JHeimbach LLC

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August 17, 2018

Paulette Gaynor, Ph.D.
Senior Regulatory Project Manager
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Gaynor:

Pursuant to 21 CFR Part 170, Subpart E, Arla Foods Ingredients Group P/S (Arla), through me as its agent, hereby provides notice of a claim that the addition of alpha-lactabumin to cow-milk-based non-exempt term infant formula is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Arla has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, one copy of the GRAS monograph and one signed copy of the conclusion from each member of the Expert Panel are provided. Additionally, I have enclosed a virus-free CD-ROM with the GRAS monograph and the signed statements of the Expert Panel.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5543 or jh@jheimbach.com.

Sincerely,

[redacted]

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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Generally Recognized As Safe (GRAS) Determination for the Intended Use of Lacprodan® ALPHA-10 Alpha-Lactalbumin

Prepared for:
Arla Foods Ingredients Group P/S
Basking Ridge NJ

Prepared by:
JHeimbach LLC
Port Royal Virginia
Written by:
Eric L. Lien and James T. Heimbach

August, 2018

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Abbreviations

AE: Adverse event

ALA: Alpha-lactalbumin

α-LAC: Alpha-lactalbumin

BUN: Blood urea nitrogen

cGMP: Current Good Manufacturing Practice

CFU: Colony-forming unit

CNS: Central nervous system

FOS: Fructooligosaccharide

GI: Gastrointestinal

GMP: Glycomacropeptide

GOS: Galactooligosaccharide

HACCP: Hazard Analysis and Critical Control Points

HM: Human milk

LNAA: Large neutral amino acids

OF: Oligofructose

SAE: Severe adverse event

SNP: Single-nucleotide polymorphism

TRP: Tryptophan

WMP: Whole milk protein

WPC: Whey protein concentrate

Part 1: Signed Statements and Certification

1.1. GRAS Notice Submission

Arla Foods Ingredients P/S submits this GRAS notification through its agent James T. Heimbach, president of JHeimbach LLC, in accordance with the requirements of 21 CFR Part 170, Subpart E.

1.2. Name and Address of Notifier

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1.3. Name of Notified Substance

The subject of this Generally Recognized as Safe (GRAS) notification is Lacprodan® ALPHA-10 brand alpha-lactalbumin. Alpha-lactalbumin is often abbreviated α-LAC or ALA in this document.

1.4. Intended Conditions of Use

The intended technical effect of the addition of Lacprodan® ALPHA-10 to infant formula is to bring the level of whey protein, including ALA, in cow-milk-based infant formula up to a level approximating that of the whey protein and ALA concentration in human milk, which a study of ALA concentrations in 452 samples of mature milk from mothers from 9 countries determined to be 2.44±0.64 g/L, with higher levels in the United States (Jackson et al. 2004). The target ALA content of formula to which Lacprodan® ALPHA-10 addition is intended is 2.5 g/L.

The protein level of food-grade Lacprodan® ALPHA-10 ranges from 81% to 87%, while the minimum alpha-lactalbumin specification as a percentage of protein is 41% alpha-lactalbumin. Thus, the minimum potential alpha-lactalbumin concentration in Lacprodan® ALPHA-10 is 41% of 81%, or 33.21%.

Assuming that there is no other alpha-lactalbumin source in an infant formula, the amount of Lacprodan® ALPHA-10 needed to provide 2.5 g alpha-lactalbumin/L formula is 2.5/.3321, or 7.5 g/L. For infant formulas, a typical target protein range of 100% to 110% is used to support label

claims. Thus, the maximum intended addition level of Lacprodan® ALPHA-10 is 1.1x7.52 g, or 8.3 g/L.

The calculations above are for formulations that use Lacprodan® ALPHA-10 as the only alpha-lactalbumin source. Typically, most infant formula manufacturers strive for whey dominant infant formulas with whey:casein ratios ranging from 60:40 to 80:20. In formulas containing some level of alpha-lactalbumin prior to addition of Lacprodan® ALPHA-10 (i.e., most infant formulas containing added whey), the addition level of Lacprodan® ALPHA-10 needed to achieve a total alpha-lactalbumin content level of 2.5 g/L throughout the product shelf-life will be less than 8.3 g/L.

1.5 Statutory Basis for GRAS Status

Arla Foods Ingredients' GRAS determination for the intended use of Lacprodan® ALPHA-10 brand alpha-lactalbumin is based on scientific procedures in accordance with 21 CFR §170.30(b).

1.6. Premarket Exempt Status

The intended use of Lacprodan® ALPHA-10 brand alpha-lactalbumin is not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on Arla Foods Ingredients' determination that it is GRAS.

Lacprodan® ALPHA-10 is a concentrated whey protein and thus is grandfathered under the definition provided in 21 CFR §184.1979c[1]. It and other sources of alpha-lactalbumin have found increasing use in infant formulas globally as a source of high-quality protein derived from whey. It is proposed for use in U.S. infant formula in accordance with current Good Manufacturing Practice (cGMP) as indicated in §184.1(b)(1). Under 21 CFR 184.1979c, whey protein concentrate is produced from whey using physical separation techniques that remove sufficient non-protein constituents from whey so that the finished dry product contains not less than 25 per cent protein. In GRAS Affirmation Petition (GRP) 1G0371, the American Dairy Products Institute (ADPI) cited the final rule that affirmed the GRAS status of whey protein concentrate: "The agency does not intend to limit the processing methods that may be used. Furthermore, the Agency has no objection to the use of newly developed physical separation techniques, if there are no new toxicants introduced as a result of these techniques, and if these techniques do not result in a concentration of natural toxicants in whey products. FDA believes that such results can be avoided by the use of good manufacturing practices and by the establishment of specifications for heavy metals" (September 4, 1981; 46 FR 44435 at 44437). This submission adequately describes the manufacturing process that introduces no new toxicants as well as providing the ALPHA-10 specification including that for trace metals and microbiology.

1.7. Data Availability

The data and information that serve as the basis for the conclusion that Lacprodan® ALPHA-10 brand alpha-lactalbumin is GRAS for its intended use will be made available to the FDA upon request. At FDA's option, a complete copy of the information will be sent to FDA in either paper or electronic format, or the information will be available for review at the home office of JHeimbach LLC, located at 923 Water Street, Port Royal VA 22535, during normal business hours.

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1. In its reply to GRN 000037, FDA stated that, "Whey protein isolate is related to whey protein concentrate, which is affirmed as GRAS (21 CFR §184.1979c). "

1.8. Freedom of Information Act Statement

None of the information in the GRAS notice is exempt from disclosure under the Freedom of Information Act, USC 552.

1.9. Certification

To the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the intended use of Lacprodan® ALPHA-10 brand alpha-lactalbumin.

1.10 FSIS Statement

Not applicable.

1.11. Name, Position and Signature of Notifier

James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC
Agent to Arla Foods Ingredients P/S

Part 2: Identity, Methods of Manufacture, Specifications, and Physical and Technical Effect

2.1. Name of the GRAS Substance

The notified substance is alpha-lactalbumin, often denominated α-lactalbumin. In this document it will often be referred to for convenience as ALA. Lacprodan® ALPHA-10 is a brand-name product marketed by Arla Foods Ingredients.

2.2. Source, Description, Manufacture, and Specifications of the GRAS Substance

2.2.1. Source

The starting material for the production of Lacprodan® ALPHA-10 is sweet whey with a pH of 5.9 – 6.6 that is created in the production of cheese and rennet caseins. The sweet whey used as raw material for the production of Lacprodan® ALPHA-10 conforms to the European Union Food Hygienic Guidelines and EU Regulation 853/2004, which allows for the use of only U.S.-approved pesticides and veterinary drugs. The raw materials – bovine milk and whey – come from processing plants that are all licensed by authorities that regulate dairy product facilities. All plants have implemented Hazard Analysis and Critical Control Points (HACCP) and comply with cGMP, which are regulated in the European Union under Regulation (EC) No 852/2004 on hygiene of foodstuffs and consistent with U.S. cGMP for infant formula.

2.2.2. Description

Alpha-lactalbumin (α-lactalbumin or ALA) is present in the milk of all mammals. The ALA of both human milk and bovine milk consists of a single polypeptide chain of 123 amino acids and contains 4 disulfide bonds (Lonnerdal and Lien 2003). The amino acid sequence homology between these species is 72% (Heine et al. 1991); the molecular weight of human-milk ALA is 14,070 dalton (Da) and of bovine milk is 14,178 Da. ALA plays 2 essential roles in humans. First, it is present in the milk producing cells of the mammary gland, where it plays a central role in lactose synthesis (Brodbeck et al. 1967). Second, following its function as a component of the lactose synthase enzyme complex, it is secreted into milk and in human milk becomes a protein of primary nutritional importance for the infant (Lien 2003). In mature human milk (>1 month lactation), the concentration of ALA is approximately 2.44±0.64 g/L (Jackson et al. 2004) and it is the predominant protein in the whey fraction. In contrast, the proportion of ALA in bovine-milk protein is much lower with a concentration of approximately 1.3 g/L (Heine et al. 1991).

Lacprodan® ALPHA-10 is a bovine derived whey protein concentrate (WPC) enriched in bovine alpha-lactalbumin (≥41% of protein) and with reduced beta-lactoglobulin content (<23%). Bovine milk and commercial whey protein concentrates have higher beta-lactoglobulin than alpha-lactalbumin; in cow's milk the beta-lactoglobulin is about 2-3 times higher than alpha-lactalbumin. In typical enrichment of alpha-lactalbumin at Arla Foods Ingredients, the Lacprodan® ALPHA-10 fraction has approximately 2-fold higher enrichment of alpha-lactalbumin, while the beta-lactoglobulin is reduced to half of what is found in a typical whey protein concentrate. A typical profile of alpha and beta levels as a percentage of protein in Lacprodan® ALPHA-10 starting material and the typical Lacprodan® ALPHA-10 fraction are provided in Table 1.

Table 1. Protein Fractions in WPC and Lacprodan® ALPHA-10.

Fractions	Alpha % {Mean±SD)	Beta % {Mean±SD)
Starting WPC material (n = 6)	18.77±0.98	51.75±1.47
Lacprodan® ALPHA-10 (n = 3)	32.2±1.55	15.5±1.04
Source: Arla Foods Ingredients		

The amino acid composition of human and bovine ALA is provided in Table 2. Both proteins are relatively rich in tryptophan (4-5%) and cysteine (6%).

Table 2. Percentage of Amino Acids in Bovine and Human ALA (Lonnerdal and Lien 2003).

Mole %	Bovine α-lactalbumin	Human α-lactalbumin
Essential		
Arginine	1.1	1.1
Cysteine	5.8	5.8
Histidine	2.9	2.0
Isoleucine	6.4	9.7
Leucine	10.4	11.3
Lysine	10.9	10.9
Methionine	0.9	1.9
Phenylalanine	4.2	4.2
Threonine	5.0	5.0
Tryptophan	5.3	4.0
Tyrosine	4.6	4.6
Valine	4.2	1.4
Non-essential		
Alanine	1.5	2.5
Aspartic acid	10.6	9.8
Glutamic acid	6.4	7.4
Glycine	2.4	2.4
Proline	1.4	1.4
Serine	4.3	5.0
Asparagine	6.4	3.2
Glutamine	5.4	6.4
Totals		
Amino Acids	100.0	100.0
Percent Coverage		

Human ALA is a globular protein with a flexible C terminus and a striking structural resemblance to C-type lysozymes (Acharya et al. 1991). The protein consists of 2 primary domains as illustrated in Figure 1 (Brew 2013).

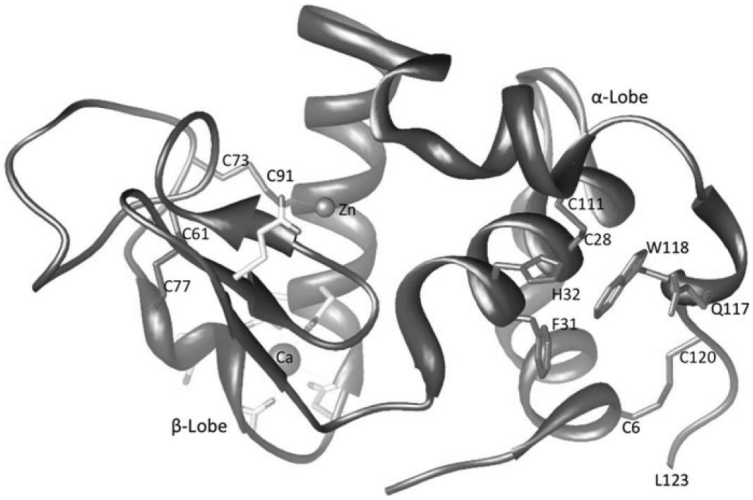

Figure 1. The 3-Dimentional Structure of the Zn/Cu Complex of Human ALA (Brew 2013).

