies, and the substantial differences in design and conduct between these studies and the larger studies with AA and DHA from native TG sources, limit a more definitive interpretation of these earlier data.

After thorough evaluation of the large, randomized, controlled trial of premature infants (O'Connor et al 2001), the large, randomized controlled trial of term infants (Auestad et al 2001), and assessment of infant mortality (including deaths reported as SIDS) in previous Ross-sponsored studies, as well as published studies conducted by others, the Ross Medical Department and the external advisory panel concur that addition of AA and DHA from the native TG sources as studied in the 1-year feeding trials (O'Connor et al 2001 and Auestad et al 2001) to formulas for preterm or full-term infants is not associated with an increased risk of SIDS or infant mortality.

E. Summary of Published Literature on Clinical Trials of Infant Formulas Supplemented with AA and DHA

Several studies (Vanderhoof et al., 1999, 2000; Foreman-Van Drogelen et al., 1996; Clandinin et al., 1997, 1999; Faldella et al., 1996; Carlson et al., 1998, Koletzko et al., 1989, 1995a) in preterm infants have shown no short term (21 days – 4 months PCA) or long term (up to 92 weeks PCA, following feeding through 48 weeks PCA) adverse effects related to AA and DHA supplementation in preterm infant formula (Table VII-68, Attachment 4). The AA and DHA were obtained from single-cell oils, which have been determined to be Generally Recognized As Safe substances (Letter from FDA in response to GRAS Notice No, GRN 000041), or egg lipids or egg phospholipids. The amount of AA and DHA used in the supplemented infant formulas ranged from 0.20 to 1.1% and 0.10 to 0.75%, respectively. The duration of AA and DHA supplementation ranged from 21 days to 52 weeks PCA. A variety of growth, developmental and monitoring assessments were conducted during or following the period of supplementation in each study. Vanderhoof et al., (1999) observed a significant increase in serum cholesterol levels in infants supplemented with AA and DHA compared to the control and the human milk group at 40 and 48 weeks PCA. However, the cholesterol concentrations in all groups were within the ranges reported in other studies for human milk and

formula-fed infants. Similarly, the preterm AG38 study conducted by Ross also showed an increase in plasma cholesterol levels. As previously discussed, the total cholesterol values were within the 95% confidence intervals (44-218 mg/dL) published by Tietz (1990) and Soldin (1997).

Several studies (Carlson et al., 1996; Birch et al., 1998, 2000; Hoffman et al., 2000; Kohn et al., 1994; Lucas et al., 1999; Willatts et al., 1998a, 1998b, 1996; Auestad et al., 1997; Scott et al., 1998; Makrides et al., 1999, 2000) in term infants were designed to study the effects of AA and DHA supplementation on growth and developmental outcomes, including visual development. Results of these studies showed no adverse effects related to the AA and DHA supplementation in term infant formula (Table VII-69; Attachment 4). The duration of the AA and DHA supplementation ranged from 3 months to 1 year. The AA and DHA were obtained from GRAS single-cell oils or egg lipids or egg phospholipids and the amount of AA and DHA used in the infant formulas ranged from 0.25 to 0.72% and 0.10 to 0.36%, respectively.

Results of these clinical trials indicate that there are no adverse effects related to the supplementation of AA and DHA in formulas designed for preterm and term infants.

Table VII-68
Preterm Infants and AA + DHA Supplemented Formulas: A Summary of Clinical Trials

								Safety Assessments		
Study	**Subjects in group** [a]	**Treatment** [b]	**AA (%FA)** [c]	**DHA (%FA)**	**AA/DHA Source** [d]	**Duration of feeding** [e]	**Time of Primary Assessments**	**Growth** [f]	**Development** [g]	**Monitoring** [h]
Carlson, 1998	85 34	FF FF_{AA+DHA}	ND 0.41	ND 0.13	-- egg	~ 5 d to 1-mo CA	~ d 23, ~ d 37	No effects on weight gains.	Not measured.	Effects on FA in RBCs. Positive effects on incidence of NEC.
Clandinin, 1997 Clandinin, 1999	25 18 18 18 12	HM FF FF_{AA+DHA} 1 FF_{AA+DHA} 2 FF_{AA+DHA} 3	0.54 -- 0.32 0.49 1.10	0.30 -- 0.24 0.35 0.75	-- -- SSO SSO SSO	to 6 wk (~42 d)	2 wks, 6 wks	No effects on weight, length and OFC compared to FF.	Not measured.	Effects on FA in RBCs and lipoprotein lipids. No effects on clinical chemistries, hematology, urinalysis.
Faldella, 1996	12 25 21	HM FF FF_{AA+DHA}	1.23 0.01 0.35	1.22 NA 0.23	-- -- egg	~d 10 to 12-mo CA	12-mo CA	No effects on weight, length and HC.	Positive effect on VEP; no effects on ERG or BAEP.	Effects on DHA in RBC membrane.
Foreman-Van Drogelen, 1996	12 14 14	HM FF FF_{AA+DHA}	0.52 ND 0.61/0.54	0.26 ND 0.30	-- -- SSO	>d 9 to 3-mo CA	3-mo CA	No effects on weight gains compared to FF.	Not measured.	Effects on FA in plasma lipids.

000326

Table VII-68
Preterm Infants and AA + DHA Supplemented Formulas: A Summary of Clinical Trials

								Safety Assessments		
Study	**Subjects in group** [a]	**Treatment** [b]	**AA (%FA)** [c]	**DHA (%FA)**	**AA/DHA Source** [d]	**Duration of feeding** [e]	**Time of Primary Assessments**	**Growth** [f]	**Development** [g]	**Monitoring** [h]
Koletzko, 1989 Koletzko, 1995a	111 10 8	HM FF FF_{AA+DHA}	0.40 ND 0.20	0.20 ND 0.10	-- -- egg/fish	d 4 to 21	d 4, d 21	No effects on weight gain.	Not measured.	No effects on tolerance. Effects on FA in plasma lipids Negative effects on - toc/total lipid ratio in RBCs.
Vanderhoof, 1999 Vanderhoof, 2000	66; 51 66; 51 60; 51	HM FF FF_{AA+DHA}	no data -- 0.50	no data -- 0.35	-- -- SSO	to 48 wk PCA	term CA 40 wk PCA 48 wk PCA 92 wk PCA	No effects on weight, length and OFC or MAC compared to FF.	Not measured.	Increase in cholesterol and decrease in TG. No effects on other clinical chemistries, tolerance, nervous system events, hematology[i], urinalysis, major system events.

0003327

Table VII-68
Preterm Infants and AA + DHA Supplemented Formulas: A Summary of Clinical Trials

								Safety Assessments		
Study	**Subjects in group** [a]	**Treatment** [b]	**AA (%FA)** [c]	**DHA (%FA)**	**AA/DHA Source** [d]	**Duration of feeding** [e]	**Time of Primary Assessments**	**Growth** [f]	**Development** [g]	**Monitoring** [h]

[a] Numbers generally refer to evaluable group; see summaries for details.
[b] HM = human milk; FF = formula fed; FF_{AA+DHA} or FF_{DHA} = AA + DHA or DHA supplemented formula, respectively.
[c] %FA values refer to mean or median values; see summaries for details. ND = not detected.
[d] SSO = single-cell oils (all manufactured by Martek Biosciences); egg = egg phospholipids and/or egg triglycerides; fish = fish oil; see summaries for details.
[e] CA = corrected age; PCA = postconceptional age.
[f] Growth refers to various measurements including weight, length, head circumference (HC), occipitofrontal circumference (OFC), or midarm circumference (MAC).
[g] Development refers to measurements of visual, cognitive, or language development.
[h] Monitoring refers to clinical chemistries, hematology, tolerance, morbidity, or adverse events.
[i] With the exception of anemia.

000328

TABLE VII-69
Term Infants and AA + DHA Supplemented Formulas: A Summary of Clinical Trials

								Safety Assessments		
Study	**Subjects in group** [a]	**Treatment** [b]	**AA (%FA)** [c]	**DHA (%FA)**	**AA/DHA Source** [d]	**Duration of feeding**	**Time of Primary Assessments**	**Growth** [e]	**Development** [f]	**Monitoring** [g]
Auestad, 1997 Scott, 1998	63 45 43 46	HM FF FF_{DHA} FF_{AA+DHA}	0.48 0 0 0.43	0.15 0 0.23 0.12	-- -- fish egg	to ≥ 4 mo	1, 2, 4, 6, 9, 12 mo	No effects on weight, length or head circumference.	No effects on visual acuity or VEP. No effects on MDI or PDI scores. DHA (without AA) had negative effects on vocabulary.	Effects on RBC levels of AA and DHA. No effects on tolerance.
Birch, 1998 Birch, 2000 Hoffman, 2000	20 21 20 19	HM FF FF_{DHA} FF_{AA+DHA}	0.56 0.00 0.02 0.72	0.29 0.00 0.35 0.36	-- -- SSO SSO	to 17 wks	6, 17, 26, 52 wks, 18 mo	No effects on growth.	Positive effects on sweep VEP acuity and ERG. No effects on FPL acuity. Positive effects on MDI at 18 mo. Early fatty acid levels in blood lipids were correlated to later visual development.	Effects on AA and DHA in RBCs. No effects on tolerance.
Carlson, 1996	19 20 19	HM FF FF_{AA+DHA}	0.6 0.0 0.43	0.1 0.0 0.1	-- -- egg	to 4 mo	2, 4, 6, 9, 12 mo	Not measured.	Positive effects on visual acuity at 2 months.	Effects on AA and DHA in plasma and RBC lipids.

000329

TABLE VII-69
Term Infants and AA + DHA Supplemented Formulas: A Summary of Clinical Trials

								Safety Assessments		
Study	**Subjects in group** [a]	**Treatment** [b]	**AA (%FA)** [c]	**DHA (%FA)**	**AA/DHA Source** [d]	**Duration of feeding**	**Time of Primary Assessments**	**Growth** [e]	**Development** [f]	**Monitoring** [g]
Kohn, 1994	No data No data No data	HM FF FF_{AA+DHA}	0.36 0.02 0.25	0.22 < 0.01 0.18	-- -- egg	to 3 mo	1 wk, 1 mo, 3 mo	No effects on weight or length gains.	Not measured.	Effects on AA and DHA in plasma and RBC lipids. No effects on tolerance.
Lucas, 1999	138 155 154	HM FF FF_{AA+DHA}	no data -- 0.30	no data -- 0.32	-- -- egg	to 6mo	6 and 12 wks 6, 9, 18 mo	No effects on growth.	No effects on MDI or PDI.	No effects on tolerance, adverse effects, infections, atopy.
Makrides, 1999 Makrides, 2000	23 21 23 24	HM* FF FF_{DHA} FF_{AA+DHA}	0.39 ND ND 0.34	0.20 ND 0.35 0.34	-- -- fish egg	to 1 yr	6, 16 and 34 wk, 1 and 2 yrs	No effects on weight, length, HC.	No effects on VEP. No effects on MDI or PDI scores.	Effects on AA and DHA in plasma and RBC lipids. No effects on tolerance.
Willatts, 1996 Willatts, 1998a Willatts, 1998b	27 24 24	HM FF FF_{AA+DHA}	no data < 0.10 0.3-0.4	no data --- 0.15-0.5	-- -- egg	to 4 mo	3, 9, 10 mo	No effects on weight, length, OFC.	Positive effects on problem solving at 10 months.	Not measured.

[a] Numbers generally refer to evaluable group; see summaries for details.
[b] HM = human milk; FF = formula fed; FF_{AA+DHA} or FF_{DHA} = AA + DHA or DHA supplemented formula, respectively.
[c] %FA values refer to mean or median values; see summaries for details. ND = not detected.
[d] SSO = single-cell oils (all manufactured by Martek Biosciences); egg = egg phospholipids and/or egg triglycerides, or egg yolk lecithin; fish = fish oil; see summaries for details.
[e] Growth refers to various measurements including weight, length, head circumference, weight-for-length, subscapular fat, or triceps fat deposition.
[f] Development refers to measurements of visual, cognitive, or language development, or problem solving skills.
[g] Monitoring refers to clinical chemistries, hematology, tolerance, morbidity, or adverse events.

0003330

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EXPERT PANEL SIGNATURES

We have reviewed all the available data on the short-term and long-term toxicity of Arachidonic acid-rich oil (AA-rich oil; SUNTGA40S, Suntory LTD, Osaka, Japan) and Docosahexaenoic acid-rich oil (DHA-rich oil from tuna; Mochida International Co., LTD, Tokyo, Japan), human exposure to AA and DHA through human milk, and human clinical safety studies evaluating safety of administered intakes of AA-rich oil and DHA-rich oil and have concluded that:

There is no evidence in the available information on AA-rich oil and DHA-rich oil that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected from the proposed applications. AA-rich oil and DHA-rich oil are GRAS for use as an ingredient in term and preterm infant formulas, as proposed by Ross Products Division, Abbott Laboratories.

It is our opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, AA-rich oil and DHA-rich oil, to be used as ingredients in term and preterm infant formulas, are GRAS, when used to supplement preterm and term infant formula at target mean concentrations of: 0.40% and 0.25% , respectively for Similac® Special Care® (at a target ratio of AA/DHA of 1.6); 0.40 % and 0.15%, respectively, for Similac NeoSure® (at a target ratio of AA/DHA of 2.7) and; 0.40% and 0.15%, respectively, for Similac® With Iron (at a target ratio of AA/DHA of 2.7).

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Dr. Michael Georgieff University of Minnesota	Signature: _____ Date: _____
Dr. M.W. Anders University of Rochester Medical Center	Signature: _____ Date: _____
Dr. Sanford Miller Georgetown Center for Food and Nutrition Policy	Signature: _____ Date: _____
Dr. Howard Sprecher The Ohio State University	Signature: _____ Date: _____

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Dr. Vasilios Frankos ENVIRON International Corporation	Signature: _____ Date: _____
Dr. Sheila Innis University of British Columbia	Signatur _____ Date: Dec 9/2001.
Dr. Michael Georgieff University of Minnesota	Signature: _____ Date: _____
Dr. M.W. Anders University of Rochester Medical Center	Signature: _____ Date: _____
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Dr. Vasilios Frankos ENVIRON International Corporation	Signature: _____ Date: _____
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Dr. M.W. Anders University of Rochester Medical Center	Signature: _____ Date: _____
Dr. Sanford Miller Georgetown Center for Food and Nutrition Policy	Signature: _____ Date: _____
Dr. Howard Sprecher The Ohio State University	Signature: _____ Date: October 11, 2001

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GRAS DETERMINATION FOR DOCOSAHEXAENOIC ACID RICH OIL DERIVED FROM TUNA AND ARACHIDONIC ACID RICH OIL DERIVED FROM *MORTIERELLA ALPINA*