Human (Hiraoka et al. 1980) and bovine (Fitzgerald and Swaisgood 1989) ALA tightly bind one calcium atom with a substantial change in tertiary structure: the protein moves from an open flexible configuration to a tight, compact structure with the Stokes radius reduced from 50 Â to 35 Â (Lonnerdal and Lien 2003). Binding of calcium to the high affinity site plays a role in the folding and disulfide-bond formation of the protein (Rao and Brew 1989). ALA may also loosely bind a second molecule of calcium (Chandra et al. 1998). The amount of calcium bound is minor in comparison to the total calcium in human milk (0.1-0.15%) and is therefore not significant in meeting calcium requirements of infants (Lonnerdal and Glazier 1985). Zinc is bound to a cleft in the ALA structure and may play a role in ensuring that the protein retains its active form in the lactose synthase enzyme complex (Ren et al. 1993).

UDP-D-galactose: D-glucose β-1, 4-galactosyltransferase is the enzyme that catalyzes the synthesis of the disaccharide lactose (Rao and Brew 1989). However, lactose synthesis is very inefficient in the absence of ALA. ALA binds in a one-to-one molar ratio with the catalytic unit (Klee and Klee 1972) and acts as a regulatory subunit of the enzyme complex lactose synthetase, The Km for glucose is increased by 3 orders of magnitude (Khatra et al. 1974), and lactose is synthesized in sufficient quantities to become the primary component of milk by weight. Lactose is synthesized in the Golgi vesicles, where the sugar is osmotically active, drawing water into these vesicles and eventually forming the secretory vesicles that contain most of the aqueous components of milk (McManaman and Neville 2003).

The gene for ALA is located at 12q13.11 in cows (Gene ID 2017) and 12q13 in humans (Davies et al. 1987). Gene deletion experiments demonstrate the importance of this gene and its product to milk production (Stacey et al. 1995). A line of mice was established in which the ALA gene was deleted (null allele). The gene deletion had no apparent adverse effects on female mice other than during lactation; null-allele homozygous female mice could not rear their offspring successfully. Milk yield was severely reduced and the milk was viscous and difficult to express. When the human ALA gene was placed in null-allele mice, mice homozygous for the human alpha-lactalbumin gene produced apparently normal milk and these dams reared offspring with weight gain similar to wild-type mice pups. Human ALA appeared in the milk of the homozygous mice. These results demonstrate the importance of ALA in mammary function and demonstrate that ALA from different species is enzymatically similar in its activity. In addition, milk containing ALA from different species is capable of nourishing young animals comparable to pups raised with milk from wild type mothers.

A single nucleotide polymorphism (SNP) in the human ALA gene has been reported by Chowanadisai et al. (2005), who evaluated an ALA variant initially identified by HPLC. The molecular weight of the variant was determined by mass spectrometry and the location of the SNP was evaluated by DNA sequencing. The genetic polymorphism was identified as replacement of guanine for adenine, which resulted in the substitution of valine for isoleucine at position 46. The frequency of this SNP was higher in milk from Asian women than in milk from European, African, North American, or South American women. There was no difference in lactose content of milk from women with or without this SNP, suggesting the activity of lactose synthase was not affected. Genetic variants of the ALA gene have also been observed in both French (Sanchez et al. 2016) and Chinese cows (Zhou and Dong 2013), but genetic variants were not associated with milk yield (Zhou and Dong 2013).

2.2.3. Manufacture

Lacprodan® ALPHA-10 is produced by whey fractionation utilizing well-accepted dairy processing methodologies which separate the ALA protein from bovine whey fractions. The starting material for Lacprodan® ALPHA-10 production is sweet whey with a pH of 5.9 – 6.6 conforming to the European Union Food Hygienic Guidelines and EU Regulation 853/2004. Furthermore, the purified water (reverse osmosis water), lactose, and sodium hydroxide used in the production of Lacprodan® ALPHA-10 are are food grade materials approved for infant formula use.

The raw materials – bovine milk and whey – come from processing plants that are all licensed by authorities that regulate dairy product facilities. All plants have implemented Hazard Analysis and Critical Control Points (HACCP) and comply with cGMP, which are regulated in the European Union under Regulation (EC) No 852/2004 on hygiene of foodstuffs and consistent with U.S. cGMP for infant formula.

The production sites that manufacture Lacprodan® ALPHA-10 are certified under DS/EN ISO 50001: 2011, ISO 22000: 2005 / TS 22002-1: 2009 and FSSC 22000, which all control: (1) Implemented quality control systems, which include cGMP and HACCP; (2) Raw material analytical control; (3) The physicochemical and microbial microbiological characteristics of the final product. All processing equipment and supporting material that is installed with the machines in the processes are suitable for food and pharmaceutical applications and in compliance with FDA rules and regulations (CFR) Title 21. Arla Foods Ingredients has been certified for the development, production, and sale of products based on whey protein and lactose by the Danish Authorities and by the FDA. The only factory that currently produces Lacprodan® ALPHA-10 was audited and approved by the FDA in August 2012.

When the raw skim milk is received at the cheese or casein production facility, it goes through a pasteurization step (72°C for 15 seconds). This is a critical control point (CCP) at the dairy facility that is lethal to all pathogens and ensures a significant reduction of contaminating microorganisms. The milk is then processed to manufacture cheese and casein, a process that yields a whey fraction (including the ALA protein).

After the whey has been drawn from the cheese process, it is kept for a short duration in storage tanks before it is clarified and separated. The whey finally undergoes an additional pasteurization (72°C for 15 seconds) and is kept cooled in storage tanks. The whey fraction is kept in the tanks until it is transported to the manufacturing site that produces Lacprodan® ALPHA-10.

At the point of arrival at the production site that produces Lacprodan® ALPHA-10, analytical controls are used to assess the temperature, pH, and nitrate-level of raw material before entry. Additionally, the level of protein and fat is measured and selected microbial analyses are carried out for analytical control of the raw material.

The raw material is kept in storage tanks at 5°C until it undergoes the separation process that yields 3 fractions. The first fraction is a high protein ALA concentrate. Second is a fraction in which lactose accounts for the majority of the dry matter content; minor amounts of components like non-protein nitrogen (NPN) and minerals are found in this fraction as well. The third fraction from the same separation is a beta-lactoglobulin-enriched fraction. The lactose-enriched fraction and the β-LG-enriched fraction are transferred to large cold storage tanks for other applications.

The ALA fraction with high protein concentration is also transferred to a new set of storage tanks where the product is again kept cold and food-grade lactose (VARIOLAC 992) can be added to achieve the desired protein percentage of the total dry matter and food-grade HCl or NaOH can be added to attain the target pH prior to protein drying.

The liquid fraction containing the concentrated ALA is sent to the spray drying tower to be dried into the Lacprodan® ALPHA-10 powder. The dried powder passes through a sieve and a rotating magnet before the powder is collected in silos and introduced into bags. These filled bags pass through a metal detector before they are put on pallets.

Finally, Arla Foods Ingredients performs a finished product analysis based on a certificate of analysis. The level of ALA in the total protein content of Lacprodan® ALPHA-10 is between 41 and 52%. The production process is summarized in Figure 2.

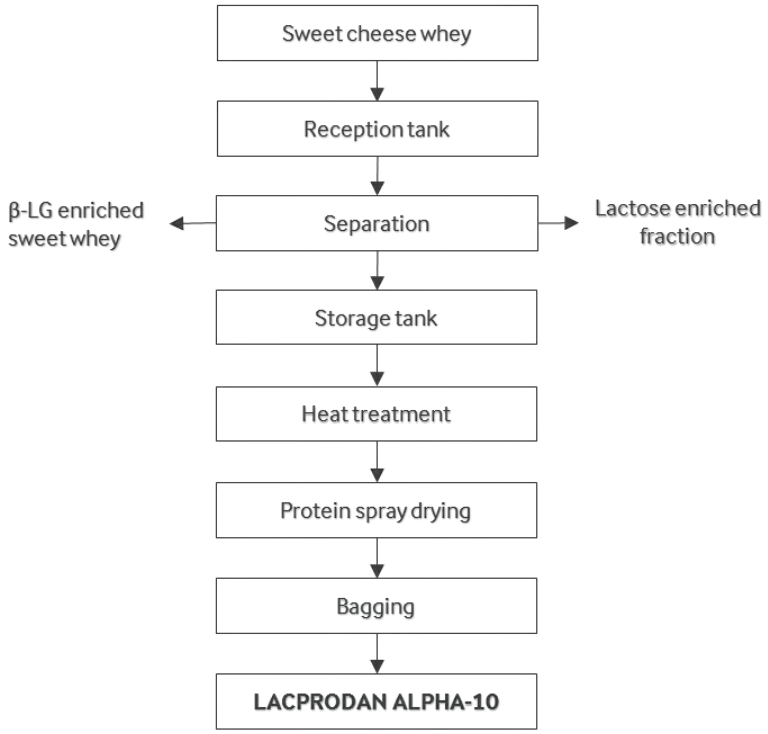

Figure 2. Process Flow Diagram of Lacprodan® ALPHA-10.

2.2.4. Specifications

Food-grade specifications for Lacprodan® ALPHA-10 are displayed in Table 3, along with the results of analyses of 5 non-consecutive lots, showing that the process is in control and consistently results in product meeting specifications.

Table 3. Food-Grade Specifications and Analytical Results for Lacprodan® ALPHA-10.

Analysis	Method	Specification	Results 5 Lot Mean	Tested Lots				
				E060251 (2014)	F530251 (2016)	H050250 (2017)	H240251 (2017)	J030250 (2018)
Protein (%)	ISO 8968-3/ IDF 20-3	81.0-87.0	83.4	82.0	82.5	84.0	84.5	84.0
Alpha-lactalbumin (% of protein)	HPLC	≥41.0	48.1	44.5	47.0	50.1	51.5	47.6
Ash (%)	NMKL 173	≤5.0	3.5	3.6	3.6	3.4	3.5	3.5
Moisture (%)	ISO 6731	≤5.5	4.5	5.1	4.5	4.0	4.6	4.5
Lactose (%)	ISO 5765-2/ IDF 79-2	≤10.0	8.0	8.5	8.0	8.5	7.5	7.5
Fat (%)	ISO 1736	≤2.0	0.4	0.5	0.4	0.5	0.1	0.5
Minerals								
Sodium (%)	ICP	0.20-0.45	0.29	0.30	0.27	0.32	0.29	0.28
Chloride (%)	ISO 5943/ IDF 88	≤0.20	0.11	<0.05	<0.01	0.12	0.10	0.12
Phosphorus (%)	ICP	0.20-0.40	0.26	0.28	0.29	0.24	0.25	0.23
Calcium (%)	ICP	0.40-0.60	0.50	0.49	0.50	0.52	0.52	0.48
Potassium (%)	ICP	0.50-0.90	0.66	0.63	0.72	0.59	0.66	0.72
Heavy metals								
Arsenic (mg/kg)	ICP-HRMS ISO 17294m:2016	<0.5	<0.1	<0.1	<0.01	<0.01	<0.01	<0.001
Cadmium (mg/kg)	ICP-MS ISO 17294m:2016	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Lead (mg/kg)	ICP-HRMS ISO 17294m:2016	<0.05	<0.01	<0.003	<0.003	0.006	<0.003	0.01
Mercury (mg/kg)	ICP-MS ISO 17294m:2016	<0.05	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Selenium (mg/kg)	ICP-MS ISO 17294m:2016	No specification	0.35	0.39	0.31	0.32	0.37	0.34
Microbiological load								
Total plate count (cfu/g)	ISO 4833-1	≤10.000	2480	300	400	700	1000	10000
Mold/yeast (cfu/g)	ISO 6611	<10	<10	<10	<10	<10	<10	<10
Bacillus cereus (cfu/g)	ISO 7932	<50	<10	<10	<10	<10	10	<10
Enterobacteriaceae (cfu/g)	ISO 21528-2	<10	<10	<10	<10	<10	<10	<10
Staphylococcus aureus (cfu/g)	ISO 6888-1	Absent/1g	Absent/1g	Absent	Absent	Absent	Absent	Absent
Salmonella (cfu/g)	ISO 6579	Absent/25 g	Absent/25 g	Absent	Absent	Absent	Absent	Absent
Cronobacter sakazakii (cfu/10 g)	ISO/TC 34/SC 9	Absent/10 g	Absent/10 g	Absent	Absent	Absent	Absent	Absent

Source: Arla Foods Ingredients

2.3. Stability

2.3.1. Raw Material Stability

From the beginning of commercialization of Lacprodan® ALPHA-10 until recently, the alpha-lactalbumin content was determined using a size-exclusion HPLC method *(Alpha Lac analysis TSK)* using a Tosoh Biosciences trademarked TSKgel g3000PWXL column. The sample extraction procedure followed the principles of non-reduced and non-denatured conditions for the quantitation of alpha-lactalbumin content, with absorbance detected at UV 214 nm. Over the years, comparison with other available techniques indicated that this method leads to over-estimation of the alpha-lactalbumin content by about 10-15%.

Three modifications were made to the method to address this problem:

1. 2-mercaptoethanol (reducing agent to break disulfide bonds) was added to the sample preparation.
2. Chromatography was done using a silica-based rather than polymer-based size exclusion TSK column, TSKgel g3000SWXL instead of TSKgel g3000PWXL, that minimizes co-elution of beta casein derived peptides along with alpha-lactalbumin.
3. The absorbance was detected at 280 nm (measuring mainly the aromatic amino acids) versus 214 nm (measuring all amino acids). Beta casein peptides are mostly devoid of aromatic amino acids and measurement at 280 nm further reduces overestimation of the alpha-lactalbumin value.

The modifications implemented minimized interference from beta-casein derived peptides and provided a more accurate value of alpha-lactalbumin content in the Lacprodan® ALPHA-10 raw material. These modifications have been validated and the modified method is called *Alpha lac analysis TSK modified.*

The stability study was performed using 4 samples manufactured during week 46 in 2015 and weeks 1, 3, and 4 in 2016, all stored at room temperature. Since the time-zero analyses of alpha-lactalbumin content were based on the old *Alpha lac analysis TSK*, analyses after 2+ years were performed using the same method in order to measure any changes over time. However, because the inaccuracy of this method was recognized, analyses of 2+ year-old material were also performed using the modified method, *Alpha lac analysis TSK modified.*

The stability data (Table 4) show that the content of alpha-lactalbumin measured at day 0 changed little after 2+ years. The data indicate that there is no degradation of alpha-lactalbumin during storage at room temperature for that period.

The third line of the table, showing the results of analyses using *Alpha lac analysis TSK modified*, do not bear on the stability study. However, they show values about 11% lower than those indicated by the original method and provide more accurate figures on the actual alpha-lactalbumin content of Lacprodan® ALPHA-10.

Table 4. Stability of the Alpha-Lactalbumin Content of Lacprodan® ALPHA-10.

Analysis	Alpha-Lactalbumin Content (%)			
	Lot G010250	Lot G030250	Lot G040250	Lot F460251
Alpha lac TSK: Day 0	49.2	48.8	50.4	51.5
Alpha lac TSK: 2+ years	48.1	49.3	49.3	51.2
Alpha lac TSK modified: 2+ years	44.4	43.0	43.2	44.9
Source: Arla Foods Ingredients				

2.3.2. Stability in Infant Formula

Analysis of the level of alpha-lactalbumin in infant formula was conducted using the *Alpha lac analysis TSK modified* method as described above. A typical infant formula with 60:40 whey casein ratio was manufactured with skim milk powder, standard whey protein concentrate, and Lacprodan® ALPHA-10 to provide 2.5g/100g alpha-lactalbumin content. The formula was manufactured according to commercial specifications.

Two batches were manufactured and stored for 360 days, half of each batch at room temperature (25°C, 60% relative humidity) and the other half of each batch at 40°C and 75% relative stability to assess accelerated stability. Samples were analyzed at 0, 30, 60, 90, 180, 270, and 360 days, with the results shown in Table 5.

Table 5. Infant Formula Stability Trial Results.

Time (days)	Alpha-Lactalbumin Content (g/100 g)			
	Room Temperature Stability		Accelerated Stability	
	Batch 1	Batch 2	Batch 1	Batch 2
0	2.505	2.47	2.505	2.47
30	2.515	2.47	2.515	2.45
60	2.485	2.405	2.455	2.395
90	2.495	2.465	2.48	2.425
180	2.385	2.32	2.34	2.285
270	2.375	2.325	2.385	2.295
360	2.385	2.31	2.385	2.33
Source: Arla Foods Ingredients				

These findings are expressed in Table 6 as percentages of starting levels. The data demonstrate that alpha-lactalbumin is stable in infant formula under both room-temperature and accelerated-stability conditions, remaining over 360 days within 10% variability to time zero.

Table 6. Infant Formula Stability as Percent of Starting Level.

Time (days)	Alpha-Lactalbumin Content (% of Starting Level)			
	Room Temperature Stability		Accelerated Stability	
	Batch 1	Batch 2	Batch 1	Batch 2
0	100.00	100.00	100.00	100.00
30	100.40	100.00	100.40	99.19
60	99.20	97.37	98.00	96.96
90	99.60	99.80	99.00	98.18
180	95.21	93.93	93.41	92.51
270	94.81	94.13	95.21	92.91
360	95.21	93.52	95.21	94.33
Source: Arla Foods Ingredients				

2.4. Technical Effect

The intended technical effect of the addition of Lacprodan® ALPHA-10 to infant formula is to bring the level of whey protein, including ALA, in cow-milk-based infant formula up to a level approximating that of the whey protein and ALA concentration in human milk.

A meta-analysis by Lonnerdal et al. (2017) showed that human milk protein composition changes dramatically during early lactation, with total protein decreasing from 20.6 g/L at days 0-5 of lactation to 15.7 g/L at days 16-30 and 11.0 g/L at days 90-360. The whey:casein ratio is approximately 90:10 in colostrum and drops to 65:35 at 2 weeks of lactation (Lonnerdal et al. 2017). By the end of the first month of lactation, the concentration of whey is nearly stable and the whey:casein ratio approaches 60:40, although this ratio is somewhat variable (Lonnerdal et al. 2017; Kunz and Lonnerdal 1992). Table 7 summarizes human milk whey to casein ratios during the first year of lactation.

Table 7. Median Values of Whey:Casein Ratio and ALA Concentration in Human Milk (Lonnerdal et al. 2017).

Time (Days)	Whey:Casein Ratio	ALA (g/L)
0-5	11:89	4.30
6-15	65:35	4.20
16-30	59:41	3.30
31-60	61:39	3.10
61-90	61:39	2.84
91-360	60:40	2.62

While ALA is only a minor fraction of the protein in bovine milk, 1.3 g/L, 3.7 % of total protein (Heine et al. 1991), it is the dominant whey protein in human milk. A multinational study of human milk ALA concentrations reported by Jackson et al. (2004) evaluated approximately 50 mature human milk samples (samples obtained 1-12 months after birth) from each of 9 countries (Australia, Canada, Chile, China, Japan, Mexico, Philippines, UK, and USA). The mean±SD concentration determined in 452 samples was 2.44±0.64 g/L (Figure 3). The concentration of ALA was significantly lower in Mexico and significantly higher in the United States than all other countries. There was no obvious explanation for these differences. The concentration of ALA and duration of lactation were negatively correlated.

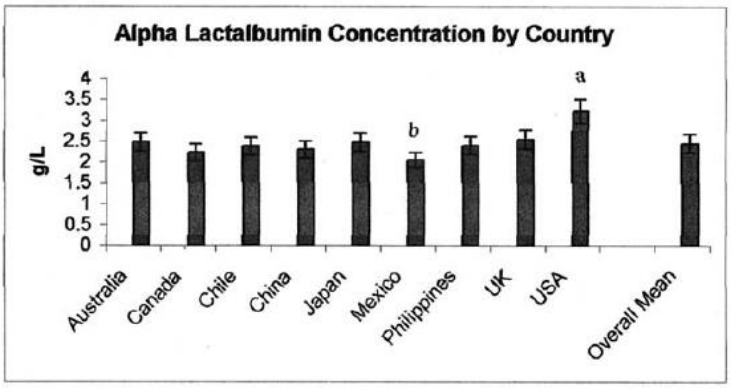

Figure 3. ALA Concentrations by Country, Mean±SD (Jackson et al. 2004).

Numerous other studies (summarized in Lonnerdal et al. 2017) have evaluated ALA concentrations in human milk. While ALA concentration is high in colostrum (mean of 4.30 g/L), it slowly decreases during the first 30 days of lactation and remains at a relatively constant level of approximately 2.6 g/L after 90 days. Unfortunately, this evaluation of data did not include the large study of Jackson et al. (2004), but did include studies with limited numbers of samples or utilizing older analytical methodologies. ALA as a percentage of total protein is 22.2% when calculated using the Jackson data for ALA and the Lonnerdal data for total protein (at lactation duration from 90-360 days of lactation); it is 23.8% when utilizing the Lonnerdal data for ALA.

Fleddermann et al. (2014b) reviewed 13 prospective, randomized, double-blind, placebo-controlled trials that examined the impact of the macronutrient composition of infant formula on the growth and energetic efficiency (i.e., growth per 100 kcal of energy intake) of apparently healthy term infants. In these studies, an increased whey:casein ratio, increased ALA content, or higher tryptophan content increased energetic efficiency by about 10-13%. The authors suggested that these findings may help in "explaining the differences in growth and metabolism between breastfed and formula-fed infants."

2.5 Regulatory Status of Alpha-Lactalbumin

A search of the website of the Food and Drug Administration did not reveal any similar substances having been approved for use in infant formula. Under section 912 of the FDA Amendments Act of 2007 amended Section 301(II) of the Federal Food, Drug, and Cosmetic Act, there is a prohibition of the introduction of any food into interstate commerce to which has been added an approved drug, licensed biological, or a drug or biological product for which substantial clinical investigations have been instituted and for which the existence of such investigations has been made public. Our search of the FDA website did not reveal any reference to alpha-lactalbumin as a drug and no published clinical studies were located that would lead us to believe a substantial clinical investigation has been conducted and made public that would indicate a contemplated drug use of alpha-lactalbumin.

In the European Union alpha-lactalbumin has been on the market before 1997, when the Novel Food regulation entered into force, and therefore alpha-lactalbumin does not need safety approval for marketing in the member states of the EU.

In China, alpha-lactalbumin falls within the definition of GB standard 11674-2010 for whey protein powder, and no approval is needed for marketing and sales in China.

Part 3: Dietary Exposure

3.1. Intended Conditions of Use

As described in Section 2.4, the intended technical effect of the addition of Lacprodan® ALPHA-10 to infant formula is to bring the level of whey protein, including ALA, in cow-milk-based infant formula up to a level approximating that of the whey protein and ALA concentration in human milk.

The total protein in cow's milk ranges from approximately 3.1 to 3.7%, with an average of about 3.5%, and has slight variability from this average number based on seasonality and species (Vincent et al. 2016). In bovine milk, casein is the predominant protein class, accounting for about 80% of the total protein content. The whey fraction constitutes only about 20% of total protein and ALA content is about 3% of the total protein content in bovine milk, or about 1.26 g/L (Vincent et al. 2016).

Infant formulas based on cow's milk came to market some 150 years ago, but only relatively recently have analytical advancements in protein analysis showed a stark contrast between cow's milk (predominantly casein) and human milk (predominantly whey). In human milk, the ratio of whey to casein varies from about 80:20 in early lactation to about 50:50 in late lactation (Lonnerdal 2003). It has long been accepted as a fundamental principle that human milk is the optimal food for human infants, and infant formula "manufacturers attempt to alter their products to imitate human milk in either composition or performance" (IOM 2004). One goal of many infant formula companies is to manufacture products with protein composition as close to human milk as possible.

Demineralized whey dominant cow's milk protein fractions became commercially available in the 1950s, leading to the first whey dominant infant formula in the early 1960s. However, whey dominant formulas (prior to addition of alpha-lactalbumin enriched, beta-lactoglobulin reduced whey) contained about 1.3 g/L of alpha-lactalbumin protein in the formulations (Lien et al. 2004). Alpha-lactalbumin enriched ingredients became commercially available in the late 1990s and the first infant formula containing about 2.2 g/L of bovine alpha-lactalbumin was launched by Wyeth Nutrition in Hong Kong in 2002 (Wyeth 2018).