ATTACHMENTS

Prepared for

Ross Products Division
Abbott Laboratories
Columbus, Ohio

Prepared by

ENVIRON International Corporation
Arlington, Virginia

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December 12, 2001

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ATTACHMENT 1

ANIMAL TOXICOLOGY STUDIES

ATTACHMENT 1

Animal Toxicology Studies

TABLE 1 Boswell et al. (1996) 4 Week Oral Gavage Study in Rats		
AA Source and/or DHA Source	AA (*Mortierella alpina*) ARASCO Oil (40% AA) DHA (*Crypthecodinium cohnii*) DHASCO Oil (40% DHA)	
Dose	Dose of Oil (mg/kg b.w.)	Dose of AA or DHA (mg/kg b.w.)
	AA Oil: 50 mg/kg AA Oil: 1000 mg/kg AA Oil: 2500 mg/kg	AA: 20 mg/kg AA: 400 mg/kg AA: 1000 mg/kg
	DHA Oil: 25 mg/kg DHA Oil: 500 mg/kg DHA Oil: 1250 mg/kg	DHA: 10 mg/kg DHA: 200 mg/kg DHA: 500 mg/kg
	AA/ DHA Oil Total = 1500 mg/kg (AA :DHA Oil = 1000:500)	AA: 400 mg/kg DHA: 200 mg/kg
	AA /DHA Oil Total =3750 mg/kg (AA:DHA Oil = 2500:1250)	AA: 1000 mg/kg DHA: 500 mg/kg
Standardization of Test Groups for Dietary Lipid	All test groups received a total of 3750 mg/kg b.w. oil, using sunflower oil to standardize the oil dose received (added to basal diet). All test and control groups received the same amount of total fat	
Control Groups	Control group received 3750 mg/kg sunflower oil added to basal diet.	
Adverse Effects		
Clinical Chemistry	Females in the high dose AA/DHA Oil group had significantly lower total protein and albumin levels compared to control; these changes were not dose related or seen in males.	
Hematology	No treatment-related abnormalities	
Organ Weight	Significant increase in absolute liver weight (<20%) of males in the high dose AA/DHA group compared to both control and to the DHA 1250 mg/kg dose group.	
Histopathology	No treatment-related abnormalities	
Other	Rats fasted prior to sacrifice	
Threshold for Effects of Oil Ingestion	AA: 2500 mg/kg (highest dose tested) DHA: 1250 mg/kg (highest dose tested) AA/DHA: 1500 mg/kg (based on increased liver weight)	

TABLE 2 Wibert et al. 1997 4 Week Dietary Study in Rats		
AA Source and/or DHA Source	AA (*Mortierella alpina*) ARASCO (41.2% AA) DHA (*Crypthecodinium. cohnii*) DHASCO (40% DHA)	
Dose	Dose of Oil *(mg/kg b.w.)	Dose of AA or DHA*(mg/kg b.w.)
	AA/ DHA Oil Total: 1600 mg/kg (AA:DHA=1067:533)	AA: 440 mg/kg DHA: 213mg/kg
	AA/ DHA Oil Total: 4500 mg/kg (AA:DHA=3000:1500)	AA: 1236 mg/kg DHA: 600mg/kg
	AA/ DHA Oil Total: 9250 mg/kg (AA:DHA=6167:3083)	AA: 2541 mg/kg DHA : 1233 mg/kg
	* mean for male and female combined	* mean for male and female combined
Standardization of Test Groups for Dietary Lipid	Diet for all three test groups matches the dietary fat of 13.1% w/w used in the high fat control.	
Control Groups	Low fat control (canola oil) (5% w/w) (3500-6500 and 3700-6900 mg/kg/day; male and female, respectively) High fat control (canola oil) (13.1% w/w) (8500-16,400 and 9600-18,100 mg/kg/day; male and female, respectively)	
Adverse Effects		
Clinical Chemistry	No adverse treatment-related abnormalities. Mean BUN values for the high dose male and female groups were increased compared to the high fat control, however, average values fell within historical control range. Serum creatinine levels, a more sensitive indicator of renal function, were consistent among all groups. Statistically significant differences in glucose, albumin, and ALT values were seen at the high dose, however, the differences were small, did not occur in both sexes, and the values were within the ranges of historical controls.	
Hematology	No adverse treatment-related effects. Slight but statistically significant increase in neutrophil count for the high dose group compared to the high fat control.	
Organ Weight	Absolute liver weights not given. Higher relative liver weights in the mid- and high dose groups compared to the high fat control, however, there was no difference compared to the low fat control. Body weight was higher although not significantly, in the high fat control group. This may have resulted in lower organ/body weight ratios.	
Histopathology	Both the high fat control and high dose AA/DHA group had hepatic periportal vacuolation indicative of fatty infiltrate in the liver. Renal corticomedullary mineralization in females of all groups is consistent with an effect of the semipurified AIN-76 diet.	
Other	Rats fasted prior to sacrifice.	
Threshold for Effects of Oil Ingestion	AA/DHA: 9250 mg/kg (highest dose tested)	

000357

TABLE 3 Hempenius et al. (1997) 4 Week Oral Gavage in Rats		
AA Source and/or DHA Source	AA (*Mortierella alpina*; contains 32.7% AA) DHA (fish oil, not specified; contains 25.5% DHA)	
Dose	**Dose of Oil (mg/kg b.w.)**	**Dose of AA or DHA (mg/kg b.w.)**
	AA Oil: 100 mg/kg	AA: 33 mg/kg
	600 mg/kg	AA: 196 mg/kg
	2000 mg/kg	AA: 654 mg/kg
	3000 mg/kg	AA: 981 mg/kg
	DHA Oil: 50 mg/kg	DHA: 13 mg/kg
	300 mg/kg	DHA : 77 mg/kg
	1000 mg/kg	DHA: 255 mg/kg
	1500 mg/kg	DHA: 383 mg/kg
	AA/ DHA Oil Total = 3000 mg/kg (AA:DHA Oil =2000:1000)	AA: 654 mg/kg DHA: 255 mg/kg
	AA/DHA Oil Total = 4500 mg/kg (AA:DHA Oil =3000:1500)	AA: 981 mg/kg DHA: 383 mg/kg
Standardization of Test Groups for Dietary Lipid	All test groups received test article diluted in corn oil to ensure a constant volume of 5 ml/kg/day.	
Control Groups	Control group received 5 ml corn oil/kg/day.	
Adverse Effects		
Clinical Chemistry	Levels of phospholipids decreased in males of the 1000 mg/kg DHA oil group and in both sexes of the 1500 mg/kg DHA oil group and low- and high-dose AA/DHA oil groups. Triglyceride levels were relatively low in males of the 2000 and 3000 mg/kg AA oil groups and the 1500 mg/kg DHA oil group and the low- and high-dose AA/DHA oil groups. Changes were not always statistically significant and did not always show a clear dose-response, however, they may reflect the effect of PUFAs on reducing plasma lipids. Females in the high dose AA/DHA group, but not males, had significantly elevated AST and ALT activity. Significantly elevated ALT activity was also seen in the males, but not females, of the 1500 mg/kg DHA group,.	
Hematology	No changes in clotting potential and total and differential white blood cell count. No corresponding hematology to corroborate an adverse effect of increased spleen weight. Slight increases in MCHC in males of the low- and high-dose AA/DHA groups; these increases were within normal range.	
Organ Weight	Relative spleen weight was increased in males of the 3000 mg/kg AA oil group and females of the high dose AA/DHA oil group. Absolute spleen weight was increased in males of the 2000 mg/kg AA oil group. Increase in relative liver weight in males of the 50 mg/kg DHA oil group only.	
Histopathology	No corresponding histopathology in spleen or liver. No adverse findings in other organs.	
Other	Fasting state prior to sacrifice not reported.	
Threshold for Effects of Oil Ingestion	AA: 2000 mg/kg (based on increased relative spleen weight) DHA: 1500 mg/kg (highest dose tested) AA/DHA: 3000 mg/kg (based on increased relative spleen weight)	

000358

TABLE 4 Hempenius et al. (2000) 13 Week Dietary Study in Rats with an *In Utero* Phase		
AA Source and/or DHA Source	AA (*Mortierella alpina*; contains 38.6% AA) DHA (fish oil, not specified; contains 26.6% DHA)	
Dose	**Dose of Oil *(mg/kg b.w.)** AA Oil: 190 mg/kg 970 mg/kg 4800 mg/kg AA/ DHA Oil Total = 8565 mg/kg (AA:DHA Oil = 4940:3625) * mean for male and female combined	**Dose of AA or DHA *(mg/kg b.w.)** AA: 73 mg/kg AA: 374 mg/kg AA: 1853 mg/kg AA: 1907 mg/kg DHA: 964 mg/kg * mean for male and female combined
Standardization of Test Groups for Dietary Lipid	All test groups, except AA/DHA group, received test article with added corn oil to ensure a consistent oil concentration in the feed of 130,000 ppm. AA/DHA oil was added at a total concentration of 130,000 and did not need additional corn oil to standardize oil content.	
Control Groups	Standard rat chow control High fat control: 130,000 ppm corn oil added to diet	
Adverse Effects		
Clinical Chemistry	Analysis compared test groups to the high fat control. Alkaline phosphatase activity was decreased in males and females of the AA high dose group. Cholesterol concentration was decreased in females in the high dose AA group and males and females of the AA/DHA group. Triglycerides were decreased in males of the AA high dose group and the AA/DHA group. Phospholipids were decreased in the AA high dose group and the AA/DHA high dose group of both sexes. Creatinine concentration was increased in males of the AA high dose group and the AA/DHA group. Urea concentration was increased in males of the AA/DHA group. In the standard rat chow control group, decreases were observed in alkaline phosphatase activity and urea concentration in both sexes, albumin/globulin ratio was decreased in males, and the bilirubin, triglyceride and phospholipid concentration and alanine aminotransferase activity were increased in females, compared to the high fat control group.	
Hematology	Packed cell volume was decreased and mean corpuscular hemoglobin concentration increased in males of the AA/DHA group compared to the corn oil control group. Mean corpuscular hemoglobin concentration was also increased in males in the high dose AA group. The changes were slight and other red blood cell variables did not show any significant changes.	
Organ Weight	Relative weight of the spleen was increased in males and females of the AA high dose group and the AA/DHA group. The relative weight of the liver was increased in females of the AA high dose group and the AA/DHA group. The relative weight of the adrenals was increased in females of the AA/DHA group. In females of the standard rat chow control, the relative weights of the kidneys and liver were increased compared to the standard rat chow control.	
Histopathology	Mesenteric lymph nodes of most males and several females of the AA high dose group and the AA/DHA group contained focal aggregates of oil droplets. Oil droplets were also seen in the tips of the villi of the small	

TABLE 4 Hempenius et al. (2000) 13 Week Dietary Study in Rats with an *In Utero* Phase	
	intestine of many animals of the AA high dose group and the AA/DHA group. Oil droplets were also observed in the Peyer's patches of the small intestine in several rats of all groups, including the corn oil control group, but not in the standard rat chow control group. In addition, a number of animals had lipogranulomas in either the Peyer's patches or in the mesenteric lymph nodes. In females, a dose-dependent increase of hepatocellular vacuolation was seen; this was statistically significant in the AA high dose group and the AA/DHA group. Hepatocellular vacuolation was also seen in some males of all AA groups and the high fat control, however, no vacuolation was seen in the livers of males from the AA/DHA group.
Other	Administration of test substances from 4 weeks prior to mating, throughout mating, gestation, lactation of parental (F0) animals and weaning of the F1 pups did not affect fertility or reproductive performance, general condition of pups, viability, sex ratio or number of pups. Pup weight gain in the AA/DHA group was lower than corn oil control group. Lower pups weights were not, however, reflected in significant effects on body weight of the F1 rats in the subchronic study. Functional observation and motor activity assessment did not reveal any indications of neurotoxicity.
Threshold for Effects of Oil Ingestion	AA: 970 mg/kg (based on increased liver and spleen weight) AA/DHA: not determined (based on increased liver and spleen weight)

TABLE 5 Wyeth (1995) 13 week dietary study in rats		
AA Source and/or DHA Source	AA (*Mortierella alpina*) ARASCO (40% AA) DHA (*Crypthecodinium. cohnii*) DHASCO (40% DHA)	
Dose	Dose of Oil *(mg/kg b.w.) Low dose: AA Oil = 2% of fat (0.1% diet) = 60 mg/kg DHA Oil = 1.3% of fat (0.07% diet) = 42 mg/kg AA/DHA = 102 mg/kg Mid dose: AA Oil = 10% of fat (0.5% diet) = 298 mg/kg DHA Oil = 6.5% of fat (0.3% diet) = 156 mg/kg AA/DHA = 454 mg/kg High dose: AA Oil = 40% of fat (2% diet) =1190 mg/kg DHA Oil = 26% of fat (1.3% diet) = 750 mg/kg AA/DHA = 1940 mg/kg * mean for male and female combined	Dose of AA or DHA *(mg/kg b.w.) Low dose: AA: 24 mg/kg DHA: 17 mg/kg AA/DHA: 41 mg/kg Mid dose: AA: 119 mg/kg DHA: 62 mg/kg AA/DHA: 181 mg/kg High dose: AA: 476 mg/kg DHA: 300 mg/kg AA/DHA: 776 mg/kg * mean for male and female combined
Standardization of Test Groups for Dietary Lipid	All groups, including test groups had diets with 5% fat blend using soybean oil to standardize oil dose received.	
Control Groups	Positive control: 5% of diet was soybean oil. Negative control: rat chow.	
Adverse Effects		
Clinical Chemistry	Decrease in LDL, cholesterol and triglycerides in mid and high doses. Slightly increased BUN values in individual female rats in all synthetic diet groups.	
Hematology	Slight decrease in hematocrit and RBC, slight increase in platelets and reticulocytes. Changes were not considered biologically relevant due to the slight magnitude of the change, lack of a time-related trend (changes not present after 13 weeks), and lack of related histopathologic findings.	
Organ Weight	Higher absolute mean kidney weight (6%) in male rats, higher absolute and adjusted mean thyroid weights (25%) in female rats, and higher adjusted mean liver weight (10%) in female rats in the high dose group compared to the positive control.	
Histopathology	All groups, except for the negative control, had hepatic fatty change diagnosed with H&E staining. Report did indicate that oil red O staining was variable but "generally confirmed the presence of hepatocellular lipid accumulation". Hepatic change was slight to moderate and greater incidence was seen in females. Renal tubule mineralization and renal tubular basophilia (females only) is attributed to the improper calcium to phosphorous ratios from the synthetic diets.	
Other	Fasting state prior to sacrifice not reported.	
Threshold for Effects of Oil Ingestion	None (based on hepatic fatty change)	

TABLE 6 Arterburn et al. (2000) 3 Month Combined Oral Toxicity and Neurotoxicity Study By Gavage in Rats)		
AA Source and/or DHA Source	DHA (*Crypthecodinium. cohnii*) DHASCO (40% DHA) DHA oil was diluted with high oleic sunflower oil to provide a constant total oil dose of 3 ml/kg/day.	
Dose	**Dose of Oil (mg/kg b.w.)** DHA Oil: 500 mg/kg DHA Oil: 1250 mg/kg	**Dose of AA or DHA (mg/kg b.w.)** DHA: 200 mg/kg DHA: 500 mg/kg
Standardization of Test Groups for Dietary Lipid	All test and control groups received the same amount of total fat; up to3 ml/kg/day. Test base diets contain 5% fat. The additional fat bolus gavaged daily varied from 33 to 52% of the total fat content depending on the time of the study as food consumption per body weight rapidly decreased during the first 4 weeks of the study.	
Control Groups	Vehicle controls receive 3 ml/kg high oleic sunflower oil. Untreated controls received basal diet only.	
Adverse Effects		
Clinical Chemistry	Mean alkaline phosphatase values were elevated in the male low and high DHA and female low DHA oil group compared to vehicle control. No dose response was reported and no other enzymes indicative of liver toxicity were elevated.	
Hematology	Prothrombin time in the female high DHA group was significantly elevated (9.9 sec. vs. 9.4 sec.) compared to vehicle control; no change was noted in the males. No change in activated partial thromboplastin time or platelet counts.	
Organ Weight	A slight (<9%) but significant increase in the relative kidney weight in male and female high dose DHA groups was noted compared to vehicle control.	
Histopathology	No corresponding histopathology in the kidney; no adverse histopathologic findings in any other organ.	
Other	Rats fasted prior to sacrifice. No significant findings observed in the neuropathology or neurobehavioral studies	
Threshold for Effects of Oil Ingestion	DHA: 1250 mg/kg (highest dose tested)	