Based on the figure of 2.44±0.64 g/L for the mean ALA concentration in human milk (Jackson et al. 2004) and the range of 4.30 to 2.62 for median ALA content in human milk between days 0 and >90 (Lonnerdal et al. 2017) cited in Section 2.2.6, the target ALA content of formula to which Lacprodan® ALPHA-10 addition is intended is 2.5 g/L. This level not only matches average human milk levels, but also permits the production of infant formula with lower total protein content (compared to non-alpha-lactalbumin dominant formulas), better protein quality, and growth characteristics similar to breast fed infants (Trabulsi et al. 2011).

The proportion of protein in food-grade Lacprodan® ALPHA-10 ranges from 81% to 87%, while the minimum alpha-lactalbumin specification as a percentage of protein is 41% alpha-lactalbumin. Thus, the minimum potential alpha-lactalbumin concentration is 41% of 81%, or 33.21%.

Assuming that there is no other alpha-lactalbumin source in an infant formula, the amount of Lacprodan® ALPHA-10 needed to provide 2.5 g alpha-lactalbumin/L formula is 2.5/.3321, or 7.5 g/L. For infant formulas, a typical protein range of 100% to 110% is used to support label claims. Thus, the maximum intended addition level of Lacprodan® ALPHA-10 is 1.1x7.5 g, or 8.3 g/L.

The calculations above are for formulations that use Lacprodan® ALPHA-10 as the only alpha-lactalbumin source. Typically, most infant formula manufacturers strive for whey dominant infant formulas with whey:casein ratios ranging from 60:40 to 80:20. In formulas containing some level of alpha-lactalbumin prior to addition of Lacprodan® ALPHA-10 (i.e., most infant formulas

containing added whey), the addition level of Lacprodan® ALPHA-10 needed to achieve a total alpha-lactalbumin content level of 2.5 g/L throughout the product shelf-life will be less than 8.3 g/L.

3.2. Estimated Daily Exposure

If Lacprodan® ALPHA-10 is added to infant formula at the maximum concentration of 8.3 g/L, an infant consuming 800 ml formula per day will receive 0.8x8.3 g = 6.6 g/day of Lacprodan® ALPHA-10. At least 33.2% for the 6.6 g Lacprodan® ALPHA-10, or 2.2 g, is alpha-lactalbumin.

According to tables of daily energy intake by formula-fed infants provided by Fomon and Bell (1993), the subpopulation of infants with the highest intake per kg body weight is boys aged 14–27 days. The mean energy intake by this group is 121.1 kcal/kg bw/day and the 90th percentile is 141.3 kcal/kg bw/day. Among girls, the highest energy intake is found in the same age group, 14–27 days, and is nearly as high as boys: the mean and 90th energy intake percentiles are 117.8 and 138.9 kcal/kg bw/day. Most term infant formulas contain 67.6 kcal/100 ml when ready to consume. Therefore, to obtain 141.3 kcal energy/kg bw, an infant boy must consume 209.0 ml formula/kg bw. To reach her 90th percentile of energy consumption, 138.9 kcal/kg bw/day, an infant girl must consume 205.5 ml formula/kg bw/day. The 90th percentile of formula intake for the 2 sexes combined is about 207 ml/kg bw/day. If Lacprodan® ALPHA-10 is added at the maximum addition level of 8.3 g/l, the estimated daily intake (EDI—the 90th percentile of intake) is 0.207x8.3 = 1.72 g Lacprodan® ALPHA-10/kg bw/day.

Part 4: Self-limiting Levels of Use

There is no meaningful technological limitation to the concentration of Lacprodan® ALPHA-10 alpha-lactalbumin in infant formula.

Part 5: Experience Based on Common Use in Food

The conclusion that the intended use of Lacprodan® ALPHA-10 alpha-lactalbumin is GRAS is based on scientific procedures rather than experience based on common use in food prior to 1958.

Part 6: Narrative

6.1. Pharmacokinetics

Jakobsson et al. (1982) evaluated digestion of bovine casein, ALA, and beta-lactoglobulin *in vitro* in duodenal juice from infants aged 3-19 months. The kinetics of digestion were evaluated using the proteins in pure form. Thirty mg/ml of casein were hydrolyzed under the same conditions in which 1 mg of either ALA or beta-lactoglobulin were hydrolyzed. When proteins were presented in the matrix of either cow's milk or infant formula, hydrolysis was slower. Pre-incubation with gastric juice at a pH of 4-5, the pH of a typical infant stomach, did not influence the results, likely because pepsin activity in this pH range is minimal.

Studies in adults demonstrated that consumption of ALA resulted in a rapid alteration in circulating amino acids (for example, elevation of tryptophan), suggesting the rapid digestion of ALA and subsequent absorption of amino acids over a period of approximately 1.5 hours (Markus et al. 2002; Markus et al. 2000). Protein digestion was evaluated in adults with short bowel who received oral ALA and beta-lactoglobulin (Mahe et al. 1991). Both proteins were found in the intestinal effluent after 30 minutes, but not at longer time points. Lonnerdal and Lien (2003) suggested that active gastric digestion occurring in adults (but perhaps not as actively in infants) increases the digestion of these whey proteins prior to their movement from the stomach to the small intestine. This suggestion is supported by assessment of the activity of pepsin-mediated digestion of infant formula at various pHs. ALA and beta-lactoglobulin were hydrolyzed at a pH of 1.5-2.5, but were resistant to proteolysis above a pH of 3.0.

The digestibility of ALA has been studied in several animal models. One hour after administration of 42 mg ALA to mature rats, only 3.9 mg of ALA remained in the stomach (as determined by an immunological technique) and only trace amounts were found in the small intestine (Fushiki et al. 1986).

Pantako and Amiot (2001) compared the digestion of isolated bovine ALA and whey protein concentrate (WPC) in the rat gastrointestinal tract. Diets containing ALA emptied faster from the stomach than WPC. Trichloroacetic acid precipitable protein levels were lower in both the stomach and small intestine with ALA than WPC. For both diets, the small intestinal contents were characterized by high levels of amino acids and small peptides. These results demonstrate that ALA is at least as digestible (and perhaps more digestible) as WPC. Pantako and Amiot (2001) also evaluated calcium, phosphorus, and amino acid absorption in rats consuming either ALA or WPC. The concentrations of calcium and phosphorus in the GI tract were similar between groups, while the amount of insoluble minerals was higher in the ALA group. The concentrations of amino acids in the portal vein were not different between rats receiving ALA and WPC while higher levels of amino acids were found in the GI tract of the WPC group.

Wada et al. (2017) evaluated human milk and infant formula digestion in a suckling rat pup model. The main sources of peptides were α-lactalbumin and β-casein in human milk, and β-lactoglobulin and β-casein in infant formula. Both human milk and infant formula ALA were rapidly digested in this model.

Studies in preterm and 6-week old infant rhesus monkeys demonstrated the relatively slow digestion of ALA and beta-lactoglobulin following formula feeding (Lindberg et al. 1997). In 6-week-old monkeys, as much as 30-50% of these proteins were detected in duodenal aspirates 60 minutes after ingestion of the proteins, and measurable amounts of ALA were found in serum of the monkeys. At 7 months of age, no measurable amounts of the proteins could be detected in duodenal contents 15 minutes after formula consumption and no ALA was found in the serum.

Evaluation in human term infants demonstrates that they can digest formulas rich in ALA. Heine et al. (1996) reported elevated plasma tryptophan levels when infants were fed ALA-enriched formula compared to a control formula. This finding strongly suggests that the tryptophan-rich ALA formula was digested and resulting amino acids were absorbed. Numerous clinical studies have been published comparing control formulas to formulas enriched with ALA. Growth rates and protein status were similar between formula groups in all studies reporting these parameters (Trabulsi et al. 2011; Sandstrom et al. 2008; Roze et al. 2012; Lien et al. 2004; Davis et al. 2008). These studies demonstrate that ALA-enriched formulas have high protein quality and are well utilized. No increased incidence of protein allergy due to appearance of intact ALA in the circulation has been reported.

6.2. Animal Studies

6.2.1. Rodent Studies

ALA is a rich source of the amino acid tryptophan (TRP), the precursor of serotonin, a neurotransmitter that plays an important role as a mediator of sleep (Zeisel 1986). Diets with elevated ALA concentrations result in an increased TRP/large neutral amino acids (LNAA) ratio and increased transport of TRP to the central nervous system. Minet-Ringuet et al. (2004) evaluated the effect of ALA on restoration of sleep after food deprivation. Three diets varying in protein content were utilized in this study: 140 g/kg whole milk protein (designated as P14), 300 g/kg whole milk protein (P30-WMP), and 300 g/kg whole milk protein enriched with >40% ALA (P30-LAC).

After surgery to implant electrodes to obtain electroencephalogram recordings, 18 male Wistar rats (age and starting bodyweight were not reported), housed individually, were given the P14 diet for 10 days, then fasted for 4 days, and finally given a 6-day re-feeding period with animals divided into 3 groups (n = 6 rats/group) receiving P14, P30-WMP, or P30-LAC. Sleep parameters were measured during all 3 phases.

Animals lost weight during food restriction and gained weight during refeeding. During refeeding, weight gain in the P30-WMP and P30-LAC groups was nearly identical and significantly more rapid than in the P14 group. During the refeeding period, animals in the P30-LAC group rapidly returned to the basal sleep pattern (reduced wakefulness and increased slow wave sleep compared to fasting). Animals in both of the milk groups were much slower to return to normal sleep patterns. No adverse effects of any of the diets were reported, and the authors concluded that, “the results of the present study are in line with the efficacy of TRP enrichment or the use of α-lactalbumin in infant milk to improve sleep, but extend these ideas by showing that this efficacy of α-lactalbumin on sleep can also be observed in adult subjects.”

Two studies have been reported that evaluated the effect of ALA on gastrointestinal (GI) mucosal defense in rats. Matsumoto et al. (2001) gave twelve 11-week-old male Wistar rats weighing 210-250 g gavage doses of 200 mg ALA /kg bw and sacrificed 3 rats each at 0, 15, 30, or 60 minutes afterwards; 3 control rats were given saline and sacrificed at 30 minutes. Six similar male Wistar rats were given ALA (n = 3) or control (n = 3); after 30 minutes, gastric mucosal injury was induced by intragastric ethanol-HCl and the rats were sacrificed an hour later. Stomachs of all rats were examined and scored for degree of necrotic injury.

The rats receiving ALA exhibited significantly less gastric injury than the controls. No adverse effects attributable to ALA administration were reported, and the authors speculated that, “The high concentration of α-LA in human milk may lead to the suggestion that this protein fulfils a biological role in the gastrointestinal protection of newborn infants.”

Ushida et al. (2003) evaluated ALA in forty-eight 11-week-old male Wistar rats weighing 220-260 g. After 24 hours without feed, they received gavage of 5 ml/kg bw providing 0, 200, 500, or 1000 mg/kg bw α-lactalbumin (12 rats/dose); 30 minutes later the rats were sacrificed and the stomachs excised for measurement of prostaglandin (PG) E2, gastric mucin, fluid volume, and pH. ALA administration dose-dependently elevated levels of PGE2, gastric fluid volume, adherent mucin, and luminal pH, and delayed gastric emptying. No adverse effects of the treatment were reported, and the authors concluded that "α-LA enhances both PG-dependent and PG-independent gastric defense mechanisms in naïve rats."

6.2.2. Rhesus Monkey Studies

Two studies in infant rhesus monkeys have employed ALA produced by Arla Foods Ingredients. The ALA employed was a product in development and not the current commercial products, Lacprodan® ALPHA-10 or Lacprodan® ALPHA 20.

Kelleher et al. (2003) reported the growth and nutritional status in infant rhesus monkeys fed a control formula, or formulas supplemented with either ALA produced by Arla Foods Ingredients or a glycomacropeptide (n = 5 monkeys/group) from birth to 4 months of age. A breast-fed comparison group was also included. All formulas had the same protein concentration. The concentrations of glycomacropeptide and ALA in the study formulas were not reported. However, tryptophan concentration was increased in the ALA formula (23.0 mg/100 ml) when compared to the control formula (19.9 mg/100 ml); this is an indication of ALA enrichment in the ALA study formula. Weight gain was similar in all formula groups and significantly higher than in the breast-fed group. The study report did not provide length or head circumference data. While formula intake was similar between the control and ALA group, the glycomacropeptide group had significantly higher formula consumption than either of the other formula groups.