TABLE 7 Koskelo et al. (1997) 3 Month Combined Oral Toxicity and Neurotoxicity Study By Gavage in Rats		
AA Source and/or DHA Source	AA (*Mortierella alpina*) ARASCO (51.4% AA) AA oil was diluted with high oleic sunflower oil to provide a constant total oil dose of 3 ml/kg/day.	
Dose	**Dose of Oil (mg/kg b.w.)** AA Oil: 1000 mg/kg AA Oil: 2500 mg/kg	**Dose of AA or DHA (mg/kg b.w.)** AA: 514 mg/kg AA: 1285 mg/kg
Standardization of Test Groups for Dietary Lipid	All test and control groups received the same amount of total fat; up to3 ml/kg/day. Test base diets contain 5% fat. The additional fat bolus gavaged daily varied from 33 to 52% of the total fat content depending on the time of the study as food consumption per body weight rapidly decreased during the first 4 weeks of the study.	
Control Groups	Vehicle controls receive 3 ml/kg high oleic sunflower oil. Untreated controls received basal diet only.	
Adverse Effects		
Clinical Chemistry	No treatment-related abnormalities.	
Hematology	No treatment-related abnormalities.	
Organ Weight	Relative spleen weights were significantly higher in males of both AA groups compared to vehicle control. There was no dose-response, however, and the weights were within normal limits compared with historical control data.	
Histopathology	No treatment-related abnormalities.	
Other	Rats fasted prior to sacrifice. No significant findings observed in the neuropathology or neurobehavioral studies	
Threshold for Effects of Oil Ingestion	AA: 2500 mg/kg (highest dose tested)	

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TABLE 8 Martek (1995) Developmental Toxicity in Rats by Oral Gavage		
AA Source and/or DHA Source	AA (*Mortierella alpina*) ARASCO (40% AA) DHA (*Crypthecodinium. cohnii*) DHASCO (40% DHA)	
Dose	**Dose of Oil (mg/kg b.w.)** AA Oil: 1000 mg/kg AA Oil: 2500 mg/kg DHA Oil: 500 mg/kg DHA Oil: 1250 mg/kg	**Dose of AA or DHA (mg/kg b.w.)** AA: 400 mg/kg AA: 1000 mg/kg DHA: 200 mg/kg DHA: 500 mg/kg
Standardization of Test Groups for Dietary Lipid	Low and high doses of AA and DHA were prepared with the appropriate concentration of sunflower oil to achieve a total dose of 2500 mg/kg to standardize oil dose received.	
Control Groups	Controls received 2500 mg/kg sunflower oil.	
Adverse Effects		
Clinical Chemistry	Not done.	
Hematology	Not done.	
Organ Weight	Not done.	
Histopathology	No test material related necropsy findings.	
Other	With the exception of high dose AA, the fetal and litter incidences of undeveloped renal papilla and dilated renal pelvis was increased in all treated groups compared with controls. No dose response; the incidences were higher in the lower dose groups compared with the higher dose groups. Treated and control incidences for these two findings were higher than mean historical control values. These findings are classified as variations (not considered to have a persistent or permanent adverse effect) and are not considered to be a toxicologically adverse effect.	
Threshold for Effects of Oil Ingestion	AA: 2500 mg/kg (highest dose tested) DHA: 1250 mg/kg (highest dose tested)	

000364

TABLE 9 Burns et al. (1999) 3 Month Dietary Study in Rats With an *In Utero* Phase		
AA Source and/or DHA Source	AA (*Mortierella alpina*) ARASCO (40% AA) DHA (*Crypthecodinium. cohnii*) DHASCO (40% DHA)	
Dose	Dose of Oil *(mg/kg b.w.) Low dose: AA/DHA Oil = 18 g/kg diet (0.42 g/100 kcal) AA Oil = 767 mg/kg DHA Oil = 383 mg/kg AA/DHA Oil = 1150 mg/kg Mid dose: AA/DHA Oil = 60 g/kg diet (1.41g/100 kcal) AA Oil = 2700 mg/kg DHA Oil = 1350 mg/kg AA/DHA Oil = 4050 mg/kg High dose: AA/DHA Oil = 120 g/kg diet (2.82 g/100 kcal) AA Oil = 5700 mg/kg DHA Oil = 2850 mg/kg AA/DHA Oil = 8550 mg/kg * mean for male and female combined	Dose of AA or DHA* (mg/kg b.w.) Low dose: AA: 307 mg/kg DHA: 153 mg/kg AA/DHA: 460 mg/kg Mid dose: AA: 1080 mg/kg DHA: 540mg/kg AA/DHA: 1620 mg/kg High dose: AA: 2280 mg/kg DHA: 1140 mg/kg AA/DHA: 3420 mg/kg * mean for male and female combined
Standardization of Test Groups for Dietary Lipid	DHA and AA were mixed with canola oil to produce a constant level of dietary lipid of 131 g/kg diet in all groups except for the low fat control.	
Control Groups	Low fat canola oil control (50 g fat/kg diet) High fat canola oil control (131 g fat/kg diet) High fat control gave a level of fat similar to the highest level of test material. The dietary fat content, 131 g/kg diet is higher than that recommended for rats (50 - 70 g/kg diet). Canola oil was chosen because it provides n-3 and n-6 fatty acids linolenic and linoleic acid in the same ratio as the DHA and AA oil test material.	
Adverse Effects		
Clinical Chemistry	Mean BUN values of high dose animals were higher than high fat controls. Mid- and high dose animals also had a decreased albumin concentration relative to high-fat controls. All clinical chemistry values were within historical control range. No other changes in clinical chemistry were dose-related. .	
Hematology	Males, but not females, in the mid- and high-dose groups had decreased hemoglobin concentrations relative to the high fat control; in both sexes, hematocrit was lower in these groups compared to the high fat control. All values fell within range of historical control data. Other findings, within normal historical control ranges, were the increased prothrombin time and decreased activated partial thromboplastin time in mid- and high-dose animals compared to high fat control.	
Organ Weight	Mean absolute and relative liver and spleen weights of the mid and high	

TABLE 9 Burns et al. (1999) 3 Month Dietary Study in Rats With an *In Utero* Phase	
	dose groups were significantly higher than weights of the high fat control group, however, not significantly different from the low fat control. These changes were dose-dependent in females, but not males. Relative spleen weight of the high-fat control was significantly greater than the low-fat control.
Histopathology	No corresponding histopathology found in liver, spleen or kidney. No adverse effects in any other organs examined. No indications that there were adverse effects on tissues related to the immune system including thymus or mesenteric lymph nodes. Hepatic periportal vacuolation seen in high dose and high fat controls; no other details given.
Other	*In Utero* and Lactation Phase: Female mating indices, pregnancy rates and male fertility indices were comparable between treated and high fat control groups. For all treated groups, mean body weights and weight gains during gestation and lactation were comparable to or slightly higher than the high fat control group. No significant differences were observed in gestation length between treated and high fat controls. A statistically significant decrease in the mean total number of pups at birth was seen in the high-fat control compared to the low-fat control. Mean number of dead pups per litter at birth was not significantly different between the control groups or treatment groups. No difference in mean number of live pups per litter surviving on day 4 and no significant difference in percentage of pups surviving weaning at day 21. No treatment related effects seen in gross postmortem evaluation of F1 pups sacrificed on day 21 of lactation or F1 pups sacrificed following selection of the F1 animals for the subchronic study.
Threshold for Effects of Oil Ingestion	AA /DHA: 1150 mg/kg (based on higher liver and spleen weights for subchronic toxicity) : 8550 mg/kg (highest dose tested, for *in utero* and lactation phase)

000366

TABLE 10 Suntory (1992) Two Week Dietary Study in Rats		
AA Source and/or DHA Source	AA (*Mortierella alpina*) SUNTGA25 (25% AA)	
Dose	Dose of Oil *(mg/kg b.w.) Low dose: AA Oil = 2.5% of diet = 2835 mg/kg Mid dose: AA Oil = 5% of diet = 5280 mg/kg High dose: AA Oil = 10% of diet = 11,845 mg/kg * mean for male and female combined	Dose of AA or DHA *(mg/kg b.w.) Low dose: AA = 709 mg/kg Mid dose: AA = 1320 mg/kg High dose: AA = 2961 mg/kg * mean for male and female combined
Standardization of Test Groups for Dietary Lipid	Dose groups were not controlled for fat content.	
Control Groups	Basal diet (4.4% fat) only used as control	
Adverse Effects		
Clinical Chemistry	No consistent, dose-related changes.	
Hematology	No consistent dose-related changes.	
Organ Weight	Absolute and relative liver weight is increased in all dose groups, however, not statistically significant.	
Histopathology	Hepatic vacuolization and fatty change of the liver (only control and high dose examined. All vacuoles found in the control and compound dosed groups were Sudan III stain positive.	
Other	Animals fasted before sacrifice.	
Threshold for Effects of Oil Ingestion	Not applicable - range finding study	

000367

TABLE 11 SUNTORY (1992b) Ninety Day Dietary Rat Toxicity Test of an AA Containing Oil (SUNTGA25)		
AA Source and/or DHA Source	AA (*Mortierella alpina*) SUNTGA25 (25% AA)	
Dose	Dose of Oil (mg/kg b.w.) Low dose: AA Oil = 0.8% of diet = 600 mg/kg Mid dose: AA Oil = 2.0% of diet = 1200 mg/kg High dose: AA Oil = 5.0% of diet = 3000 mg/kg	Dose of AA or DHA (mg/kg b.w.) Low dose: AA: 150 mg/kg Mid dose: AA = 300 mg/kg High dose: AA = 750 mg/kg
Standardization of Test Groups for Dietary Lipid	Dose groups were not controlled for fat content.	
Control Groups	Control group had basal diet with 4.4% fat.	
Adverse Effects		
Clinical Chemistry	Increased BUN in males at the high dose and females at the mid and high doses. No other consistent treatment related changes	
Hematology	No consistent treatment related changes	
Organ Weight	Increased absolute liver weight at high dose; increased relative liver weight at mid- and high dose. Decreased relative kidney weight at mid and high doses in males and high dose in females.	
Histopathology	Six cases of fatty changes (small droplets) in the liver of high dose male rats. One case in low dose females, nine cases in mid dose females and four cases in high dose females of fatty changes (small droplets), as seen by H&E staining. Sudan III stain showed fatty changes in all male and female rats, including control. Re-analysis of photomicrographs of liver sections indicated that the lesions depicted were of the sort and degree of severity that might be observed in control rats of this age. There was an apparent increase in the incidence of vacuolar degeneration (fatty change) in the high dose males and in mid and high dose females. This increase in incidence was marginal in the high dose groups compared to controls. The increase was most evident in the mid dose females which dose not correlate well with the increases in liver weights. The severity of vacuolar degeneration observed in the photomicrographs was recorded as minimal to slight in all dose groups and would be considered a reversible change. Based on the Sudan III stained photomicrographs, there appeared to be a very slight increase in the severity of the Sudan III positive staining (indicating the presence of fats) in the high dose females only. In all dose groups, the degree of positive staining was generally minimal to slight. Based on the photomicrographs, fatty change may have contributed slightly to the increased liver weight, but the data is contradictory and inconclusive at best.	
Other	Animals were fasted before sacrifice.	
Threshold for Effects of Oil Ingestion	600 mg/kg (based on liver weight increases)	

TABLE 12 SUNTORY (1997) Thirteen Week Dietary Rat Toxicity Test of an AA Containing Oil (SUNTGA40S)		
AA Source and/or DHA Source	AA (*Mortierella alpina*) SUNTGA40S (40% AA)	
Dose	Dose of Oil* (mg/kg b.w.) Low dose: AA Oil = 0.5% of diet = 312 mg/kg Mid dose: AA Oil = 1% of diet = 626 mg/kg High dose: AA Oil = 2% of diet = 1255 mg/kg * mean for male and female combined	Dose of AA or DHA *(mg/kg b.w.) Low dose: AA: 125 mg/kg Mid dose: AA: 250 mg/kg High dose: AA: 502 mg/kg * mean for male and female combined
Standardization of Test Groups for Dietary Lipid	All test groups received, in addition to basal diet with 4.4% fat, a total of 2% oil, using soybean oil to standardize the volume of test oil received by the 0.5% and 1% test groups.	
Control Groups	Basal diet control (4.4% fat) Soybean oil control (2% oil added to diet) total of 6.4% fat in diet (Comparative AA + Fish Oil test group using SUNTGA 1% + Unspecified Fish Oil 1%) total of 2% oil added to diet. This group is not a relevant control for the AA test groups and is not included in the analysis)	
Adverse Effects		
Clinical Chemistry	No treatment-related adverse changes. Clinical chemistry values within normal physiologic fluctuation	
Hematology	Of the low, middle and high test groups, male middle dose had significantly longer prothrombin time compared to oil control. Low, middle and high dose males had significantly longer APTT compared to basal diet control; only mid dose was significantly different from oil control. No significant differences in females for prothrombin time or APTT. All values for prothrombin time and APTT were within historical control range.	
Organ Weight	No treatment related changes	
Histopathology	No treatment related changes	
Other	Not reported if animals were fasted prior to sacrifice. In a follow-on study of serum hormone levels from female rats of this study, it was determined that serum progesterone and estradiol levels in the test groups were not significantly different from either the basal diet or soybean oil control groups.	
Threshold for Effects of Oil Ingestion	1255 mg/kg (highest dose tested)	

000369

ATTACHMENT 2

INFANT MORTALITY INCLUDING REPORTS OF SUDDEN INFANT DEATH SYNDROME (SIDS) IN CLINICAL STUDIES WITH FORMULAS CONTAINING LONG-CHAIN POLYUNSATURATED FATTY ACIDS

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ATTACHMENT 3

REVIEW OF PLASMA CHOLESTEROL

ATTACHMENT 3

Review of Plasma Cholesterol

In the Similac NeoSure® phase of the preterm LCP-supplementation study (CP-AG38, AA and DHA Supplementation Study with LBW Infants – Similac NeoSure® Phase) a statistically significant main effect (p=0.0001) of study feeding on total plasma cholesterol was found at 4- and 12-months CA. As shown in Table VII-36, infants in the AA + DHA (fungal/fish) group had significantly higher plasma cholesterol than those in the control and AA + DHA(egg-DTG/fish) groups. At 4- and 12-months CA plasma total cholesterol levels in the three formula groups were (mg/dL, mean ± SD): control, 128 ± 25, 150 ± 45; AA + DHA(fungal/fish), 147 ± 27, 172 ± 34; and AA + DHA (egg-DTG/fish), 133 ± 25, 153 ± 26. In the exclusive human milk (EHM) reference group, plasma cholesterol levels were 137 ± 22 and 154 ± 34 mg/dL, respectively. Note, however, that by 4- and 12-months CA many of the infants in the EHM group had weaned partially or completely to one of the three formulas. Thus, the EHM reference values represent a mixed group of infants fed varying amounts of human milk and one of three formulas, rather than infants fed human milk and no formula.

The preplanned analyses for plasma cholesterol used a repeated measures approach and controlled for site, gender, visit, birth weight group, feeding*visit, feeding*gender, and feeding*birth weight group (PROC MIXED). However, only about one-third of the infants had cholesterol determinations at both 4- and 12-months CA. Ross-Abbott subsequently conducted a *post hoc* statistical analysis at both 4- and 12-months CA using an analysis of covariance approach (ANCOVA; General Linear Models Procedure) again controlling for site, gender, birth weight group, feeding*gender, feeding*birth weight group. Adjustment for human milk intake was also included since infants in the three formula groups were permitted feedings with human milk. Numerous studies have reported higher plasma cholesterol in infants fed human milk than those fed infant formulas (e.g., Fomon et al., 1960; Friedman et al., 1975; Van Biervliet et al., 1981; Huttunen et al., 1983; Fomon et al., 1984; Lane & McConathy, 1986; Wagner & von Stockhausen, 1988; Jooste et al., 1991; Wong et al., 1993; Cruz et al., 1994). Human milk intake was categorized at term CA as no human milk (i.e., exclusively formula fed), ≥50% of milk intake as human milk, and < 50% of milk intake as human milk. Adjustment for the duration of human milk feedings was also made in one of the analyses.

Consistent with the results of the preplanned analyses, the *post hoc* analyses found a main effect of formula group on plasma cholesterol concentrations (p=0.0016) at both 4- and 12-months with higher plasma cholesterol in the AA + DHA(fungal/fish) group than in the control (p=0.0005) and AA + DHA (egg-DTG) (p=0.01) groups. At 4-months, the least squares means (95% confidence interval [CI]) for plasma cholesterol in the control, AA + DHA (fungal/fish), and AA + DHA (egg-DTG/fish) groups were 131 (124 - 138), 149 (141 - 156), and 135 (126 - 143) mg/dL, respectively. At 12-months cholesterol levels were 152 (163 - 141), 178 (166 - 190), and 158 (147 - 170) mg/dL, respectively. Similar results were obtained when the duration of human feedings was used as a covariate instead of human milk intake as categorized at term CA. There were no statistically significant effects of any of

the covariates with one exception. Human milk intake (p=0.06) and the duration of human milk feedings (p=0.07) had marginal effects at 12-months CA.

In this study, blood samples for lipid analyses were obtained voluntarily and the number of samples at one or both time points varied from site to site. Additionally, there was an early introduction of cholesterol-rich foods (e.g., eggs) to the infant diet, indicated by examination of the diet record from two sites, Chile and the U.K., compared to any of the sites in the U.S. (unpublished observation). The absence of significant site effects suggests that the differences in plasma cholesterol levels were not explained by differences in the timing and types of weaning foods provided.

As noted above, only about one-third of the infants in the formula groups (n=88) had cholesterol determinations at both 4- and 12-months CA. Although not analyzed statistically and highly variable, plasma cholesterol levels generally increased in this cohort between 4- and 12- months CA. The median (25th, 75th quartile) changes in plasma cholesterol levels in the control, AA + DHA (fungal/fish), and AA + DHA (egg-DTG) groups were 11 (-15, 37),19 (4, 52), and 13 (4, 39) mg/dL, respectively. These observations are consistent with the overall results for all available samples (n=300). Infants with the highest cholesterol levels at 4-months also appeared to have the highest cholesterol levels at 12 months (Fig 1).

This is the first study examining plasma cholesterol in preterm infants through 12-months CA. Blood samples were obtained voluntarily from about one-third of the infants enrolled in this AA + DHA supplementation study (CP-AG38, AA and DHA Supplementation Study with LBW Infants). Plasma total cholesterol levels were determined, but apolipoprotein or lipoprotein cholesterol levels were not measured. Total cholesterol levels were about 10-25 mg/dL higher in infants fed the AA + DHA (fungal/fish) formula than those fed the control or AA + DHA(egg-DTG/fish) formulas at both 4- and 12-months CA.

The results of the present study were compared with reference ranges for total cholesterol as summarized in Table 1, Attachment 3 (Tietz 1990; Soldin 1997; Fomon 1993). Among the three formula groups, with the exception of 12 of 300 samples, all cholesterol values were within the 95% confidence intervals (CI), 44-218 mg/dL, published by Tietz (1990) and Soldin (1997). Two of 108 samples in the control group were below the lower limit of the 95% CI (44 mg/dL), and 2 of 108 samples in the control group, 7 of 103 samples in the AA + DHA(fungal/fish) group, and 1 of 89 samples in the AA + DHA(egg-DTG) group were above the upper CI limit (218 mg/dL). Fomon (1993) reported *mean* cholesterol levels in 1 to 4 month-old breast fed infants in the range of 119 to 143 mg/dL. Others, however, have reported substantially higher mean cholesterol levels. Innis and Hamilton (1992) reported mean cholesterol levels of 200 mg/dL in 2 month old infants and Agostoni et al. (1994) reported mean values of 178 mg/dL in 4 month old infants. Analytical variability among different laboratories, nutrient differences in human milk, formula and weaning foods in the first year, and/or genetic differences among the populations studied may explain the wide range of total cholesterol concentrations across the different studies.

A recent study with term infants fed formulas supplemented with AA + DHA from egg-lipid from birth to 4 months of age found higher total- and non-HDL cholesterol on day 5, but not thereafter (Desci et al. 1997). In another study, total cholesterol, HDL-cholesterol and LDL-cholesterol levels were not different between infants fed an unsupplemented formula and those fed a formula with the same egg lipid source of AA + DHA (Agostoni et al. 1994).

Adding egg lipid to formula to provide AA and DHA also increases the amounts of cholesterol and phospholipid in the formula. In these studies cholesterol levels were less than 1.5 mg/dL in the standard formulas and 25-30 mg/dL in the supplemented formulas. Phospholipid levels were 10 mg/dL and 200 mg/dL in the standard and supplemented formulas, respectively. In piglets fed formula supplemented with egg phospholipid, similarly providing increased amounts of AA + DHA and phospholipid, biliary AA and DHA, bile acid, and phospholipid levels were increased as was plasma HDL (Devlin and Innis 1999).

Human milk cholesterol levels range from 20 to 50 mg/dL, considerably higher than typical for infant formulas manufactured with vegetable oil blends (<5 mg/dL; Innis 1992 [review]). The higher plasma cholesterol levels in breast fed compared with formula fed infants (Fomon et al., 1960; Goalwin & Pomeranze, 1962; Darmady et al., 1972; Friedman et al., 1975; Van Biervliet et al., 1981; Carlson et al., 1982; Jarvenpaa et al., 1982; Huttunen et al., 1983; Fomon et al., 1984; Van Biervliet et al., 1986; Lane et al., 1986; Wagner et al., 1988; Jooste et al., 1991; Cruz et al., 1994; Bayley et al. 1998), however, do not seem to be related to the higher cholesterol content of human milk (Innis and Hamilton 1992 [review]). Dietary factors other than cholesterol itself have been shown to affect plasma cholesterol response in infants. The ratio of casein to whey and the whey source influence plasma total- and LDL-cholesterol (Weizman et al. 1997), dietary nucleotides increase plasma cholesterol (Sanchez-Pozo et al. 1995), and high dietary monounsaturated fatty acids through the first year increase total- and HDL-cholesterol levels (Mize et al. 1995).

Studies on healthy adults have shown no adverse effects of AA (Nelson et al., 1997) or DHA (Conquer and Holub, 1996) supplementation on the total plasma cholesterol levels. Conquer and Holub (1996), observed a favorable decrease in the total cholesterol:HDL cholesterol ratio and in LDL cholesterol:HDL cholesterol ratio without any changes in total cholesterol levels in 24 healthy adults supplemented with 1.62 g/d of DHA from algal source. Nelson et al. (1997) observed no effects of AA (1.5 g/d) supplementation for 50 days on cholesterol levels in 10 healthy adults. Innis (1996b) and Hansen (1996) observed an increase (statistical significance reported at $p<0.1$) in total cholesterol in healthy subjects supplemented with 2.2 g/d AA and 1.7 g/d DHA (4.90 ± 0.48 mmol/L), and with 3.6 g/d AA and 2.9 g/d DHA (4.86 ± 0.68 mmol/L) for 14 days compared to the control group (4.03 ± 0.84 mmol/L). The VLDL+LDL fraction was higher in these groups compared to the control group but not significantly. The HDL fraction was higher in all AA and DHA groups but it was significant only in the group supplemented with 2.2 g/d AA and 1.7 g/d DHA ($p<0.05$). Although, this study showed an increase in total cholesterol levels, the findings may not represent a clinically significant adverse finding, because the sample size was small (8/group) and the total cholesterol values were within the reference range (3.10 – 5.69 mmol/L) (Thomas, 1985) for healthy adults. In addition, in the final rule for menhaden oil, the FDA concluded that omega-3 fatty acids (EPA and DHA) from fish oils may reduce blood triglycerides but have no effect on serum cholesterol, LDL and HDL (Federal Register, 1993).

Approximately 30 years ago, Reiser and Sidelman (1972) suggested a relationship between cholesterol intake during infancy and later susceptibility to coronary artery disease. Studies with animals designed to test this hypothesis have found inconsistent results (Reiser & Sidelman, 1972. Kris-Etherton et al., 1979; Innis, 1985; Lewis et al., 1988; Mott et al., 1990) and the role of dietary factors other than cholesterol in the infant diet on susceptibility to vascular disease has not been tested. Studies with human infants are limited to

comparisons between children fed human milk or formula as infants (Huttunen et al., 1983; Fomon et al., 1984; Lapinleimu et al., 1995; Plancoulaine et al, 2000) or retrospective observations of men who died from ischemic heart disease (Fall et al., 1992). The prenatal diet (Lucas 1996), maternal cholesterol status during pregnancy (Napoli et al., 1999) and genetic factors (MacCluer et al., 1988; Pesonen et al., 1990; Kushwaha et al., 1991; Kallio et al. 1997) also play significant roles in the susceptibility to vascular disease later in life.

Formula-related effects on plasma cholesterol in children are mixed (Fomon et al., 1984; Huttunen et al., 1983; Plancoulaine et al., 2000). Fomon et al. (1984) measured serum cholesterol concentrations in approximately 150 healthy 8 year-old children who where fed human milk or infant formulas available in the early 1980s. At 8 years of age, there was no difference in serum cholesterol concentrations between previously breast fed and formula-fed infants. Huttunem et al. (1983) similarly reported no differences in serum cholesterol levels in 3- and 5-year-old children who were fed human milk or formula to at least 6 months of age. In another study with 5- to 11-year old prepubertal children (boys, n=251; girls, n=223) in northern France, Plancoulaine et al. (2000) reported higher cholesterol in boys who had been formula-fed than those who had been breast-fed (181 vs. 170 mg/dL, respectively). In another recent study, 1062 healthy 7-month old infants were randomized to a control group or an intervention group whose parents were given dietary counseling (Lapinleimu et al. 1995). The dietary advice was given as: formula or human milk encouraged to one year of age; energy to accommodate the child's hunger; 30-35% of energy from fat; a ratio of polyunsaturated to monounsaturated to saturated fatty acids of 1:1:1; cholesterol intake of less than 200 mg/day; 15% of energy from protein; and 55% from carbohydrates. At 13 months of age serum cholesterol was 3.96 mmol/L (SD, 0.70) in the intervention group and 4.22 mmol/L (SD, 0.78) in the control group ($p < 0.05$). The authors suggested that this difference constituted evidence of a change in atherogenic potential, a statement which led to several letters to the editor (Olson, 1995; Shahar et al., 1995; Michaelsen & Hernell, 1995) noting insufficient scientific evidence to support the premise of the study that moderation of serum cholesterol concentrations in infants or children reduces risk for coronary artery disease. Newman & Garber (2000) similarly noted that there is inadequate scientific justification to advocate cholesterol screening in young children.

It can be concluded that AA and DHA supplementation in adults and infants has not been shown to have clinically adverse effects on total plasma/serum cholesterol levels. The slight increase observed in some infant and human studies is within the normal range. A relationship between cholesterol status in the first year and later susceptibility for vascular disease has not been established.

TABLE 1
Total Cholesterol (mg/dL)* in Term Infants: Published Reference Values

Reference	Age	Gender	Human milk/formula	Total Cholesterol 95% Confidence Interval (n)
Tietz et al., 1990	0 - 4 y	M F		114 - 203 (NA) 112 - 200 (NA)
From Soldin, 1996[a]	0 - 1 m	M F		45 - 177 (37) 63 - 198 (27)
	2 - 6 m	M F		60 - 197 (354) 66 - 218 (243)
	7 - 12 m	M F		89 - 208 (401) 74 - 218 (252)
From Lockitch, 1993[a]	1 - 3 y	M F		44 - 181 (49) 44 - 181 (49)
From Hicks, 1996[a]	1 - 30 d	M F		54 - 151 (62) 62 - 155 (74)
	31 - 182 d	M F		81 - 147 (77) 62 - 141 (75)
	183 - 365 d	M F		76 - 179 (53) 76 - 216 (45)
Fomon, 1984[b]	28 d	M F	Human milk	136 ± 27 (15) 140 ± 33 (28)
	56 d	M F	Human milk	121 ± 18 (15) 135 ± 43 (30)
	84 d	M F	Human milk	119 ± 18 (13) 133 ± 24 (26)
	112 d	M F	Human milk	127 ± 24 (16) 143 ± 49 (30)
	28 d	M F	Formula	104 ± 18 (41) 106 ± 20 (36)
	56 d	M F	Formula	100 ± 23 (74) 105 ± 28 (61)
	84 d	M F	Formula	100 ± 22 (57) 105 ± 25 (34)
	112 d	M F	Formula	107 ± 23 (80) 107 ± 23 (63)

[a] data obtained from Soldin et al., 1997
[b] data obtained from Fomon, 1993
* Serum or plasma cholesterol

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ATTACHMENT 4

HUMAN STUDIES

ATTACHMENT 4

Human Studies

Table 1 Clinical Studies on AA and DHA in Preterm Infants	
Carlson et al. 1998 ***Pediatric Research* 1998;44:491-498**	
Study design	Randomized, double-masked, clinical trial
Population	Preterm infants ≤ 32 weeks gestation, weighing 725-1375 g, in the ≥ 5th percentile of weight at birth Memphis, TN
Source	Egg phospholipid
Treatment	AA (%FA) / DHA (%FA) / N = 119 (in-hospital phase) FF (control): --- / --- / 85 FF_{AA+DHA}: 0.41 / 0.13 / 34 A total of 119 infants were stratified into three groups based on birth weight (725-925 g; 926-1150 g; 1151-1375 g). Infants were randomized to one of three feeding regimens during the in-hospital phase at < 8 days of age (a mean of 4.9 days of age). This report combines data from the two groups fed the control formula in-hospital. Infants were fed by nasogastric infusion until they weighed 1.5 kg, then at 3 hours intervals by nipple or bolus infusion until hospital discharge. None of the infants received human milk. Preterm formula used in all feedings was Similac Special Care (Ross Products Division). The supplemented formula and control formula contained 682 mg and 174 mg total choline per liter, respectively.
Feeding duration	from first feedings (< 8 days) until discharge from the hospital (34.5 ± 1.4 vs 38.4 ± 1.4 days (control vs experimental formula)
Formula intake	Mean intake at week 7 (kcal/kg/d): FF: 107 ± 27 FF_{AA+DHA}: 128 ± 13 (data available for wks 1 through 6) No difference in in-hospital energy intake from parenteral and enteral formulas (weeks 1 through 7).
Clinical chemistries	Plasma phospholipid and fatty acid concentration at enrollment, full feedings (~ 23 d after birth) and 2 weeks after full feedings (~ 37 d after birth) The FF_{AA+DHA} infants had higher total plasma PC at 2 weeks after full feedings, and higher plasma PE at full feedings and 2 weeks after full feedings. Plasma PC and PE concentrations of AA and DHA were higher in the FF_{AA+DHA} group at the time of full formula feedings and 2 weeks after full feedings as compared to the control group.
Hematology	No mention
Urinalysis	No mention
Growth	No difference in weight gains measured in-hospital between feeding groups (weeks 1 through 7)
Tolerance	No mention
Other	The incidence of NEC was significantly lower among infants consuming the AA and DHA supplemented formula as compared to infants receiving the control formula. There were no differences in incidence of septicemia, retinopathy of prematurity, bronchopulmonary dysplasia or deaths.
Dropouts	A total of 120 infants were randomized to the study; one infant transferred to another hospital after 7 d due to insurance requirements. Five infants in the control group died; four of the infants were diagnosed with NEC. One infant in the AA+DHA formula group developed NEC and died.
Authors' comments	"One or more of the egg phospholipid components (AA, DHA, choline) may have enhanced one or more immature intestinal functions to lower the incidence of NEC in this study."