Hemoglobin concentrations were not different among groups, while hematocrit was lower in the ALA- and breast-fed groups than the control group at 3 months of age. No hematocrit differences between the ALA group and the control group were found at birth or months 1, 2 or 4. Mineral absorption was determined by the use of radiotracers; plasma copper and zinc concentrations did not differ among the formula groups; absorption of calcium and iron did not differ among groups, while zinc absorption was significantly greater in the ALA and glycomacropeptide groups than the breast-fed group. Plasma amino acids were determined monthly. With few exceptions, amino acid concentrations did not differ between the ALA formula and the breast-fed groups. Plasma threonine, isoleucine, valine, and methionine concentrations in the breast-fed and ALA-fed animals were significantly lower than the other groups, but most of these differences occurred only at the one-month time point. Plasma insulin and blood urea nitrogen (BUN) concentrations were not different between the control and ALA-enriched formula groups. This study demonstrated that an ALA-enriched formula can support normal growth and the ALA formula group had only a few biologically insignificant differences compared to the control and breast-fed groups in hemoglobin, hematocrit, mineral status, amino acid concentrations, insulin and BUN. The authors concluded that, "α-lactalbumin-supplemented formula has no adverse effects on nutritional status in infant monkeys . . . and α-lactalbumin supplementation promotes a plasma amino acid pattern similar to that of breastfed infant monkeys."

In a second primate study using ALA produced by Arla Foods Ingredients (Bruck et al. 2003), 20 infant rhesus monkeys were fed formulas from birth to 4.5 months of age and a breast-fed group was included (n = 5 monkeys/group). These formulas were similar to the ones that Kelleher et al. (2003) fed in the above study, as shown in Table 8.

Table 8. Protein Composition of Test Formulas (Bruck et al. 2003).

Protein Composition (%)	Control Formula	α-LA Supplemented	GMP-Supplemented
α-lactalbumin	13	25	20
β-lactoglobulin	36	25	15
Glycomacropeptide	10	10	25
Other	41	40	40

Infants were exclusively breast-fed or bottle-fed *ad libitum*, with no solid food given throughout the study. Monkeys were single-caged for 1 month, then caged in pairs. Blood was drawn monthly and rectal swabs were collected weekly throughout the study and pooled to allow calculation of an average cell count per month. At 4.5 months of age the animals were orally administered an infectious dose of *E. coli* O127 (EPEC). Swabs were taken at 7 and 14 days post infection. Bacterial populations were assessed through fluorescent in-situ hybridization (FISH).

After EPEC administration no diarrhea was reported in either the breast-fed or ALA-enriched formula-fed monkeys. In contrast, the group that received control formula had acute diarrhea and animals in the GMP-enriched formula had diarrhea intermediate between the control group and the ALA group (Figure 4). Monkeys receiving the ALA-enriched and control formulas had no significant changes in bacterial levels before and after EPEC administration, while the breast-fed animals had significant increases in *E. coli*, bifidobacteria, and *Bacteroides* as well as lower *Clostridia* post-EPEC. At numerous time points, white blood cell populations varied among groups with breast-fed and ALA groups being similar to each other and significantly different from the other 2 groups.

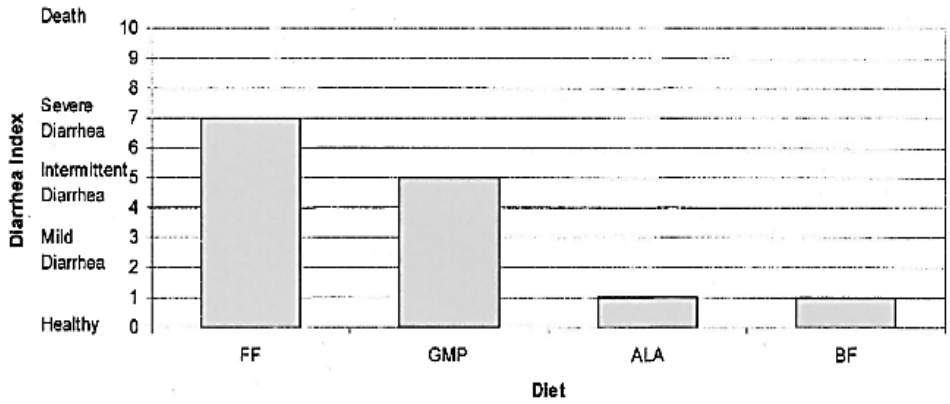

Figure 4. Diarrhea Scores Following EPEC Administration (Bruck et al. 2003).

The authors concluded:

> ". . . infant monkeys fed formula supplemented with α-lactalbumin had a gastrointestinal microflora population that was more similar to that of breastfed infant monkeys than to that of infants fed control or GMP-supplemented formula. Additionally, breast-fed infants and infants fed α-lactalbumin-supplemented formula had a similar and higher number of circulating lymphocytes compared to infants fed other formula. Our results indicate that the combination of these factors may have contributed to the ability of these infants to resist EPEC-induced diarrhea and suggest that the supplementation of infant formula with α-lactalbumin may help formula-fed infants attain similar health benefits now only afforded to infants that are breast-fed" (Bruck et al. 2003).

6.3. Human Studies

6.3.1. Studies in Adults

A number of acute studies in adult humans have evaluated the effects of ALA consumption on central nervous system (CNS) function. These studies predominantly assessed single-dose administration of ALA. The neurotransmitter serotonin (involved in stress reduction and cognitive performance) is synthesized from tryptophan and an increased ratio of tryptophan to other large neutral amino acids (TRP:LNAA) in the circulation may lead to higher CNS tryptophan (and serotonin) levels. Since ALA contains a higher level of tryptophan than other bovine milk proteins, test meals containing ALA compared to an appropriate protein control should lead to higher blood levels of tryptophan, higher TRP:LNAA ratios, and increased production of CNS serotonin.

Markus et al. (2000) provided breakfast and lunch enriched in either ALA or sodium caseinate to 29 stress-prone subjects and 29 relatively stress-resistant subjects (based on responses to a test measuring neuroticism) in a prospective, randomized, double-blind, placebo-controlled crossover study. Subjects included 19 men and 39 women aged 17-34 years; mean age was 20.7±3.14 years. The ALA-enriched diet provided 20 g ALA-enriched whey protein. After the second meal, the TRP:LNAA ratio was 48% higher following ALA consumption than following casein consumption. In stress-prone subjects the ALA diet resulted in higher prolactin and lower cortisol concentrations and reduced depressed feelings under stress. These results suggest that providing a diet with an elevated TRP:LNAA ratio improves coping ability under stress, most likely through increases in CNS serotonin levels. No adverse effects were reported.

Utilizing a similar design (23 stress-prone subjects and 29 relatively stress-resistant subjects aged 17-33 years participating in a prospective, randomized, double-blind, placebo-controlled, crossover study) memory function was assessed comparing ALA and casein meals (Markus et al. 2002). The plasma TRP:LNAA ratio followed a similar pattern to the previous study and memory function was improved in the stress-prone individuals who received the ALA meals. The authors concluded that increased brain serotonin levels may lead to improved cognitive function in stress-prone individuals. No adverse events associated with treatment were reported.

Following the initial studies by Marcus et al. (2000 and 2002), additional research evaluated acute administration of ALA on mood following a stress test (an unsolvable mental arithmetic task with loud noise) in both recovered depressed subjects (n = 23) and controls (n = 20) in a prospective, randomized, double-blind, placebo-controlled crossover trial (Merens et al. 2005). No significant differences following ALA and casein consumption occurred in mood or plasma levels of the stress related hormone cortisol. The authors attributed this lack of effect to the ALA and casein diets having been consumed on only one day; no adverse effects were reported from the acute intervention.

In a prospective, randomized, double-blind, placebo-controlled study evaluating 28 apparently healthy women receiving a single 40-g dose of either ALA or casein, emotional processing and cortisol levels were not different between groups (Scrutton et al. 2007). The authors suggested that the modest increase in tryptophan availability resulting from the single dose of ALA may have been insufficient to produce significant effects. No adverse effects were reported.

Memory function has also been assessed in several studies. In one prospective, randomized, double-blind, placebo-controlled crossover trial (Schmitt et al. 2005), 20 premenstrual women were given ALA or placebo to assess the effect on short- and long-term memory function. Administration of ALA improved long-term memory for abstract figures but not for words, an effect attributed to amelioration of serotonergic hypofunction.

ALA also improved abstract visual memory but impaired motor performance in a simple test given to 23 recovered depressed patients and 20 healthy controls in a prospective, randomized, double-blind, placebo-controlled crossover study (Booij et al. 2006). However, ALA had no adverse effect on the performance of more difficult versions of the motor performance test, suggesting that ALA may impair cognitive and physical performance when tasks are easy and monotonous, perhaps due to the sleep-inducing properties of ALA (likely due to increased brain serotonin).

This suggestion was supported by a prospective, randomized, double-blind, placebo-controlled study (Markus et al. 2005) which provided ALA (at a concentration of 4.8 g tryptophan/100 g protein) or a low tryptophan protein (n = 14/condition) in the evening and resulted in higher bed-time TRP:LNAA ratio in the ALA group. The following morning ALA administration decreased sleepiness and improved attention processes with no reported adverse effects.

The effect of ALA on satiety was determined in a prospective, randomized, single-blind crossover study in which 24 apparently healthy adults aged 19-37 years received 10%-protein breakfasts containing ALA, gelatin, or gelatin+tryptophan (Nieuwenhuizen et al. 2009). Suppression of hunger at lunchtime was stronger after the ALA breakfast than the other meals. Plasma tryptophan was higher after the ALA meal than either the gelatin or gelatin+tryptophan meals. Hormones related to satiety, GLP-1 and ghrelin, were not related to the type of breakfast consumed. The authors concluded that the study did not identify the mechanism of action of ALA satiety-related activity. No adverse effects were reported.

In a second prospective, randomized, single-blind crossover satiety study with 24 apparently healthy subjects aged 25±2 years, Veldhorst et al. (2009) evaluated energy intake at lunch following a breakfast 3 hours earlier containing one of a variety of proteins: ALA, gelatin, casein, soy, whey, whey-GMP, or gelatin+tryptophan. The ALA, gelatin and gelatin+tryptophan breakfasts resulted in reduced energy intake at lunch compared to experimental conditions in which the subjects consumed breakfasts containing the other proteins. No adverse effects were reported, but further study is warranted to fully evaluate the satiety-related effects of ALA. It should be noted that feeding infant formula enriched in ALA does not reduce formula intake (see Section 6.3.2.).

6.3.2. Studies in Infants

6.3.2.1. Studies in Infants Using Alpha-Lactalbumin Produced by Arla Foods Ingredients

Five publications have reported outcomes of prospective, randomized, double-blind clinical studies employing Lacprodan® ALPHA-10 or ALPHA 20, summarized in Table 14. Different aspects of the data from one prospective, randomized, double-blind, placebo-controlled intervention trial were reported in 3 publications—Bruck et al. (2006), Sandstrom et al. (2008), and Andersson et al. (2009). Each of these reports is discussed in detail in this section as well as results of other studies utilizing alpha-lactalbumin preparations manufactured by Arla Foods Ingredients.

In a prospective, randomized, double-blind, placebo-controlled clinical intervention, Bruck et al. (2006) evaluated the effect of feeding a formula enriched in ALA and GMP on fecal microbiota in healthy term infants aged 6±2 weeks with mean birth weight 3512.3±559.8 g. The protein concentration was 13.1 g/L in all formulas. The formulas evaluated in the study, and their ALA and GMP content as percent of protein, were:

1. Standard whey-dominant formula (11% ALA, 14% GMP),
2. ALA-enriched formula (25% ALA, 15% GMP), and
3. GMP-reduced formula (25% ALA, 10% GMP).

Infants received study formulas exclusively from 6 weeks of age to 4 months, at which time weaning foods could be introduced. Infants continued to receive study formulas until 6 months of

age. Fecal samples were collected at the start of the study (baseline) as well as at 2 and 6 months of age and analyzed for predominant population of gut microbiota using FISH. In this first report, data from 17 infants receiving standard formula, 21 receiving ALA-enriched formula, and 16 receiving GMP-reduced formula were reported, along with 31 breast-fed infants.

This publication did not present anthropometrics at birth or study entry and no growth data were provided; this information appeared in a later report, Sandstrom et al. (2008; see below). Bifidobacteria were the predominant bacterial population in all infants and no significant changes in bifidobacteria or lactobacilli levels were observed in any group throughout the study. (Note that all infants were breast-fed before study initiation, which might have established a *Bifidobacterium*-dominant microbiota.) Bifidobacteria levels were similar in all groups by 6 months of age. The ALA group had significantly lower bacteroides counts at 4 months than either the breast-fed or control groups. Between 4 and 6 months, all formula-fed infants had an increase in *Clostridium*. The breast-fed group had higher Clostridia counts than any of the formula-fed groups at 4 months.

The results of this study suggest some advantages of formulas enriched in ALA and GMP; large variations in bacterial populations may mask other beneficial effects of these materials on infant microbiota. The authors reported that there were no differences in gastrointestinal or other symptoms of disease among the groups, and that parents did not express any concern regards the formulas and were generally satisfied.

Sandstrom et al. (2008) reported other measures from the same prospective, randomized, double-blind, placebo-controlled intervention as was reported by Bruck et al. (2006), above. The number of infants for which these data were reported were n = 20 for ALA-enriched formula, n = 21 for each of the other formulas, and n = 34 for the breast-fed reference group. Anthropometrics were measured periodically and blood drawn at enrollment as well as 4 and 6 months of age. An experienced research nurse visited each infant enrolled in the study at months 2, 3, 4, 5 and 6. Structured interviews were conducted to collect information on morbidity during the entire study as well as information on general well-being and sleeping time. Questionnaires concerning formula intake, bowel habits, and stool consistency on the 3 days prior to the study were also completed.

Table 9 presents baseline characteristics of the infants. Gestational age at birth was similar for all groups (approximately 39 weeks) and all groups had normal weight as well as length at birth. A significantly greater proportion of girls were recruited to the ALA-enriched group than to the other groups; sex was used as a covariate in the analysis of those parameters which vary by sex (such as weight and length).

Table 9. Baseline Characteristics of Participating Infants (Sandstrom et al. 2008).

	Study group				
	Standard (n = 21)	α-LAC (n = 20)	RGMP (n = 21)	Breastfed (n = 34)	P
Birth weight (g)	3487 ± 475[2]	3407 ± 496	3638 ± 662	3713 ± 433	0.205
Birth length (cm)	50.4 ± 2.6	49.9 ± 2.8	50.8 ± 2.4	51.1 ± 2.4	0.473
Gestational age at birth (wk)	39.8 ± 1.3	39.7 ± 1.5	39.2 ± 1.8	39.9 ± 1.3	0.532
Girls (%)	35[a]	76[b]	41[a]	34[a]	0.038

[1] Groups were compared by using ANOVA and a Bonferroni post hoc test. Values with different superscript letters are significantly different ($P < 0.05$).
[2] $\bar{x}$ ± SD (all such values). α-LAC, α-lactalbumin enriched (15% glycomacropeptide); RGMP, α-lactalbumin enriched (10% glycomacropeptide).

Table 10 presents the infant disposition; there were no significant differences among groups in dropout rates or the reasons for discontinuation. Sandstrom et al. (2008) reported that "no serious adverse events were recorded for any of the groups."

Table 10. Infant Disposition (Sandstrom et al. 2008).

Disposition	Study Group			
	Standard	ALA-Enriched	GMP-Reduced	Breast-fed
Total enrolled	21	20	21	34
Developed allergy to cow milk protein	2	2	1	
Families chose to discontinue participation	2	1	3	
Discontinuation due to inadequate milk supply				5

Table 11 provides data related to formula intake, growth characteristics and infection incidence. Formula intake, length, head circumference, and knee-heel length were not significantly different among groups. Although weight gain was not significantly different among any of the formula groups, it was significantly greater in the standard formula group than the breast-fed group. Fever and episodes of airway infections were not significantly different among the groups.

Table 11. Formula Intake, Growth and Infection Incidence from Enrollment to Six Months of Age (Sandstrom et al. 2008).

	Study group				
	Standard	α-LAC	RGMP	Breastfed	*P*
Formula intake (mL)					
Entry	880 ± 199	820 ± 172	792 ± 143	—	0.385
2 mo	938 ± 130	870 ± 304	838 ± 90	—	0.372
3 mo	960 ± 151	906 ± 156	893 ± 110	—	0.361
4 mo	1062 ± 193	984 ± 262	928 ± 171	—	0.206
5 mo	1009 ± 176	991 ± 208	943 ± 141	—	0.590
6 mo	930 ± 136	866 ± 304	852 ± 230	—	0.640
Growth (gain/mo)					
Length (cm)	2.57 ± 0.32	2.50 ± 0.29	2.46 ± 0.26	2.48 ± 0.89	0.961
Weight (g)	730 ± 134[a]	707 ± 145[a,b]	697 ± 123[a,b]	595 ± 144[b]	0.014
Head circumference (cm)	1.25 ± 0.29	1.12 ± 0.24	1.16 ± 0.15	1.09 ± 0.13	0.118
Knee-heel length (cm)	0.35 ± 0.08	0.33 ± 0.07	0.35 ± 0.08	0.32 ± 0.07	0.753
Infections (*n*/mo)					
Fever episodes	0.14 ± 0.23	0.12 ± 0.16	0.16 ± 0.24	0.13 ± 0.17	0.908
Days of fever	0.36 ± 0.53	0.27 ± 0.40	0.33 ± 0.58	0.36 ± 0.49	0.908
Episodes of airway infections	0.36 ± 0.28	0.36 ± 0.25	0.38 ± 0.34	0.39 ± 0.33	0.956

[1] All values are $\bar{x} \pm$ SD. α-LAC, α-lactalbumin enriched (15% glycomacropeptide); RGMP, α-lactalbumin enriched (10% glycomacropeptide). Groups were compared by using ANOVA and a Bonferroni post hoc test. Values with different superscript letters are significantly different ($P < 0.05$).

Bowel habits and stool consistency were similar among groups. Sleep patterns and daytime sleep did not differ among the formula groups. There were no differences among the formula groups in red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, platelets, serum iron or serum ferritin at entry, 4 months, or 6 months. Total iron binding capacity was significantly lower in the ALA-enriched group than in the standard formula or GMP-reduced groups at 6 months, but the ALA-enriched group was not different from the breast-fed comparison group at any time point. Total iron binding capacity of all groups was within normal ranges (Samour and King 2005). Plasma insulin, leptin, and blood urea nitrogen were measured at 4 and 6 months. The groups did not differ in insulin or leptin at either time point; blood urea nitrogen was higher at both time points in all formula groups than in the breast-fed group, an expected result due to the higher protein concentrations in formula compared to breast-fed infants.

Weight, length and head circumference data were reported as Z-scores (Figure 5). At enrollment, the weight-for-age z-score (WAZ) was significantly higher in the breast-fed group than

the ALA-enriched formula group ($p<0.05$). From enrollment to 6 months of age, WAZ decreased in the breast-fed group, increased in the control formula group, and marginally increased in the experimental formula groups (Figure 5). Although WAZ was similar among the breast-fed group and the experimental groups at 6 months, when adjusted for entry weight, the WAZ of the breast-fed group was significantly lower than any of the formula groups. The only difference in length-for-age z-score (LAZ) occurred at 6 months: the control formula group was significantly longer than the GMP-reduced group. A rapid increase in head circumference-for-age z-score occurred in the control group from 3 to 6 months of age and was significantly greater than the breast-fed or GMP-reduced formula groups at 6 months. However, due to the small sample size, the study did not have sufficient statistical power to determine equivalence of growth between the groups randomized to intervention and control formulas, based on the generally accepted goal of detecting a 0.5-SD difference in growth measures (Scientific Committee on Food 2003).

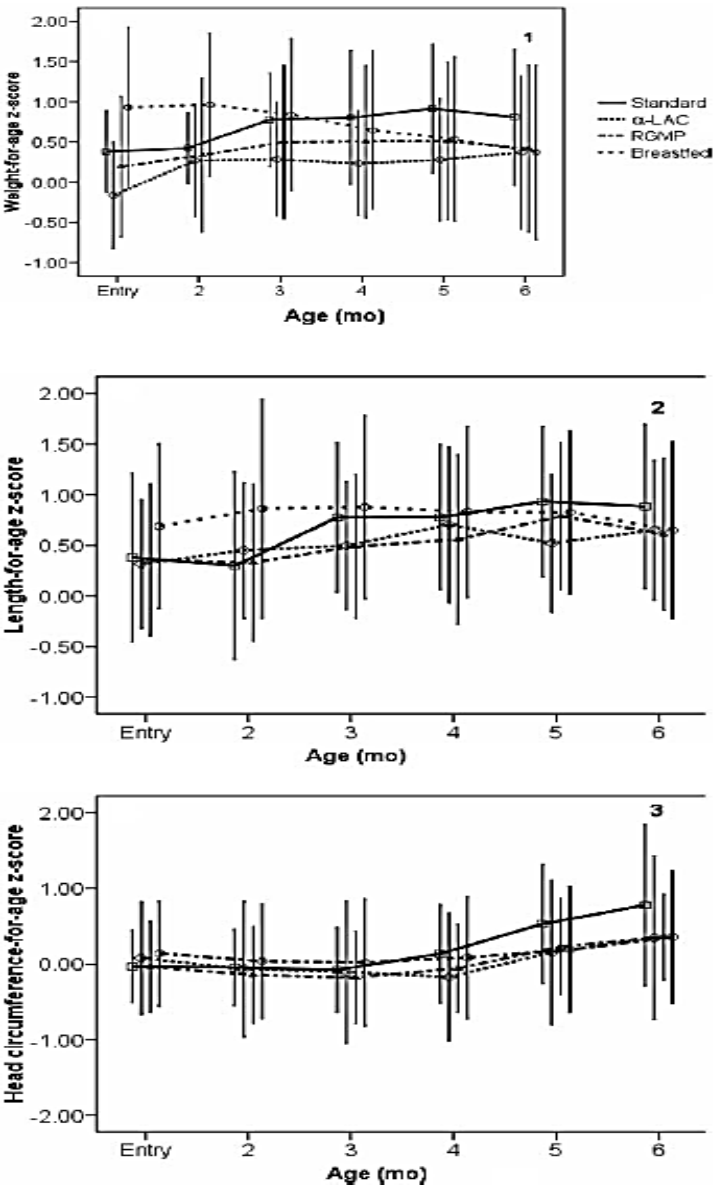

Figure 5. Weight-, Length- and Head Circumference-for-Age Z-Scores[1]. (Sandstrom et al. 2008).

[1] Bars represent means±1 SD.

Plasma concentration of the essential amino acids threonine, isoleucine, valine, methionine, isoleucine, lysine, and phenylalanine were higher in the formula-fed groups than in breast-fed infants at 6 months of age.

This study did not suggest any adverse effects of the tested infant formula enriched in ALA (with either high or lower GMP) when fed to term infants from 6 weeks to 6 months of age, although growth equivalence could not be demonstrated due to lack of statistical power. Adverse events and infections were not different among formula groups. Plasma essential amino acids and blood urea nitrogen in the ALA-enriched formula groups were greater than in the breast-fed group, demonstrating satisfactory amino acid and total protein intake in these formula groups. Growth was similar (not significantly different) among the control and ALA-enriched formula groups. Although total iron binding capacity was significantly lower in the ALA-enriched formula group than the control formula group at 6 months, it was within normal limits for infants. Red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, platelets, serum iron, and serum ferritin were not different between the ALA-enriched and control formula groups, demonstrating that the ALA-enriched group had normal iron status. The authors concluded that all formulas "were well tolerated and caused no adverse effects."

A third publication (Andersson et al. 2009) addressed other aspects of the same prospective, randomized, double-blind, placebo-controlled intervention as discussed above (Bruck et al. 2006; Sandstrom et al. 2008). As reported above, blood was drawn at enrollment and at 4 and 6 months of age; peripheral blood mononuclear cells were isolated by density gradient centrifugation. White blood cell counts and frequencies of lymphocytes and immune cells of the myeloid lineage were determined for all groups. As expected, frequencies of white blood cells were not significantly different between breast-fed and any of the formula-fed groups at study entry (most infants were breast-fed prior to the study). From entry to 4 months there was a small but statistically significant decline in WBC counts in the ALA-enriched and GMP-reduced groups compared to the breast-fed group and also a decline in the ALA-enriched group compared to control (Figure 6A). However, at 6 months all groups were equivalent. The functional significance of the brief, modest decrease in WBC counts (Figure 6A) is not obvious, especially since no difference in disease incidence was reported (Sandstrom et al. 2008).