Table 2
Clinical Studies on AA and DHA in Preterm Infants

Clandinin et al. 1997 ***Pediatric Research* 1997;42:819-825**	
Study design	Randomized clinical trial
Population	Preterm infants who were medically table, weight appropriate for gestational age; birth weight < 2300 g; infants received 100% of daily fluid and energy requirements enterally by day 14 of life Albert, Canada
Source	AA and DHA from single cell oils (Martek Biosciences Corporation, MD)
Treatment	

	AA (%FA)	DHA (%FA)	N=(6 wks)
HM*	0.54	0.30	25
FF	---	---	18
FF_{AA+DHA} (low)	0.32	0.24	18
FF_{AA+DHA} (medium)	0.49	0.35	18
FF_{AA+DHA} (high)	1.1	0.76	12

* human milk data appears to be taken from a published paper

Preemie SMA ® (Wyeth Nutritional International, Radnor, PA) was used as the control formula and as the base formula to which the LCPUFAs were added.

This report presents only the assessments of RBC membrane phospholipids, growth, hematology and urinalysis; see Clandinin 1999 for additional results.

Feeding duration	from first enteral feedings to 6 weeks
Formula Intake	No mention
Clinical chemistries	Blood samples collected at approximately 14 and 42 days of life (2 and 6 weeks, respectively) Fatty acid composition of erythrocyte membrane phospholipids; serum creatinine RBC PC: Significant diet x age effects in concentrations of C18:1*n*-9; C18:1*n*-7; C22:6*n*-3; C18:2*n*-6; C22:4*n*-6 RBC PE: Significant diet x age effects in concentrations of C16:0; C18:0; C18:1*n*-9; C18:1*n*-7; C22:5*n*-3; C22:6*n*-3; C18:2*n*-6 RBC PS: Significant diet x age effects in concentrations of C14:0; C18:3*n*-3; C20:5*n*-3; C22:5*n*-3; C22:6*n*-3; C20:4*n*-6 ; C22:4*n*-6 Erythrocyte phosphatidylinositol: Significant diet x age effects in concentrations of C16:0; C22:5*n*-3; C22:6*n*-3; C18:2*n*-6; C22:4*n*-6; C22:5*n*-6 Total plasma phospholipid: No significant diet x age effects observed Infants fed the control formula had lower AA and DHA levels as compared to human milk fed infants and infants fed any of the LCPUFA supplemented formulas. Infants in the low and medium LCPUFA supplemented formula groups had plasma phospholipid AA and DHA levels similar to infants in the human milk fed group. Infants in the high LCPUFA supplemented formula group had plasma phospholipid levels of AA and DHA that were higher than levels in the human milk fed group. No differences in blood clinical values at 2 and 6 weeks among the groups (all were within normal ranges; though not all data reported)
Hematology	Red blood cell, white blood cell and platelet counts; hemoglobin; hematocrit; mean corpuscle volume; mean corpuscular hemoglobin concentration; white blood cell differential count: All values observed were within the normal range for all study groups.
Urinalysis	No mention of specific results, though they report no differences at 2 and 6 weeks among the groups (all were within normal ranges; though data not presented)
Growth	Body weight measured daily during hospitalization, crown-heel length measured weekly, occipitofrontal head circumference measured weekly. At birth, length of infants in the low AA+DHA group and in the HM group was lower as compared to length of infants in the high AA+DHA formula group; length of infants in the control group was

Table 2 Clinical Studies on AA and DHA in Preterm Infants	
	greater as compared to the HM group. No differences in growth parameters at 2 weeks among all feeding groups. At 6 weeks, formula fed infants exhibited greater rates of growth (weight and length) as compared to HM infants. No differences in weight and length gains at 6 weeks among the formula feeding groups.
Tolerance	No mention
Other	No mention
Dropouts	A total of 26 infants enrolled in the study did not complete the study due to protocol violations (17), failure to return for second blood sampling period (7), or exclusion per physician's request (2)
Authors' Comments	"Based on comparisons of the fatty acid erythrocyte membrane phospholipids and the plasma phospholipid pool between AA and DHA supplemented formulas and human milk fed infants, supplementation of formula with 0.6% AA and 0.4% DHA provides sufficient and perhaps optimal levels of these fatty acids for this population of infants."

Table 3
Clinical Studies on AA and DHA in Preterm Infants

Clandinin et al. 1999
***Acta Paediatr* 1999 Aug;88(8):890-896**

Study design	Randomized clinical trial
Population	Preterm infants who were medically table, appropriate for gestational age mean 31.8 wks); birth weight < 2300 g; infants received 100% of daily fluid and energy requirements enterally by day 14 of life Albert, Canada
Source	AA and DHA from single cell oils (Martek Biosciences Corporation, MD)
Treatment	

	AA (%FA)	DHA (%FA)	N = (6 weeks)
HM	0.54	0.30	25
FF	---	---	18
FF_{AA+DHA} (low)	0.32	0.24	18
FF_{AA+DHA} (medium)	0.49	0.35	18
FF_{AA+DHA} (high)	1.1	0.76	12

* human milk data appears to be taken from a published paper

Preemie SMA ® (Wyeth Nutritional International, Radnor, PA) was used as the control formula and as the base formula to which the LCPUFAs were added.

This report presents only the assessments of lipoprotein lipids; see Clandinin 1997 for additional results.

Feeding duration	from first enteral feedings to 6 weeks
Formula intake	No mention
Clinical chemistries	Blood samples collected at approximately 14 and 42 days of life (2 and 6 weeks, respectively) FA content in lipoprotein lipids (content of HDL, LDL, VLDL and chylomicrons in each of the triglyceride (TG) cholesterol ester (CE), and phospholipid fractions) at 6 weeks <u>Triglyceride fraction:</u> There were no dietary interactions in the main effects on HDL, LDL, VLDL, or chylomicron phospholipids. In general, supplementation with increasing amounts of AA and DHA tended to increase the levels of AA and DHA in the LDL, VLDL and chylomicron triglyceride. <u>Cholesterol ester fraction:</u> There were no dietary interactions in the main effects on HDL, LDL, VLDL, or chylomicron phospholipids. The AA content of the CE fraction of the lipoproteins was greater as compared to the CE concentration in the TG fraction, and responded to the level of AA in the formulas. The DHA content of the CE fraction wais relatively smaller as compared to the TG content, and did not follow a clear response to the level of DHA supplemented in the diet as observed for AA. The saturated FA content of the CE fraction was lower for infants fed the control formula. <u>Phospholipid fraction:</u> There were significant diet x age effects: HDL: DHA, AA LDL: DHA, AA VLDL: C16:0, C18:0, C18:1n-9, DHA, total fatty acid Chylomicrons: no significant differences The AA and DHA content of the HDL and LDL phospholipid contained the largest pool of these FA and this pool generally increased when these FA were supplemented into the formulas. The phospholipids in the VLDL and chylomicrons did not show a clear dose response effect of supplementation with AA and DHA, though DHA was higher in supplemented compared to unsupplemented formulas. The AA level was higher in the phospholipid of the chylomicron fraction for infants fed AA. On a quantitative basis, the phospholipid fraction contains > 75% of the AA and DHA pool present in the circulating plasma lipoproteins. Almost half of the total lipoprotein pool of AA and DHA is

Table 3 Clinical Studies on AA and DHA in Preterm Infants	
	present in the HDL phospholipid fraction.
Hematology	*See Clandinin 1997*
Urinalysis	*See Clandinin 1997*
Growth	*See Clandinin 1997*
Tolerance	No mention
Other	No mention
Dropouts	The dropout rate (1 to 2 infants per group) due to protocol violations was similar between groups.
Authors' Comments	"From comparison of the fatty acid levels present in the lipoproteins it appears that a formula level of 0.49% AA and 0.35% DHA provides sufficient levels of these fatty acids to achieve a similar fatty acid content of that of infants fed human milk for the major lipoprotein fractions examined."

Table 4
Clinical Studies on AA and DHA in Preterm Infants

Faldella et al. 1996
***Archives of Disease in Childhood* 1996;75:F108-F112**

Study design	Prospective, controlled, single blind study
Population	Healthy preterm infants, < 33 weeks gestational age, of appropriate weight, no malformations that could interfere with somatic and/or psychomotor development Bologna, Italy
Source	egg lipids (Milupan Ltd., also contains milk fat and vegetable oils)
Treatment	

	AA* (%FA)	DHA*(%FA)	N= (enrolled)	N = (completed)
HM	1.23	1.22	17	12
FF	0.01	---	26	25
FF_{AA+DHA}	0.35	0.23	23	21

* AA and DHA values based on analysis performed by study investigators; HM data based on 5 samples. FF_{AA+DHA} also contained 0.08% EPA.

Infants in the formula groups received less than 25% of their caloric intake from human milk, and HM infants received at least 75% of their own mother's milk.
The preterm formula used for both formula groups was Preaptamil.

Feeding duration	All infants in the study received at least 50% of their caloric intakes through enteral feedings by the 10th day of life, and continued the feedings until 52 weeks postconceptional age.
Formula intake	No mention
Clinical chemistries	Fatty acid composition of red blood cell membranes at 52 weeks PCA. no differences in AA significant differences in DHA (2.4%, 2.5%, 1.3% for HM, FF_{AA+DHA} and control infants, respectively), total *n*-6 (29.3%, 30.8%, 33.7% for HM, FF_{AA+DHA} and control formula infants, respectively) total *n*-3 (3.9%, 3.3%, 2.4% for HM, FF_{AA+DHA} and control formula infants, respectively)
Hematology	No mention
Urinalysis	No mention
Growth	Weight, length and head circumference examined weekly until 40 weeks PCA, then every two weeks; no differences were observed in postnatal growth velocity to 52 weeks PCA.
Tolerance	No mention.
Other	Visual evoked potential (VEP) (N-44), electroretinography (ERG) (N=53), and brainstem acoustic evoked potentials (BAEPs) (N=52). Infants fed the control formula had two longer wave latencies as compared to infants in the LCP supplemented group and the breast fed infants. There were no feeding effects on the ERG and BAEPS tests.
Dropouts	8 infants dropped due to deviations from feeding protocols; not all infants were in ideal behavioral state during the visual tests and some were sleepy, resulting in variable samples.
Authors' comments	The study supports previous findings in the importance of dietary LCP for an optimal visual development of preterm newborn infants.

Table 5
Clinical Studies on AA and DHA in Preterm Infants

Foreman-Van Drongelen et al. 1996 ***British J Nutrition* 1996;76:649-667**	
Study design	Randomized, double-blind, controlled trial
Population	Preterm infants born before 37th week of gestation, birth weight between 900-1795 g, and free from metabolic disease and major congenital malformations. Maastricht, Netherlands
Source	single cell oils (Martek Biosciences Corporation)
Treatment	<u>AA (%FA)</u> <u>DHA (%FA)</u> <u>N= (enrolled)</u> <u>N= (3 mo CA)</u> HM 0.43-0.64* 0.22-0.42* 4.5 d to 3-mo CA 12 12 FF (control) ND ND d 4.5 to 3-mo CA 16 14 FF_{AA+DHA} 0.61 0.30 d 7 to term CA 15 FF_{AA+DHA} 0.54 0.30 term to 3-mo CA 14 * range based on data from 6 subjects Infants started consuming preterm formula; consumed term formula when body weight reached 2000 g (approximately term CA. Fat-free human milk fortifier was added to the human milk for 5 infants. Energy sources such as fruits were given to both groups during the third month of corrected age. Some infants received intravenous RBC and plasma infusions and since these infusions contain AA and DHA, the time and amount of infusions were recorded. 30 mg -tocopherol/L was added to the AA+DHA enriched formulas.
Feeding duration	d 4.5 to 3 months corrected age for control group, d 7 to 3 months corrected age for LCP group (all infants began feeding by d 9 of life)
Formula intake	Full enteral feeding (420-500 kJ/d) by 12th day of life. Median formula intake: <u>0-35 d</u> <u>0-3 mo CA</u> HM NA NA FF 364.1 kJ/kg/d 494.8 kJ/kg/d FF_{AA+DHA} 357.2 kJ/kg/d 478.8 kJ/kg/d No differences in formula intake
Clinical chemistries	Plasma and RBC phospholipids were measured on d 0, 7, 14, 21, 35, and 3-mo CA: Plasma and RBC phospholipid AA and DHA concentrations increased significantly in the FF_{AA+DHA} group compared to the control group and were comparable to the HM (days 0-35), and at 3-mo CA. At 3-mo CA, the average absolute value of AA was > 3 times and DHA was > 2 times higher in the FF_{AA+DHA} group than in the control group.
Hematology	No mention
Urinalysis	No mention
Growth	Median daily weight gain (days 0-35) of control group was significantly greater than HM group; no difference in median weight gain from term to 3-mo CA between the formula groups.
Tolerance	No mention
Other	No mention
Dropouts	One infant in each formula groups was discharged before the required term study formulas were ready, and one infant in the FF group turned out to have been fed on a non-study term formula from 2-mo CA.
Authors' comments	The authors concluded that, "adding DHA and AA to artificial formulas in balanced ratios and in amounts similar to those found in preterm human milk raises both the DHA and the AA status of formula fed preterm infants to values found for human-milk-fed preterm infants".

Table 6
Clinical Studies on AA and DHA in Preterm Infants

Koletzko et al. 1989
***European Journal of Pediatrics* 1989;148:669-675**

Study design	Randomized, double-masked, clinical trial
Population	Preterm infants weighing ≥ 1300 g, clinically stable, tolerated full enteral feedings, mean weight = 1681 g; mean gestational age 34 weeks Memphis, TN
Source	Egg lipids and fish oil
Treatment	

	AA (%FA)	DHA (%FA)	N = (d 21)
HM	0.4	0.2	11
FF (control)	ND	ND	10
FF_{AA+DHA}	0.2	0.1	8

Infants were fed exclusively HM or one of the formulas. No infant received parenteral nutrition after the 3rd day of life; no infants received intravenous lipids. Infants were randomized to one of two feedings during the in-hospital phase at < 8 days of age (a mean of 4.9 days of age). No infants received human milk.
Preterm formula used in all feedings was Pre-Aptamil (Milupa, Friedrichsdorf, Germany). The control and supplemented formulas contained 13.2% LA, 1.0% ALA, 1.7 mg α-tocopherol-equivalents, and 11.8% LA, 0.7% ALA, and 1.4 mg α-tocopherol-equivalents, respectively.

This publication reports only the assessment of fatty acid status; see Koletzko 1995a for additional results on vitamin E status.

Feeding duration	d 4 to d 21 of life
Formula intake	No mention
Clinical chemistries	Fatty acid concentrations of plasma triglycerides, sterol esters, and phospholipds were measured on days 4 and 21.

Plasma triglycerides:
AA decreased significantly between d4 and d21 among infants in the control group and was significantly lower as compared to the AA+DHA group
DHA decreased significantly between d4 and d21 among infants in the HM, control and AA+DHA groups; levels were significantly lower in the control group as compared to the AA+DHA group
total n-6 LCP and total LCP concentrations decreased significantly between d4 and d21 among infants in the control group; total n-6 LCP and total LCP concentrations were significantly lower in the control and AA+DHA groups as compared to the HM group, and concentrations were significantly higher in the AA+DHA group as compared to the control group
Plasma sterol esters:
AA decreased significantly between d4 and d21 among infants in the control and AA+DHA groups, concentrations were significantly lower as compared to the HM group, and the AA+DHA group was significantly higher as compared to the control group
DHA decreased significantly between d4 and d21 among infants in the control group; no significant differences in %DHA among the groups
total n-6 LCP and total LCP concentrations decreased significantly between d4 and d21 in the control and AA+DHA groups, were significantly lower in the control and AA+DHA groups as compared to the HM group, and were significantly higher in the AA+DHA group as compared to the control group
Plasma phospholipids:
AA decreased significantly between d4 and d21 among infants in the control and AA+DHA groups, concentrations were significantly lower as compared to the HM group, and the control group was significantly lower as compared to the AA+DHA group
DHA decreased significantly between d4 and d21 among infants in the control and AA+DHA groups, control group was significantly lower as compared to the HM group
total LCP decreased significantly between d4 and d21 among infants in the control and AA+DHA

Table 6 Clinical Studies on AA and DHA in Preterm Infants	
	groups, controls were significantly lower as compared to the HM group, and the AA+DHA group was significantly higher as compared to the control group total n-6 LCP decreased significantly between d4 and d21 among infants in the control and AA+DHA groups, were significantly lower in the control and AA+DHA groups as compared to the HM group, and concentrations were significantly higher in the AA+DHA group as compared to the control group.
Hematology	No mention
Urinalysis	No mention
Growth	No mention
Tolerance	Investigators state that all infants tolerated the feeds well, and no side effects were noted.
Other	No mention
Dropouts	No mention
Authors' comments	"Our results demonstrate that small concentrations of dietary LCP have marked effects on plasma lipid composition, particularly on phospholipids, suggesting that dietary LCP are preferentially channeled into structural lipids. We conclude that the essential fatty acid status of formula-fed premature infants can be improved by a supplementation of omega-6- and omega-3LCP."

Table 7 Clinical Studies on AA and DHA in Preterm Infants	
Koletzko et al. 1995a ***International Journal of Vitamin Nutrition Research* 1995a;65:101-104**	
Study design	Randomized, double-masked, clinical trial
Population	Preterm infants weighing ≥ 1300 g, clinically stable, tolerated full enteral feedings, mean weight = 1681 g; mean gestational age = 34.0 weeks Memphis, TN
Source	egg lipids (Milupan Ltd., also contains milk fat and vegetable oils) and fish oil
Treatment	<u>AA* (%FA)</u> <u>DHA* (%FA)</u> <u>N = (d 21)</u> HM 0.4 0.2 15 FF (control) ND ND 8 FF_{AA+DHA} 0.2 0.1 9 * Information on fatty acid content was taken from a previous publication of the study (Koletzko 1989). Infants were fed exclusively HM or one of the formulas. No infant received parenteral nutrition after the 3rd day of life. Infants were randomized to one of two feedings during the in-hospital phase at < 8 days of age (a mean of 4.9 days of age). No infants received human milk. Preterm formula used in all feedings was Pre-Aptamil (Milupa, Friedrichsdorf, Germany). The control and supplemented formulas contained 13.2% LA, 1.0% ALA, 1.7 mg α-tocopherol-equivalents, and 11.8% LA, 0.7% ALA, and 1.4 mg α-tocopherol-equivalents, respectively. *This publication reports only the assessment of vitamin E status; see Koletzko 1989 for additional results on plasma fatty acids.*
Feeding duration	d 4 to d 21 of life
Formula intake	No mention
Clinical chemistries	Plasma and RBC α-tocopherol and total lipids concentrations were measured on days 4 and 21 <u>Plasma levels:</u> α-tocopherol: concentration significantly increased between d 4 and 21 in HM group; no changes in either formula group α-tocopherol/total lipid ratio: no significant differences <u>RBC levels:</u> α-tocopherol: no significant differences α-tocopherol/total lipid ratio: concentration significantly decreased between d 4 and 21 in FF_{AA+DHA} group; no changes in control or HM group
Hematology	No mention
Urinalysis	No mention
Growth	No mention
Tolerance	No mention
Other	No mention
Dropouts	No mention
Authors' comments	"[These results] raise the possibility that some forms of LCP-supplementation may increase -tocopherol requirements in low birth weight infants. All products for infant feeding containing preformed LCP should be carefully evaluated with respect to their possible effects on infantile antioxidant status"

Table 8
Clinical Studies on AA and DHA in Preterm Infants

Vanderhoof et al. 1999
***J Pediatric Gastroenterology Nutrition* 1999;29:318-326**

Study design	Randomized, double-blind, controlled trial
Population	Preterm infants 0-28 days old, birth weight between 750-2000 g, received enteral feeding for less than 24 hours 16 centers in the USA
Source	single cell oils (Martek Biosciences Corporation)
Treatment	

	AA (%FA)	DHA (%FA)	N= (enrolled)	N= (40 wk PCA)	N = (48 wk PCA)
HM	no data	no data	133	66	53
FF (control)	---	---	78	66	50
FF_{AA+DHA}	0.50	0.35	77	60	48

Human milk feedings were fortified with a powdered form of a human milk fortifier (Enfamil Human Milk; Fortifier, Mead-Johnson). Infants in the HM group who weaned at 40 wk consumed the unsupplemented Preemie SMA until 48 wk PCA.
Wyeth Preemie SMA formula (Phase I: 24-cal/oz formula; Phase II: 20-cal/oz formula)

This report presents findings through 48 wks; see Vanderhoof 2000 for additional results.

Feeding duration	Enrollment to 48 wks postconceptional age (PCA) Phase I: enrollment to 40 wks PCA (term CA) Phase II: 40 – 48 wks PCA (term to 2-mo CA) In phase II, HM infants weaned at 40 weeks PCA were fed the control formula, the formula fed infants received their respective formulas.
Formula intake	data collection started on the day when the intake was 145 ml/kg/d or more
Clinical chemistries	Serum albumin, alkaline phosphatase, total bilirubin, serum calcium, serum creatinine, serum potassium, phosphorous, urea nitrogen, alanine aminotransferase, aspartate aminotransferase, serum bicarbonate, serum chloride, direct bilirubin, serum sodium, TC, TG, fatty acid analysis were assessed at 40 and 48 wks Serum albumin: significant difference between the human milk and formula groups (FF_{AA+DHA} = control > HM) at 40 weeks, no difference at 48 weeks. Alkaline phosphatase: significant difference between the human milk and formula groups at 40 and 48 weeks (HM > control = FF_{AA+DHA}). Total bilirubin: significant difference between the HM and formula groups at 40 (HM > control = FF_{AA+DHA}) and 48 (HM > FF_{AA+DHA} = control) weeks. Serum calcium: significant difference between the HM and formula groups (control = FF_{AA+DHA} > HM) at 40 weeks, no difference at 48 weeks. Serum creatinine: significant difference between the HM and formula groups (control = FF_{AA+DHA} > HM) at 40 weeks, no difference at 48 weeks. Serum potassium: significant difference between the HM and formula groups (control > FF_{AA+DHA} > HM) at 40 weeks, no difference at 48 weeks. Phosphorous: significant difference between the HM and formula groups (FF_{AA+DHA} = control > HM) at 40 weeks, no difference at 48 weeks. Urea nitrogen: significant difference between the HM and formula groups (FF_{AA+DHA} = control > HM) at 40 weeks, no difference at 48 weeks. TC: higher in FF_{AA+DHA} group compared to the other groups at 40 and 48 weeks TG: Significant difference among the groups. FF_{AA+DHA} group had lower TG compared to other groups but the values were within the normal range. No significant difference between groups was observed for alanine aminotransferase, aspartate aminotransferase, serum bicarbonate, serum chloride, direct bilirubin, and serum sodium at 40 wks. Phosphotidylcholine and phosphotidylethanolamine AA and DHA concentrations were higher in the

Table 8 Clinical Studies on AA and DHA in Preterm Infants	
	FF_{AA+DHA} and HM group compared to the control group.
Hematology	Bands, basophils, eosinophils, hemoglobin, hematocrit, WBC, lymphocytes, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, monocytes, neutrophils, platelets, RBC at 40 and 48 weeks. No significant differences between groups were observed for bands, basophils, eosinophils, hemoglobin, hematocrit, WBC, lymphocytes, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, monocytes, neutrophils, platelets, RBC at 40 and 48 wks. Significant increase in anemia in the control (n=25/78) compared to the FF_{AA+DHA} group (n=12/77).
Urinalysis	NS difference between groups in urine pH and specific gravity at 40 and 48 wks.
Growth	NS difference in weight, length, and occipitofrontal and midarm circumference between the FF_{AA+DHA} and control groups. Significant difference in weight (40 wks/48 wks), length (40 wks) and midarm circumference (full feedings/40 wks/48 wks) between the FF_{AA+DHA} and HM groups (FF_{AA+DHA} > HM). NS difference in occipitofrontal circumference.
Tolerance	Differences at 48 wk PCA: Significant difference in diarrhea: FF_{AA+DHA} (n=10/77) > HM (n= 4/132); [control n=8/78]. Significant difference in flatulence: FF_{AA+DHA} (n=12/77) > control (n=3/78) and HM (n=7/132). Significant difference in intolerance to cow's milk: control (n=3/78)> FF_{AA+DHA} (n=0) and HM (n=0).
Other	NS differences between groups in respiratory, cardiovascular, GI, hemic and lymphatic, or urogenital system events. Significantly fewer nervous system events in the FF_{AA+DHA} (3.9 %) and HM (2.3%) groups compared to the control group (11.5%) at 48 wks. The numbers of infants with jaundice were 5, 1 and 13 in FF_{AA+DHA}, control and HM groups, respectively at 48 wks. Higher percent of infants had cutaneous moniliasis in the control group (5.25%) compared to the FF_{AA+DHA} (0%) or HM (0.8%) groups at 40 wks. Bradycardia and apnea were common in all 3 feeding groups and there was no significant difference between the groups at 40 wks. One infant on FF_{AA+DHA} formula died of SIDS (physician judged the death to be unrelated to the feeding of the study formula). A second infant on human milk for 1 day and then on standard infant formula died from necrotizing enterocolitis. The death was judged as unrelated to the participation in the study.
Dropouts	11 infants in the FF_{AA+DHA}, 10 in the control formula group and 9 infants in the HM group dropped out due to adverse events. The occurrence of adverse events was not significantly different among groups. The reasons for withdrawal were diarrhea (n=6), vomiting (n=8), abdomen enlarged (n=5) and pseudomembranous necrotizing enterocolitis (n=5) at 48 wks.
Authors' comments	The authors concluded that, "the results of this study demonstrate the safety and efficacy of a preterm formula supplemented with LCPUFAs from single-cell oil".

Table 9
Clinical Studies on AA and DHA in Preterm Infants

Vanderhoof et al. 2000
***J Pediatric Gastroenterology Nutrition* 2000;31:121-127**

Study design	Randomized, double-blind, controlled trial
Population	Preterm infants 0-28 days old, birth weight between 750-2000 g, received enteral feeding for less than 24 hours 16 centers in the USA
Source	single cell oils (Martek Biosciences Corporation)
Treatment	

	AA (%FA)	DHA (%FA)		N=(enrolled)	N=(92wk PCA)
HM	NA	NA	< 8d to 40 wk PCA	133	50
FF (control)	---	---	< 8d to 92 wk PCA	78	51
$FF_{+AA+DHA}$ (exp)	0.50	0.35	< 8d to 48 wk PCA	77	
FF (exp)	---	---	48 to 92 wk PCA		50

Wyeth Preemie SMA formula used until 48 wk PCA for both FF groups.
Infants on either formula feeding consumed term SMA from 48 to 92 wk PCA. Infants in the HM group who weaned at 40 wk consumed the unsupplemented Preemie SMA until 48 wk PCA. Infants in the HM group who weaned at 48 wk or later consumed the term SMA.

This report presents findings at 92 wks; see Vanderhoof 1999 for additional results.

Feeding duration	Treatment period from enrollment to 48 weeks PCA Follow-up period up to 92 weeks PCA (This publication concerns only the 48 to 92 wk PCA phase)
Formula intake	See Vanderhoof 1999
Clinical chemistries	Plasma phospholipid fatty acid levels at 92 wks PCA: RBC-PC, RBC- PE, total phospholipid, plasma PC, plasma PE. At 92 weeks PCA, the AA levels in plasma and RBC phospholipid subfractions were not different between the groups. The DHA levels in RBC-PC, RBC-PE, total plasma phospholipids and plasma PC subfractions were significantly different among the three groups; mean values for these subfractions were similar between the HM and experimental groups, and higher than means values in the control group.
Hematology	*See Vanderhoof 1999*
Urinalysis	*See Vanderhoof 1999*
Growth	<u>From 40 to 92 weeks PCA:</u> The increase in length was higher in the human milk and control group compared to the experimental formula group. The head circumference growth rate between 48 to 92 weeks was higher in the human milk group compared to the experimental group although the absolute head circumference was not different at 92 weeks. Changes in weight and midarm circumference from 40–92 weeks were not different between the groups. <u>92 weeks PCA:</u> There were no significant differences between the groups in weight, length or head circumference. Midarm circumference was similar between the control and experimental formula group but significantly lower in the human milk group.
Tolerance	*See Vanderhoof 1999*
Other	Seventy four (96.1%), 73 (93.6%) and 115 (86.5%) infants in the experimental, control and human milk group, respectively had at least one adverse event during the study. Bradycardia, apnea and infection were the most common adverse events.
Dropouts	The number of infants who were withdrawn were 82, 26 and 27 in the human milk, experimental formula and control group, respectively. The majority of infants in the HM group were withdrawn because of violation of the protocol. One infant in the experimental group died of SIDS (physician judged the death to be unrelated to the feeding of the study formula). A second infant on human milk for 1 day and then on standard infant formula died from necrotizing enterocolitis. The death was judged as unrelated to the participation in the study.
Authors' comments	The authors concluded that, "the results of this study demonstrate the efficacy and long-term safety of preterm formula supplemented with LCPUFAs".

Table 10
Clinical Studies on AA and DHA in Term Infants

Auestad et al. 1997 *Pediatric Research* 1997;41(1):1-10	
Study design	Longitudinal, prospective, randomized, double blinded
Population	Healthy term infants <7 days old; □37 weeks of gestation with weight appropriate for gestational age Kansas City, MO; Portland, OR; Seattle, WA
Source	egg yolk phospholipid (FF_{AA+DHA}), low-EPA tuna oil (FF_{DHA})
Treatment	

	AA	DHA	N = (12 mo)
HM*	0.48±0.10	0.15±0.09	63
FF (control)	0	0	45
FF_{DHA}	0	0.23	43
FF_{AA+DHA}	0.43	0.12	46

*mean values from human milk samples collected from 29 mothers in the study at the Portland site

Infants in the formula groups were to receive formula as the sole source of nutrition for a minimum of 4 months. Infants in the breast feeding group were to receive human milk as the sole source of nutrition for a minimum of 3 months; beyond 3 months the infants were permitted a commercial formula. The fish oil contained 0.07 % EPA.

This report deals only with assessments of growth, fatty acid composition of RBCs, tolerance and visual acuity during the first 12 months; see Scott 1998 for additional results.