Mean lymphocyte counts were equivalent among the groups and did not change over time (data not shown). Monocytes initially decreased in all groups (Figure 6B). Compared to the breast-fed group, the ALA-enriched group had statistically significantly lower mean neutrophil counts at both 4 and 6 months (Figure 6C), although these differences were not clinically significant. This same trend was noted when the breast-fed group was compared to the other formula groups, but the differences did not reach statistical significance. All formula groups had lower eosinophil granulocytes at 4 months compared to the breast-fed group, and these remained statistically significantly lower at 6 months in both the control group and the ALA-enriched group (Figure 6D), but the difference was not clinically significant.

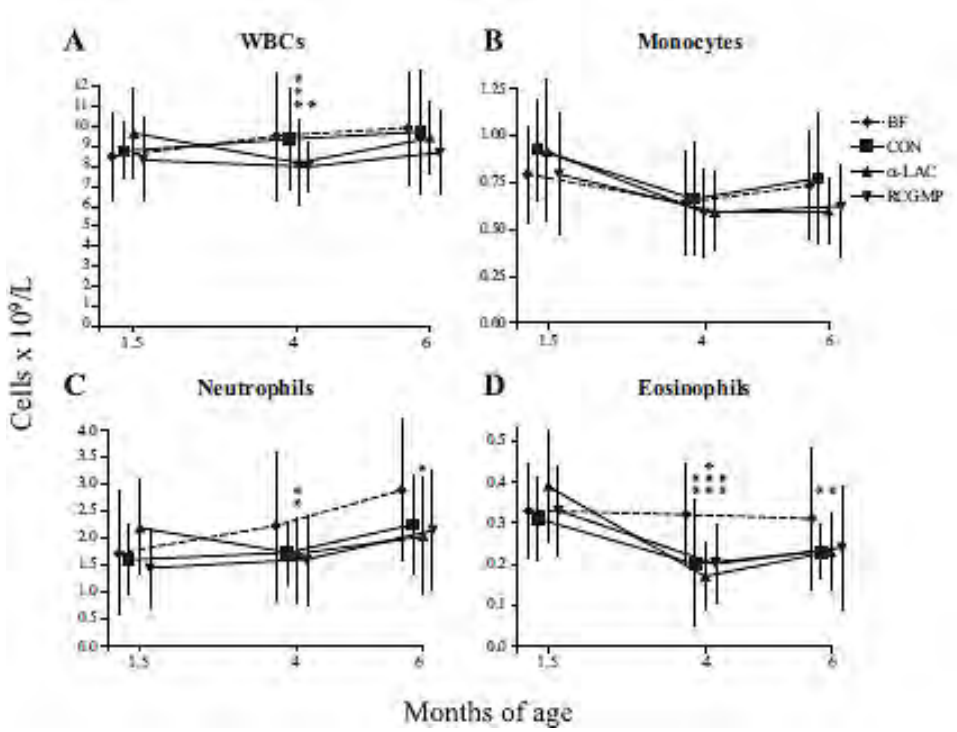

Figure 6. WBC, Monocyte, Neutrophil Granulocyte, and Eosinophil Granulocyte Counts in Whole Blood of Infants (Andersson et al. 2009)[1].

At 4 and 6 months, the proportion of T-cells (CD3+) in the formula-fed groups increased significantly with age and were higher than in breast-fed infants at both time points. In contrast, NK-cell proportions decreased in all formula groups and were significantly lower than in breast-fed infants at both 4 and 6 months. No significant differences in B-cells were found. A more detailed analysis of T-cells (naïve T-cells, memory/activated T-cells, and activated T-cells) revealed additional differences between infants receiving formula and breast-fed infants although no significant differences were found among the formula groups. The authors concluded that, since no significant differences were found among the 3 formula groups, the modulation of ALA and GMP concentrations in formula had no, or only minor, effects on the distribution of immune cells in peripheral blood during the first 6 months of life.

Dupont et al. (2010) evaluated a formula enriched in ALA with added probiotics compared to a control formula in a prospective, randomized, double-blind, placebo-controlled, multi-center study. The experimental formula (EF) contained 2.9 g/L ALA and both *Lactobacillus rhamnosus* and *Bifidobacterium infantis*. The EF formula had a protein concentration of 14 g/L while the control formula (CF) had a protein concentration of 15 g/L. In addition, the EF had reduced lactose content and no glucose-syrup, but contained malto-dextrin and corn starch. The energy level was lower in the EF (680 kcal/L) than control formula (720) kcal/L). The compositions of the 2 formulas are shown in Table 12.

[1] Cell counts were done by flow cytometry. Bars represent means ±1SD, and statistically significant differences between the BF group and a FF group are indicated: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

Table 12. Composition of Experimental and Control Formulas (Dupont et al. 2010).

Composition *Mean analysis (for 100 ml)*	*Experimental formula*	*Control formula*
Reconstitution rate (%)	13.7	14.3
Energy (kcal/kJ)	68/286	72/303
Casein (g)	0.55	0.75
Whey proteins (g)	0.85	0.75
α-Lactalbumin (g)	0.29	0
Lactose (g)	4.2	6.3
Malto-dextrin (g)	2.7	0
Glucose syrup (g)	0	2.6
Precooked corn starch (g)	1.6	0
Linoleic acid (mg)	502	550
α-Linolenic acid (mg)	48	51
Calcium (mg)	85	54
Iron (mg)	0.8	0.8
Lactic ferment		
Lactobacillus rhamnosus LCS-742 (UFC/g)	10^7	0
Bifidobacterium infantis M63 (UFC/g)	10^7	0

Sixty-six apparently healthy term infants were enrolled between the ages of 3 weeks and 3 months. Enrollment criteria included ≥ 3 weeks of crying periods of ≥ 3 hours duration/day, ≥ 3 days/week; these symptoms were defined as indicating colic. Periods of crying, irritability and agitation without crying, peaceful alertness, peaceful eating, sucking, and sleep were recorded the day preceding initiation of study formula as well as at days 15 and 30. Infants completing the study included 30 in the EF group and 32 in the control formula (CF) group. Numbers of drop-outs and the causes for drop-outs were similar between the groups.

Age at study entry was similar between groups: 52.5 days for the EF-group and 50.3 days for the CF group. At study entry the EF infants' mean weight and length were slightly, but statistically significantly, greater than the CF infants. Weight and length gain during the study were not significantly different and were similar to WHO reference growth charts. Weight gain was 1023.4±360.4 g and 1047.4±372.1 g in the EF and CF groups, respectively. Length gain was 4.2±1.4 cm (EF) and 4.3±1.9 cm (CF). However, this study did not have sufficient statistical power to determine equivalence of growth between the groups based on its inability to detect a 0.5-SD difference in growth measures due to the limited sample size (Scientific Committee on Food 2003).

The number of infants with a reduction in daily crying duration of more than 25% between enrollment and day 15 did not differ between formula groups. However, irritability and agitation without crying decreased significantly more with EF (decrease of 53.2 minutes/day) compared to CF (21.1 minutes). Crying duration decreased significantly in both groups and parents' satisfaction related to improvement of GI symptoms increased significantly in both groups during the course of the study, with no significant difference between groups.

Side effects, defined as any event temporally associated with the consumption of the study formula (also termed Study Events), were classified by the investigators as "any causality" or "feeding related." "Any causality" effects did not differ significantly between groups, while "feeding related" study events were significantly lower in the EF group (Figure 7). In the EF-fed group, "feeding-related" GI events were vomiting (one infant) and colitis (one infant). In the CF-fed group,

"feeding related" GI events were constipation (5 infants), vomiting (4 infants), colitis (one infant), regurgitation (3 infants), and flatulence (one infant).

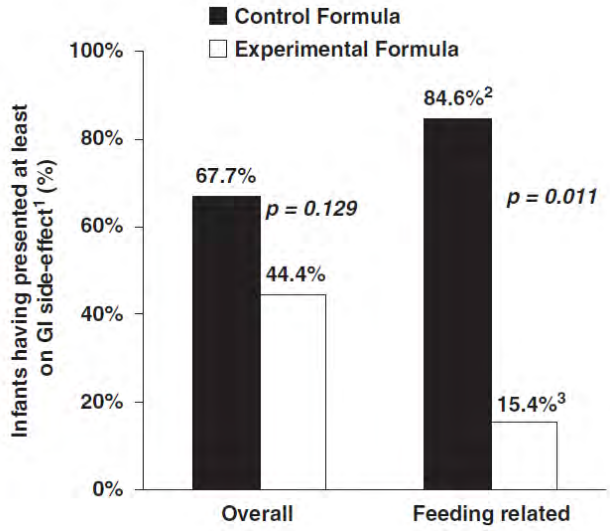

Figure 7. Distribution of Overall and "Feeding-Related" GI Side Effects (Dupont et al. 2010)[1].

This study did not demonstrate abnormal growth in infants with colic fed a formula enriched in ALA and with a slightly lower protein concentration than the CF, although the sample size was not large enough to demonstrate equivalent growth. The study did not demonstrate a reduction in colic in the infants receiving the EF; however, gastrointestinal events were less frequent in the EF group. This effect may be due to ALA (as demonstrated in the studies by Lien et al. (2004) and Davis et al. (2008) discussed in the next section, and/or by the introduction of probiotics, a result previously reported by Savino et al. (2006). The authors' primary conclusion was that "an α-lactalbumin-enriched and probiotic-supplemented formula proved to be adequate for infants with colic in terms of growth and of reduction in GI side effects."

In a prospective, randomized, double-blind, placebo-controlled study, Szymlek-Gay et al. (2012) determined the effect of ALA- and GMP-enriched formula on iron absorption in term infants. The formulas employed in this study were the same formulas as evaluated by Sandstrom et al. (2008), referred to here as Standard formula, α-LAC (high ALA and GMP), and α-LAC/RGMP (high ALA and reduced GMP). The formulas each contained 4 mg iron/L provided as $FeSO_4$. The 31 infants in this study were a subsample of the infants in the Sandstrom study, enrolled at age 4-8 weeks. The study was conducted at 5.5 months of age. Prior to the study, breast feeding mothers expressed breast milk and one day prior to the study ^{58}Fe was added to either the HM sample or to an equivalent sample of each of the formulas. Following refrigeration overnight, infants received the radioactive feedings. Iron absorption was calculated as iron incorporation into erythrocytes 14-28 days after isotope administration. Table 13 provides data concerning iron absorption in the 4 groups of infants.

[1] Tolerance population: 29% of infants presented at least one GI side effect and 72.2 presented at least one "feeding-related" GI side effect. [2] In the EF-fed group, "feeding-related" GI side effects were vomiting (one infant) and colitis (one infant). [3] In the CF-fed group, "feeding-related" GI side effects were constipation (5 infants), vomiting (4 infants), colitis (one infant), regurgitation (3 infants), and flatulence (one infant).

Iron status was determined at baseline (approximately one month of age), 4, and 6 months. Mean iron status indices were similar among all groups at all time points. No infants were iron deficient at baseline. At 4 months, depleted iron stores were found in one infant in the control group and one infant in the α-LAC/RGMP group. At 6 months, 2 infants in the standard formula group, one in the α-LAC/RGMP group, and 2 in the breast-fed group had depleted iron stores. At 6 months, the chance of developing iron deficiency was not different among any of the groups.

Iron absorption was inversely correlated with serum ferritin concentrations and was not significantly different among groups. The authors concluded that "α-lactalbumin and casein-glycomacropeptide do not affect iron absorption from infant formula in infants."

Table 13. Fractional Iron Absorption from an Isotopically Labeled Test Meal (Szymlek-Gay et al. 2012).