Feeding duration	from < 7 days to 4 mo, and potentially to 1 year
Formula intake	Formula intake did not differ among the formula groups at 1, 2, or 4 months.
Clinical chemistries	Fatty acid composition in RBC PC and RBC PE at 2, 4, 6, and 12 mo (only the results for 4 and 12 mo are presented in this report). The results presented are limited to AA, DHA, and the *n*-6/*n*-3 and AA/DHA ratios and the relationship of the AA+DHA supplemented infants to the other groups. RBC-PC fatty acids measured at 4 and 12 months: AA and DHA concentrations did not differ FF_{AA+DHA} and HM infants at 4 and 12 months. At 4 and 12 months, AA levels were higher in the FF_{AA+DHA} group as compared to the control and FF_{DHA} groups. At both 4 and 12 months, DHA levels in the FF_{AA+DHA} infants were higher as compared to the control group and lower as compared to the FF_{DHA} group. The *n*-6/*n*-3 ratio was higher in FF_{AA+DHA} infants as compared to HM and FF_{DHA} infants at 4 months, while it was lower as compared to control infants. At 12 months, infants in the FF_{AA+DHA} group had a higher *n*-6/*n*-3 ratio as compared to breast fed infants and FF_{DHA} infants, and lower as compared to the control formula infants. There were no differences in the AA/DHA ratio between the FF_{AA+DHA} group and the HM infants; but at both 4 and 12 months, the AA/DHA ratio in FF_{AA+DHA} infants was lower as compared to control formula infants and higher as compared to FF_{DHA} infants. RBC-PE fatty acids measured at 4 and 12 months: Infants fed FF_{AA+DHA} did not differ from HM infants in AA levels at 4 or 12 months; AA levels were higher as compared to control formula and FF_{DHA} infants at 4 months, and higher than FF_{DHA} infants at 12 months. DHA levels were lower as compared to HM and FF_{DHA} infants at 4 months while levels were higher as compared to the control formula fed infants. At 12 months, DHA levels in infants in the FF_{AA+DHA} group were higher than levels in control formula infants and lower as compared to FF_{DHA} infants. The *n*-6/*n*-3 ratio in FF_{AA+DHA} infants at 4 months was higher as compared to HM and FF_{DHA} infants, and lower as compared to control formula infants. At 12 months, the *n*-6/*n*-3 ratio in FF_{AA+DHA} infants was higher as compared to breast fed and FF_{DHA} infants. The AA/DHA ratio at 4 months was lower as compared to the control group and higher as compared to the HM and FF_{DHA} groups; at 12 months, the FF_{AA+DHA} group was lower as compared to the control group and higher as compared to the FF_{DHA} group.

Hematology	No mention
Urinalysis	No mention
Growth	No significant differences were found among feeding groups, or as compared to HM infants, in weight, crown-heel length, or head circumference at birth, study entrance, or 1, 2, 4, 6, 9, and 12 months of age.
Tolerance	Formula tolerance, based on occurrence of spit-up and vomiting did not differ among the formula groups at 1, 2, or 4 months. Infants in the FF_{AA+DHA} group had firmer stools as compared to infants in the control, FF_{DHA}, and HM groups at 2 and 4 months.
Other	Visual function (Teller Acuity Card procedure [behavioral method] and sweep VEP method [electrophysiologic method]) at 2, 4, 6, 9, and 12 months No differences between feeding groups at any time points.
Dropouts	Poor compliance, early cessation of breast feeding, formula intolerance, or occurrence of an illness that compromised study participation; 1 SIDs death in the DHA supplemented group.
Authors' comments	"These data show that provided 0.4 wt% AA and 0.12 wt% DHA from egg yolk phospholipid in formula results in RBC membrane AA and DHA levels similar to those of infants fed human milk. However, the fact that visual function was not different among any of the groups in this study does not support adding DHA or AA to infants formulas."

Table 11
Clinical Studies on AA and DHA in Term Infants

Scott et al. 1998
***Pediatrics* 1998;102(5). URL: http://www.pediatrics.org/cgi/content/full/102/5/e59**

Study design	Longitudinal, prospective, randomized, double blinded
Population	Healthy term infants <7 days old; ≥ 37 weeks of gestation with weight appropriate for gestational age Kansas City, MO; Portland, OR; Seattle, WA
Source	egg yolk phospholipid (FF_{AA+DHA}), low-EPA tuna oil (FF_{DHA})
Treatment	

	AA (% FA)	DHA (% FA)	N = (12 mo)	N = (14 mo)
HM*	0.48±0.10	0.15±0.09	63	60
FF (control)	0	0	45	42
FF_{DHA}	0	0.23	43	33
FF_{AA+DHA}	0.43	0.12	46	38

*mean values from human milk samples collected from 29 mothers in the study at the Portland site

Infants in the formula groups were to receive formula as the sole source of nutrition for a minimum of 4 months. Infants in the HM group were to receive human milk as the sole source of nutrition for a minimum of 3 months; beyond 3 months the infants were permitted a commercial formula. Beginning at 4 months of age, all feeding groups were permitted solid food supplementation. The fish oil contained 0.07 % EPA. (Some of this information was obtained from an earlier publication of the same study.)

This report presents only the assessments of MDI, PDI, and language development; see Auestad 1997 and for additional results.

Feeding duration	from < 7 days to 1 year
Formula intake	*See Auestad 1997*
Clinical chemistries	Post hoc correlation analyses of red cell or plasma phospholipid concentrations of DHA or AA and vocabulary production or vocabulary comprehension. In the HM group: negative correlations at 4 months between red cell DHA concentrations and vocabulary production and comprehension. Among the formula groups: negative correlations at 4 months between red cell and plasma phospholipid DHA concentrations and vocabulary production, and between plasma phospholipid DHA concentrations and vocabulary comprehension. No consistent correlations were found between 4 month AA levels and vocabulary results at 14 months.
Hematology	No mention
Urinalysis	No mention
Growth	*See Auestad 1997*
Tolerance	*See Auestad 1997*
Other	Mental and Motor Scales of the Bayley Sales of Infant Development were assessed at 12 months of age No differences in MDI or PDI scores across all feedings or across the three formula groups. Language was assessed (MacArthur Communicative Development Inventories) at 14 months of age. Infants in the DHA supplemented formula group had lower vocabulary comprehension as compared to HM infants, and lower vocabulary production as compared to control formula fed infants.
Dropouts	Not detailed
Authors' comments	"[The authors] found no evidence that infants in the unsupplemented control formula group performed less well than breast-fed infants or that supplementation conferred any developmental benefits."

Table 12
Clinical Studies on AA and DHA in Term Infants

Birch et al. 1998 ***Pediatric Research* 1998;44(2):201-209**						
Study design	Randomized, double-masked, controlled trial					
Population	Term infants born 37-40 week PCA, singleton births, birth weight appropriate for gestational age Dallas, Texas (2 sites)					
Source	single cell oils (Martek Biosciences)					
Treatment		AA (%FA)	DHA (%FA)	N = (enrollment)	N = (17 wks)	N = (52 wks)
	HM*	0.56	0.29	29	21	20
	FF (control)	0.00	0.00	26	23	21
	FF_{DHA}	0.02	0.35	26	22	20
	FF_{AA+DHA}	0.72	0.36	27	23	19
	*HM data reflects composition of 10 samples obtained approximately 6 wks postpartum Assigned formulas were fed exclusively for the duration of the feeding period (17 wk); beyond 17 wk, formula fed infants received commercial formula, and HM infants consumed human milk and/or formula. All study formulas were Enfamil® with Iron (Mead Johnson). *This publication concerns assessments made within 52 wks of age; see Birch 2000 for results of mental development assessments; see Hoffman 2000 for results of ERG measures and correlations between fatty acid measures and visual development responses.*					
Feeding duration	0 to 4 days through 17 weeks.					
Formula intake	No mention					
Clinical chemistries	RBC lipid analyses at enrollment, 17 wk, and 52 wk At 17 wks, RBC DHA levels in both supplemented formula groups and the HM group were significantly higher compared to the control group; the FF_{DHA} group had significantly lower AA levels as compared to the HM and FF_{DHA+AA} groups. At 52 wks, there were no differences in RBC AA levels among the groups; RBC levels of DHA were higher in the FF_{DHA} and HM groups as compared to the control group.					
Hematology	No mention					
Urinalysis	No mention					
Growth	No significant effects of treatment on weight, length and weight-for-length. No significant differences among groups were observed for head circumference, subscapular fat or triceps fat deposition.					
Tolerance	All diets were well tolerated (no data presented)					
Other	Sweep VEP acuity assessed at 6, 17, 26, and 52 wk Significant main effects of diet and of age: Sweep VEP was better in the supplemented groups as compared to the control group at 6, 17 and 52 wks; there were no differences between the supplemented groups and the HM group. FLP Acuity assessed at 6, 17, 26, and 52 wk There was no treatment effect on FPL acuity. Relationship between sweep VEP at 6, 17, 26 and 52 wk and RBC FA concentrations at 17 wk: The strongest correlation was observed between higher DHA and n-3/n-6 ratio in RBCs and better sweep VEP acuity.					
Dropouts	By 17 weeks 3, 4, 4 and 8 subjects dropped out in the control, DHA, DHA + AA and human milk groups, respectively due to reasons not related to the treatments. At 6 weeks 3, 2, 1 and 2 subjects dropped out in the control, DHA, DHA + AA and human milk groups, respectively due to symptoms associated with lactose or cow's milk protein intolerance.					
Authors' comments	The authors concluded that "early dietary intake of preformed DHA and AA appears necessary for optimal development of the brain and eye of the human infant".					

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Table 13
Clinical Studies on AA and DHA in Term Infants

Birch et al. 2000 ***Developmental Medicine & child Neurology* 2000;42:174-181**	
Study design	Randomized, double-masked, controlled trial
Population	Term infants born 37-40 week PCA, singleton births, birth weight appropriate for gestational age Dallas, Texas (2 sites)
Source	Single cell oils (DHASCO® and ARASCO®, Martek Biosciences)
Treatment	

	AA (%FA)	DHA (%FA)	N = (enrollment)	N = (52 wks)	N = (18 months)
FF (control)	0.00	0.00	26	21	20
FF_{DHA}	0.02	0.35	26	20	17
FF_{AA+DHA}	0.72	0.36	27	19	19

Note: infants in the HM group were not mentioned in this publication

Assigned formulas were fed exclusively for the duration of the feeding period (17 wk); beyond 17 wk, formula fed infants received commercial formula, and HM infants consumed human milk and/or formula.
All study formulas were Enfamil® with iron (Mead Johnson)

This publication concerns only the mental development assessment made at 18 months; see Birch 1999 for results of growth, clinical chemistries, and visual assessments; see Hoffman 2000 for results of ERG measures and correlations between fatty acid measures and visual development responses.

Feeding duration	0 to 4 days through 17 weeks
Formula intake	No mention
Clinical chemistries	See Birch 1999
Hematology	No mention
Urinalysis	No mention
Growth	*See Birch 1999*
Tolerance	*See Birch 1999*
Other	Bayley Scales of Infant Development at 18 months (± 2 weeks) of age: Mental Development Index (MDI): Infants in the AA+DHA group had higher MDI scores compared to the control group at 18 months. MDI score at 18 months correlated positively to plasma DHA and RBC DHA levels at 4 months. There was no correlation between AA at 4 months and MDI at 18 months, or any plasma measures at 12 months and MDI at 18 months. Psychomotor development index (PDI): No effect of LCP was observed on PDI scores, and PDI score was not correlated to plasma or RBC AA or DHA levels at 4 or 12 months. Cognitive, language and motor subscales: The DHA and AA+DHA groups performed significantly better on the cognitive and motor subscales as compared to the control group. The three groups did not differ significantly on the language subscale. Behavioral rating scale (BRS): No significant difference between the groups.
Dropouts	By 18 months 6/26, 9/26 and 8/27 infants were withdrawn from the control, DHA and AA+DHA groups, respectively. The withdrawals were either due to intolerance to formula (3 in control, 2 in the DHA and 1 in AA+DHA group), illness unrelated to treatment or were lost to follow-up.
Authors' comments	"Early dietary intake of DHA and AA appear necessary for optimal development of the human brain and eye."

Table 14 Clinical Studies on AA and DHA in Term Infants	
Carlson et al. 1996 ***Pediatric Research* 1996;39:882-888**	
Study design	Randomized, double-blind study
Population	Term infants, born 37-43 wk PMA Memphis, TN
Source	egg yolk lecithin
Treatment	AA (%FA) / DHA (%FA) / N = 58 (4 mo) HM* 0.6 / 0.1 / 19 FF (control) 0.0 / 0.0 / 20 FF_{AA+DHA} 0.43 / 0.1 / 19 * values from published data for women in the United States after ~ 8 wk of lactation A total of 94 infants were enrolled into the study. Infants in the HM group were exclusive breastfed for at least 3 mo. The formula used in the study was Similar with Iron (Ross Products)
Feeding duration	from d 1 to 4 mo
Formula intake	No mention
Clinical chemistries	Fatty acid composition of plasma PC and RBC PC and PE at 0, 2, 4, 6, and 12 mo Plasma PC AA and DHA levels were significantly lower in the control group as compared to the FF_{AA+DHA} group at 2, 4, and 6 mo RBC PE levels of DHA were greater in the FF_{AA+DHA} group as compared to controls at 4, 6, and 12 mo RBC PE levels of AA were greater in the FF_{AA+DHA} group as compared to controls at 2, 6 and 12 mo RBC PE levels of AA and DHA were similar in HM and FF_{AA+DHA} supplemented infants at 2 mo
Hematology	No mention
Urinalysis	No mention
Growth	No mention
Tolerance	No mention
Other	Binocular visual acuity assessments (Teller Acuity Cards) at 2, 4, 6, 9, and 12 mo Significantly higher visual acuity in HM and FF_{AA+DHA} infants at 2 mo as compared to control infants; no differences among feeding groups at other times
Dropouts	36 infants lost before 4 mo: 16 HM, 11 control, 9 FF_{AA+DHA} due to failure to return for visits (21), decide not to participated or quit breastfeeding (14), or because mother restricted infant's feeding (1). 1 SIDs death in the AA+DHA supplemented group.
Authors' comments	"Despite normal intrauterine accumulation of DHA and AA, infants fed formula with 2% α-linolenic acid and 0.1% DHA had better 2-mo visual acuity that infants fed formula with 2% α-linolenic acid."

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Table 15 Clinical Studies on AA and DHA in Term Infants	
Kohn et al 1994 ***European Journal of Clinical Nutrition* 1994;48(S2):S1-S7**	
Study design	Randomized trial
Population	Healthy, term infants, gestational ages 38.5-41.5 wks, birth weights 2800-4000 g Brugge, Belgium
Source	egg yolk lipids
Treatment	AA / DHA / N = (enrollment) HM* 0.36 0.22 No data FF (control) 0.02 < 0.01 No data FF_{AA+DHA} 0.25 0.18 No data * values based on published data The experimental formula contained 18 mg cholesterol per 100 mL.
Feeding duration	birth to 3-mo
Formula intake	No mention
Clinical chemistry	Fatty acid analyses of plasma and RBC phospholipids at d 0, 7, 30, and 90 Plasma phospholipids: AA: significant higher in HM group as compared to control or FF_{AA+DHA} at d 7, and control at d 30; the AA+DHA group was higher than the control group at d 7, d 30 and d 90 DHA: significantly higher in HM group as compared to control group on d 30 and d 90, and FF_{AA+DHA} group significantly higher than control group on d 30 and d 90 (changes are plasma cholesterol ester and plasma triglycerides are reportedly similar) RBC PC: AA: significantly higher in HM group as compared to both formula groups at d 7, and as compared to control group at d 30; FF_{AA+DHA} group significantly higher than control group at d 30 and d 90 DHA: significantly lower in control group at d 30 and d 90 as compared to both HM and FF_{AA+DHA} groups RBC PE: AA: no significant differences at any time point DHA: control group significantly lower than HM and FF_{AA+DHA} group at d 90
Hematology	No mention
Urinalysis	No mention
Growth	No differences in increase of weight or length during the study
Tolerance	All infants tolerated the feeds well; no side effects observed (no data presented)
Other	No mention
Dropouts	No mention
Authors' comments	"The introduction of milk-feeding leads to marked differences in the blood lipid composition of term infants during the first months of life, independent of the feeding regimen. The supplementation of an infant formula with LCP based on egg lipids seems to result in a blood lipid pattern similar to that observed in breastfed infants."

Table 16
Clinical Studies on AA and DHA in Term Infants

Lucas et al. 1999
***The Lancet* 1999;354:1948-1954**

Study design	Double-blind, randomized, controlled trial
Population	Healthy, term infants (37 weeks of gestation), singletons, appropriate size for gestational age Nottingham and Leicester, UK (4 sites)
Source	AA and DHA obtained from purified egg phospholipid and triglyceride fractions Lipid Teknic, Norway
Treatment	

	AA (%FA)	DHA (%FA)	N = (enrolled)	N = (9 mo)	N = (18 mo)
HM	no data	no data	138		104
FF (control)	--	---	155	115	125
FF_{AA+DHA}	0.30	0.32	154	126	125

The standard formula used in all feedings was Nestec Ltd, (Vevey, Switzerland)

Feeding duration	from the first week of life to 6 months
Formula intake	

	6 wks (mL/d)	12 wks (mL/d)	26 wks (mL/d)
FF	688	708	481
FF_{AA+DHA}	699	710	498

No differences in formula intake between groups

Clinical chemistries	No mention
Hematology	No mention
Urinalysis	No mention
Growth	Weight, occipitofrontal head circumference, and length were measured at 6 weeks, 12 weeks, and 6, 9, and 18 months. No differences in growth or growth rate between formula groups at 6, 9 or 18 mo Head circumference of infants at baseline and at 18 months was lower in both formula fed groups as compared to the HM group Mid upper-arm circumference and triceps and subscapular skinfold thickness were measured at 6, 9, and 18 months. No differences between formula groups
Tolerance	Volume of formula consumed, age starting solids, stool characteristics (presence of blood (not reported but declared as data collected in the method), constipation, consistency), medical histories (infection, atopy, medications) No differences in any measures between formula groups
Other	Bayley Scales of Infant Development (MDI) and (PDI) at 18 months: No differences in Bayley MDI and PDI scores (intent-to-treat and evaluable groups of formula fed infants); no differences among formula groups and breast fed infants Knobloch, Passamanik, and Sherrads Developmental Screening Inventory at 9 months: No differences between formula groups; no differences among formula groups and breast fed infants Occurrence of adverse events and infection-related events: No differences between control and AA+DHA supplemented groups; no differences among formula groups and HM infants
Dropouts	Withdrew secondary to adverse events, lost to follow up, stopped breast feeding before 6 weeks of age (breast fed group of infants); no significant difference is dropout numbers to 6 months
Authors' comments	"There was no evidence of a beneficial or adverse effect on cognitive and motor development or growth up to 18 months. [This] trial does not provide support for addition of LCPUFA to standard infant formula."

TABLE 17
Clinical Studies on AA and DHA in Term Infants

Makrides et al. 1999
***Pediatrics* 1999;104(3):468-475**

Study design	Randomized, double-blind trial
Population	Term infants, white, > 37 weeks' gestation, appropriate weight for gestational age Adelaide, Australia
Source	egg yolk phospholipid (FF_{AA+DHA}), low-EPA tuna oil (FF_{DHA})
Treatment	

	AA (% FA)	DHA (% FA)	N = (enrolled)	N = (34 wks)	N = (2 yr)
HM*	0.39±0.07	0.20±0.07	63	23 **	
FF (control)	ND	ND	28	21	20
FF_{DHA}	ND	0.35	27	23	23
FF_{AA+DHA}	0.34	0.34	28	24	19

*mean values from human milk samples collected from 7 human milk samples taken on 7 consecutive days during the 16th week of lactation.
** fully breastfed at 34 weeks

Infants were considered to be full breast feeding if they consumed 200 mL of formula per week in the first 16 weeks of life, and = 200 mL of formula per day between the ages of 16 weeks and 1 year. The formulas used in the study were supplied by Nestec Ltd (Konolfingen, Switzerland) in a powder form. The fish oil contained 0.10 % EPA.

This report presents only the assessments of growth and fatty acid status; see Makrides 2000 for additional results.

Feeding duration	from the first week of life until 1 year of age
Formula intake	No mention
Clinical chemistries	plasma and erythrocyte phospholipid FA were assessed at 6 wk, 16wk, 34 wk and 1 yr plasma phospholipid DHA: at d 5-7, control < all other groups at 6 and 34 weeks, control < HM < FF_{AA+DHA} < FF_{DHA} at 16 wk and 1 yr, control < HM < FF_{AA+DHA} = FF_{DHA} plasma phospholipid AA: at d 5-7, control < FF_{AA+DHA} < HM and FF_{DHA} < HM at 6 and 16 weeks, FF_{DHA} < control < FF_{AA+DHA} < HM at 34 wk FF_{DHA} < control < FF_{AA+DHA} = HM at 1 yr, FF_{DHA} < all other groups At 16 weeks of age, plasma phospholipid EPA levels were similar in infants fed FF_{DHA} and HM but were lower in control and FF_{AA+DHA}. Similar trends in fatty acid levels were observed in RBC-phospholipids (data not presented in the paper).
Hematology	No mention
Urinalysis	No mention
Growth	Weight, length and head circumference were measured at 6 wk, 16wk, 34 wk, 1 yr and 2 yr No significant differences in weight, length and head circumference were observed between the formula groups even after adjusting for gender, gestational age and postnatal age at assessment. The differences in weight and length between the human milk and the formula groups at 34 weeks and at 1 and 2 years of age were within the limits of expected standard growth and more consistent among girls than boys. Breast fed infants were shorter compared to the formula fed infants.
Tolerance	The frequency of parents reporting restlessness, rash, vomiting, diarrhea, and constipation was similar in all formula fed groups at 6 and 16 weeks of age (data not presented in the paper).
Other	*see Makrides 2000*
Dropouts	By 34 weeks, 15 infants in the formula groups and 17 in the HM group were withdrawn. Between 34 weeks and 2 years of age, 7 infants were lost to follow-up.
Authors' comments	The authors suggest that, "dietary LCPUFA do not influence growth of healthy term infants to a clinically significant degree".

Table 18
Clinical Studies on AA and DHA in Term Infants

Makrides et al. 2000 ***Pediatrics* 2000;105(1):32-38**	
Study design	Randomized, double-blind trial
Population	Term infants, white, > 37 weeks' gestation, appropriate weight for gestational age Adelaide, Australia
Source	egg yolk phospholipid (FF_{AA+DHA}), low-EPA tuna oil (FF_{DHA})
Treatment	

	AA (% FA)	DHA (% FA)	N = (enrolled)	N = (34 wks)	N = (2 yr)
HM*	0.39±0.07	0.20±0.07	63	23 **	22-23
FF (control)	ND	ND	28	21	18-19
FF_{DHA}	ND	0.35	27	23	19-22
FF_{AA+DHA}	0.34	0.34	28	24	19

*mean values from human milk samples collected from 7 human milk samples taken on 7 consecutive days during the 16th week of lactation.
** fully breastfed at 34 weeks

Infants were considered to be full breast feeding if they consumed 200 mL of formula per week in the first 16 weeks of life, and = 200 mL of formula per day between the ages of 16 weeks and 1 year. The formulas used in the study were supplied by Nestec Ltd (Konolfingen, Switzerland) in a powder form. The fish oil contained 0.10 % EPA.

This report presents only the assessments of VEP, MDI, and PDI; see Makrides 1999 for additional results.

Feeding duration	from the first week of life until 1 year of age
Formula intake	No mention
Clinical chemistries	*see Makrides 1999*
Hematology	No mention
Urinalysis	No mention
Growth	*see Makrides 1999*
Tolerance	*see Makrides 1999*
Other	VEP acuity assessed at 16 and 34 weeks No differences among formula groups at 16 or 34 weeks of age At 34 weeks, HM infants had better VEP acuity as compared to all formula fed infants Bayley's MDI and PDI assessed at 1 and 2 years of age No differences among formula groups at 1 or 2 years At 2 years, HM infants had higher MDI scores as compared to formula fed infants
Dropouts	7 lost, no explanation
Authors' comments	"LCPUFA supplementation did not influence VEP acuity development in these well-nourished, formula fed infants."

Table 19 Clinical Studies on AA and DHA in Term Infants	
Willatts et al. 1996 ***Major Controversies in Infant Nutrition* (David TJ, ed) pp 57-70, Royal Society of Medicine Press, London. 1996**	
Study design	Randomized, blinded
Population	Term infants, birth weight 2500-4000 g; gestation 37-42 weeks Dundee, UK
Source	egg lipids (Milupan Ltd., also contains milk fat and vegetable oils)
Treatment	AA (%FA)* / DHA (%FA)* / N = (3 mo) HM / / / 27 FF (control) / < 0.10 / --- / 24 FF_{AA+DHA} / 0.30-0.40 / 0.15-0.25 / 24 * these fatty acid composition data were obtained from a related publication by these investigators The standard term formula used was Aptamil (Milupa Ltd.); the LCPUFA formula was supplemented with Milupan. Infant formulas were supplied as ready-to-feed. *This paper presents results of assessments completed in the first 3mo; see Willatts 1998a and Willatts 1998b for additional outcomes.*
Feeding duration	Formula fed from birth until age 4 months
Formula intake	3 mo intake (mL/d) FF / 832 FF_{AA+DHA} / 828 No differences in formula intakes at 1, 2, or 3 mo (not all data are presented)
Clinical chemistries	No mention
Hematology	No mention
Urinalysis	No mention
Growth	Weight, crown-heel length, occipito-frontal head circumference, subscapular skinfold, triceps skinfold, and midarm circumference were measured at birth and at 3 months. Infants in the control group had greater triceps skinfold at birth as compared to the FF_{AA+DHA} group. HM infants had greater OFC compared to controls, and greater length compared to both formula groups.
Tolerance	No mention
Other	Habituation tested using the Infant Control Procedure at 3 mo: No significant differences between the formula groups. Infants were then categorized into early or late peak fixation groups: Control infants with late peak fixation had significantly longer peak fixation times as compared to the control infants, though after adjusting for mid-upper arm circumference at birth as a covariate, there were no differences in any of the habituation measures.
Dropouts	26 who met criteria for acceptance into study did not complete it: mother did not respond to recruitment letter (13) ; didn't attend assessment (6), infant unable to complete (7)
Authors' comments	"These findings suggest that term infants with evidence of intrauterine growth retardation may experience more efficient information processing if supplemented with LCPUFA."

Table 20
Clinical Studies on AA and DHA in Term Infants

Willatts et al. 1998a ***Lipids* 1998a;33(10):973-980**	
Study design	Randomized, blinded
Population	Term infants, birth weight 2500-4000 g; gestation 37-42 weeks Dundee, UK
Source	egg lipids (Milupan Ltd., also contains milk fat and vegetable oils)
Treatment	

	AA(%FA)	DHA (%FA)	N = (randomized)	N = (9 mo)
FF (control)	< 0.10	---	31	20
FF_{AA+DHA}	0.30-0.40	0.15-0.25	27	20

The standard term formula used was Aptamil (Milupa Ltd.); the LCPUFA formula was supplemented with Milupan. Infant formulas were supplied as ready-to-feed.

This paper presents results of the test performed at 9 mo; see Willatts 1998b and Willatts 1996 for additional outcomes.

Feeding duration	Formula fed from birth until age 4 months
Formula intake	*See Willatts 1996*
Clinical chemistries	No mention
Hematology	Mo mention
Urinalysis	No mention
Growth	No mention
Tolerance	No mention
Other	Problem solving assessment at 9 months of age (involved infants who demonstrated either an early or a late peak fixation on the habituation assessment at 3 mo): There were no differences in intention score between the groups. There was a significant diet x peak fixation at 3 mo interaction covaried with gestation and birth weight for the number of intentional solutions. Infants in the control group who had late peak-fixation at 3 months had a lower number of intentional solutions. There was no difference when early- and late- fixation infants were grouped together. Infants in the control group had a lower mean number of weeks of gestation at birth as compared to the FF_{AA+DHA} group.
Dropouts	Failed to complete first assessment: mother failed to attend (n=4), infant distressed (n=6); Failed to complete second assessment – mother failed to attend (n=7), data lost through error (n=1)
Authors' comments	"These findings suggest that in term infants who have reduced growth parameters at birth and who show evidence of impaired attention control, information processing and problem-solving ability in infancy may be enhanced by LCPUFA supplementation."

Table 21
Clinical Studies on AA and DHA in Term Infants

Willatts et al. 1998b ***The Lancet* 1998b;352:688-691**	
Study design	Randomized, blinded
Population	Term infants, birth weight 2500-4000 g; gestation 37-42 weeks Dundee, UK
Source	egg lipids (Milupan Ltd., also contains milk fat and vegetable oils)
Treatment	

	AA (%FA)	DHA (%FA)	N = (randomized)	N = (completing study)
FF (control)	< 0.10	---	38	23
FF_{AA+DHA}	0.30-0.40	0.15-0.25	34	21

The standard term formulas were Aptamil (Milupa Ltd.); the LCPUFA formula was supplemented with Milupan (Milupa Ltd.).

This paper presents results of test performed at 10 mo; see Willatts 1998a and Willatts 1996 for additional outcomes.

Feeding duration	from birth until age 4 months
Formula intake	*See Willatts 1996*
Clinical chemistries	No mention
Hematology	No mention
Urinalysis	No mention
Growth	See Willatts 1996
Tolerance	No difference reported in formula intake between 1 and 3 months (data not shown)
Other	Three-step problem solving ability was assessed at 10 months Infants in the LCPUFA supplemented formula group had higher intention scores and a higher number of intentional solutions for both the entire problem and the third step as compared to infants fed the control formula.
Dropouts	Failed to attend problem solving assessment (n=24), distressed (n=1), consumed formula < 3 months (n=3)
Authors' comments	Term infants may benefit from LCPUFA supplementation, and effects may persist beyond the period of supplementation. Supplementation may be important for the development of childhood intelligence.

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Table 22
Clinical Studies on AA and DHA in Term Infants

Hoffman et al., 2000
***Journal of Pediatric and Gastroenterology and Nutrition* 2000;31:540-553**

Study design	Double-blind, randomized, clinical trial
Population	Healthy, term infants (37-40 weeks PCA) Dallas, TX (2 sites)
Source	Single-cell oils (Martek Biosciences)
Treatment	

	AA (%FA)	DHA (%FA)	N = ERG arm 17 wk (33 enrolled)	N = VEP arm 17 wk (79 enrolled)
HM	no data	no data	no data	21
FF (control)	--	---	8	20
FF_{DHA}	0	0.35	7	21
FF_{AA+DHA}	0.72	0.36	9	22

Assigned formulas were fed exclusively for the duration of the feeding period (17 wk); beyond 17 wk, formula fed infants received commercial formula, and HM infants consumed human milk and/or formula.
All study formulas were Enfamil® with Iron (Mead Johnson).

This publication presents results of analyses of blood lipid fractions as related to information on infant fatty acid status, dietary compliance, and correlations with visual development. Results of electroretinography measurements are also presented. See Birch 1999 for results of growth, clinical chemistries, and visual assessments; see Birch 2000 for result of mental development assessments.

Feeding duration	within the first 4 days of life through 17 weeks of age
Formula intake	No mention
Clinical chemistries	*See Birch 1999*
Hematology	No mention
Urinalysis	No mention
Growth	*See Birch 1999*
Tolerance	*See Birch 1999*
Other	Significant correlations were found between the DHA content in various lipid fractions and ERG responses at 6 weeks, and VEP responses at 17 weeks. RBC PC was most sensitive to dietary n-6 fatty acids, and total RBC and RBC PE were most responsive to variations in dietary DHA. At 6 weeks, the electroretinographic (ERG) response (log k) of infants fed the AA+DHA supplemented formula reflected a more mature response as compared to control formula infants; ERG responses at 17 weeks were similar in all formula groups. See Birch 1999, 2000
Dropouts	ERG arm: 9 infants dropped out before 6 wk, 5 primarily due to lactose intolerance VEP arm: 16 infants dropped out by 17 weeks, 8 of whom had symptoms of lactose or cow's milk protein intolerance.
Authors' comments	"These data support the concept that early nutrition influences proper development of visual function later in life."

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SUBMISSION END

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