Group	*n*	Absorption	
		%	
Standard formula	10	10.3 ± 7.0	7.9 (4.4, 14.3)
α-LAC	10	8.6 ± 3.8	7.9 (5.5, 11.1)
α-LAC/RGMP	11	9.2 ± 6.5	7.2 (4.3, 12.1)
All formula groups	31	9.4 ± 5.8	7.6 (5.9, 9.8)
Formula-fed infants with adequate iron status	28	8.6 ± 4.4	7.4 (5.8, 9.3)
Formula-fed infants with iron deficiency[2]	3	16.4 ± 12.4*	10.6 (0.3, 100.0)
Breast-fed	9	12.9 ± 6.5	11.8 (8.5, 16.4)
All infants	40	10.1 ± 6.1	8.4 (6.8, 10.4)

[1] Values are mean ± SD or geometric mean (95% CI). *Different from the formula-fed infants with adequate iron status, $P = 0.023$. α-LAC, α-lactalbumin–enriched infant formula group; α-LAC/RGMP, α-lactalbumin–enriched/casein-glycomacropeptide–reduced infant formula group; Standard formula, commercially available whey-predominant standard infant formula group.
[2] Iron deficiency is defined as depleted iron stores, iron-deficient erythropoiesis, or iron deficiency anemia.

Table 14. Studies in Infants Using Alpha-Lactalbumin Produced by Arla Foods Ingredients.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Bruck et al. (2006)	Prospective, randomized, double-blind, placebo-controlled clinical intervention. Infants received study formulas exclusively from 6 weeks of age to 4 months, when weaning foods could be introduced. and the Infants continued to receive study formulas until 6 months of age.	Healthy term infants aged 6±2 weeks with mean birth weight = 3512.3±559.8 g This report included data from 17 infants receiving standard formula, 21 receiving ALA-enriched formula, and 16 receiving GMP-reduced formula, along with 31 breast-fed infants.	1. standard whey-dominant formula (11% ALA, 14% GMP) 2. ALA-enriched formula (25% ALA, 15% GMP) 3. GMP-reduced formula (25% ALA, 10% GMP).	Bifidobacteria were the predominant bacterial population in all infants and no significant changes in bifidobacteria or lactobacilli levels were observed in any group throughout the study. The ALA group had significantly lower bacteroides counts at 4 months than either the breast-fed or control groups. Between 4 and 6 months, all formula-fed infants had an increase in clostridia. The breast-fed group had higher clostridia counts than any of the formula-fed groups at 4 months. The authors reported that there were no differences in gastrointestinal or other symptoms of disease among the groups, and that parents did not express any concern regarding the formulas and were generally satisfied.

Table 14. Studies in Infants Using Alpha-Lactalbumin Produced by Arla Foods Ingredients.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Sandstrom et al. (2008)	The same prospective, randomized, double-blind, placebo-controlled intervention as was reported by Bruck et al. (2006)	This report included data from 21 infants receiving standard formula, 20 receiving ALA-enriched formula, and 21 receiving GMP-reduced formula, along with 34 breast-fed infants.	The same formulas as were reported by Bruck et al. (2006)	Formula intake, weight, length, head circumference, knee-heel length, febrile and infection episodes, sleep patterns, bowel habits, and stool consistency were not significantly different among formula groups, nor were red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, platelets, serum iron, serum ferritin, insulin, leptin, or blood urea nitrogen at entry, 4 months, or 6 months. Total iron binding capacity was significantly lower in the ALA-enriched group than in the standard formula or GMP-reduced groups at 6 months, but the ALA-enriched group was not different from the breast-fed comparison group at any time point. Total iron binding capacity of all groups was within normal ranges (Samour and King 2005). Plasma concentration of the essential amino acids threonine, isoleucine, valine, methionine, isoleucine, lysine, and phenylalanine were higher in the formula-fed groups than in breast-fed infants at 6 months of age. There were no significant differences between groups in dropout rates or the reasons for discontinuation. “No serious adverse events were recorded for any of the groups.” This study did not reveal any adverse effects of the tested infant formula enriched in ALA (with either high or lower GMP) when fed to term infants from 6 weeks to 6 months of age. Adverse events and infections were not different among formula groups. The authors concluded that all formulas “were well tolerated and caused no adverse effects.”
Andersson et al. (2009)	The same prospective, randomized, double-blind, placebo-controlled intervention as was reported by Bruck et al. (2006)	This report included data from 21 infants receiving standard formula, 20 receiving ALA-enriched formula, and 21 receiving GMP-reduced formula, along with 34 breast-fed infants.	The same formulas as were reported by Bruck et al. (2006)	From entry to 4 months there was a statistically significant decline in white blood cells in the ALA-enriched and GMP-reduced groups compared to the breast-fed group. However, at 6 months all groups were equivalent. Frequencies of lymphocytes were equivalent among the groups and did not change over time. Monocytes initially decreased in all groups. Compared to the breast-fed group, the ALA-enriched group had significantly lower frequencies of neutrophil granulocytes at both 4 and 6 months. All formula groups had lower eosinophil granulocytes at 4 months compared to the breast-fed group, and these remained significantly lower at 6 months in both the control group and the ALA-enriched group.

Table 14. Studies in Infants Using Alpha-Lactalbumin Produced by Arla Foods Ingredients.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
				At 4 and 6 months, the proportion of T-cells (CD3+) in the formula-fed groups increased significantly with age and were higher than in breast-fed infants at both time points. In contrast, NK-cell proportions decreased in all formula groups and were significantly lower than in breast-fed infants at both 4 and 6 months. No significant differences in B-cells were found. A more detailed analysis of T-cells (naïve T-cells, memory/activated T-cells, and activated T-cells) revealed additional differences between infants receiving formula and breast-fed infants although no significant differences were found among the formula groups. The authors concluded that, since no significant differences were found among the 3 formula groups, the modulation of ALA and GMP concentrations in formula had no, or only minor, effects on the distribution of immune cells in peripheral blood during the first 6 months of life.
Dupont et al. (2010)	Prospective, randomized, double-blind, placebo-controlled, multi-center study for 30 days to assess influence of experimental formula with ALA and pro-biotics on colic	66 apparently healthy term infants (33 per formula) aged 3 weeks to 3 months enrolled between the ages of 3 weeks and 3 months. Enrollment criteria included ≥ 3 weeks of crying periods of ≥ 3 hours duration/day, ≥ 3 days/week; symptoms defined as colic.	Experimental formula (EF) contained 14 g/L total protein and 2.9 g/L ALA and the probiotics *L. rhamnosus* and *B. infantis*. Control formula (CF) had a protein concentration of 15 g/L. The energy level was lower in the EF (680 kcal/L) than control formula (720 kcal/L)	Infants completing the study included 30 in the EF group and 32 in the (CF) group. Numbers of drop-outs and the causes for drop-outs were similar between the groups. Weight and length gain during the study were not significantly different and were similar to WHO reference growth charts. The number of infants with a reduction in daily crying duration of more than 25% between enrollment and day 15 did not differ between formula groups. However, irritability and agitation without crying decreased significantly more with EF (decrease of 53.2 minutes/day) compared to CF (21.1 minutes). Crying duration decreased significantly in both groups and parents' satisfaction related to improvement of GI symptoms increased significantly in both groups during the course of the study, with no significant difference between groups. Side effects, defined as any event temporally associated with the consumption of the study formula (also termed Study Events), were classified by the investigators as "any causality" or "feeding related." "Any causality" effects did not differ significantly between groups, while "feeding related" study events were significantly lower in the EF group. In the EF-fed group, "feeding-related" GI events were vomiting (one infant) and colitis (one infant). In the CF-fed group,

Table 14. Studies in Infants Using Alpha-Lactalbumin Produced by Arla Foods Ingredients.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
				"feeding related" GI events were constipation (5 infants), vomiting (4 infants), colitis (one infant), regurgitation (3 infants), and flatulence (one infant). This study did not demonstrate abnormal growth in infants with colic fed formula enriched in ALA and with a slightly lower protein concentration than the CF, although the small sample size did not permit demonstration of equivalent growth. The study did not demonstrate a reduction in colic in the infants receiving the EF; however, gastrointestinal events were less frequent in the EF group. The authors' primary conclusion was that "an α-lactalbumin-enriched and probiotic-supplemented formula proved to be adequate for infants with colic in terms of growth and of reduction in GI side effects."
Szymlek-Gay et al. (2012)	The same prospective, randomized, double-blind, placebo-controlled intervention as was reported by Bruck et al. (2006) to determine influence of formula on iron absorption at 5.5 months	This report included data from 11 infants receiving standard formula, 10 receiving ALA-enriched formula, and 10 receiving ALA-enriched GMP-reduced formula, along with 9 breast-fed infants.	The same formulas as were reported by Bruck et al. (2006), each containing 4 mg iron/L provided as $FeSO_4$.	Mean iron status indices were similar among all groups at all time points. No infants were iron deficient at baseline. At 4 months, depleted iron stores were found in one infant in the control group and one infant in the α-LAC/RGMP group. At 6 months, 2 infants in the standard formula group, one in the α-LAC/RGMP group, and 2 in the breast-fed group had depleted iron stores. At 6 months, the incidence of iron deficiency was not different among any of the groups. Iron absorption was inversely correlated with serum ferritin concentrations and was not significantly different among groups. The authors concluded that "α-lactalbumin and casein-glycomacropeptide do not affect iron absorption from infant formula in infants."

6.3.2.2. Studies in Infants Using Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

This section deals with clinical evaluation of ALA-enriched formulas employing sources other than Arla Foods Ingredients products; studies are summarized in Table 26. A typical whey dominant formula contains 1.2-1.3 g/L ALA. Formulas intended to more closely mimic human milk contain 2.3-2.5 g/L ALA, approximately double the amount found in traditional formula. A number of high quality studies have evaluated the safety and efficacy of formulas enriched in ALA. Most studies were conducted with formula containing ALA isolated via standard dairy technology; an exception is the study by Heine et al. (1996) discussed below. As opposed to the Arla Foods Ingredients studies discussed in Section 6.3.2.1, these studies evaluated formula with a single modification: a revision in the protein system in which ALA concentration is increased (with a concomitant decrease in total protein and beta lactoglobulin). Arla Foods Ingredients studies typically involved manipulation of both ALA and GMP or addition of both ALA and probiotics.

Heine et al. (1996) studied the effect of ALA enrichment of infant formulas on serum tryptophan levels of infants receiving the formulas in a prospective, randomized, double-blind, cross-over study carried out with 20 heathy term infants under 3 months of age. Three formulas with various amounts of added ALA were tested, providing different concentrations of tryptophan[1]. This was an initial metabolic study and not intended to be a safety study. The formulas were: control formula (whey: casein 60:40, protein concentration 18 g/L, tryptophan content 1.66 g/16 g nitrogen), a tryptophan intermediate formula (TRP-Int, whey:casein 59:41, protein concentration 13.4 g/L, tryptophan content 1.88 g/16 g nitrogen) and a high tryptophan formula (TRP Plus, whey:casein 75:25, protein concentration 13.4 g/L, tryptophan content 2.21 g/16 g nitrogen). All infants were fed the CF for at least 2 weeks, then were randomized so that half of the infants received each of the experimental formulas (10 infants per group). In a cross-over design, after 2 weeks the formulas of each of the groups were switched to the alternate experimental formula. Blood was drawn prior to the introduction of the experimental formulas and at 2 and 4 weeks of experimental formula feeding. A breast-fed comparison group was also included. Infants in the TRP Plus group had plasma tryptophan concentrations similar to the breast-fed infants, while those fed formulas with lower tryptophan did not. These results demonstrated that formula tryptophan is bioavailable and plasma levels depend on the amount of ALA provided. No anthropometric data were provided concerning infants at study entry or following consumption of formula. The authors concluded that, “The supplementation of ALA resulting in a higher TRP supply to low-protein diets is a further step towards the production of infant formulas more closely adapted to human breast milk.”

A series of prospective, randomized, double-blind, placebo-controlled clinical studies from Wyeth Nutrition (Lien et al. 2004, Davis et al. 2008, Trabulsi et al. 2011, Wernimont et al. 2015, and Hays et al. 2016), conducted to pharmaceutical safety standards with all adverse events rigorously collected, evaluated formulas enriched with ALA and containing modestly lower protein levels than traditional formulas.

Lien et al. (2004) evaluated a control formula (CF) with 15.1 g/L total protein and an experimental formula (EF) containing 14.4 g protein/L. The ALA content of the EF was 2.2 g/L, similar to human milk, while the CF had an ALA content of 1.2 g/L. Figure 8 illustrates the substantial increase in ALA content of the EF compared to the CF and the concomitant decrease in beta-lactoglobulin. The formula concentrations of all other nutrients were similar, with one exception: the calcium content of the EF was 420 mg/L and the CF was 460 mg/L.

[1] Heine et al. (1996) reported the tryptophan contents of the formulas, but not the amounts of ALA added to achieve them.

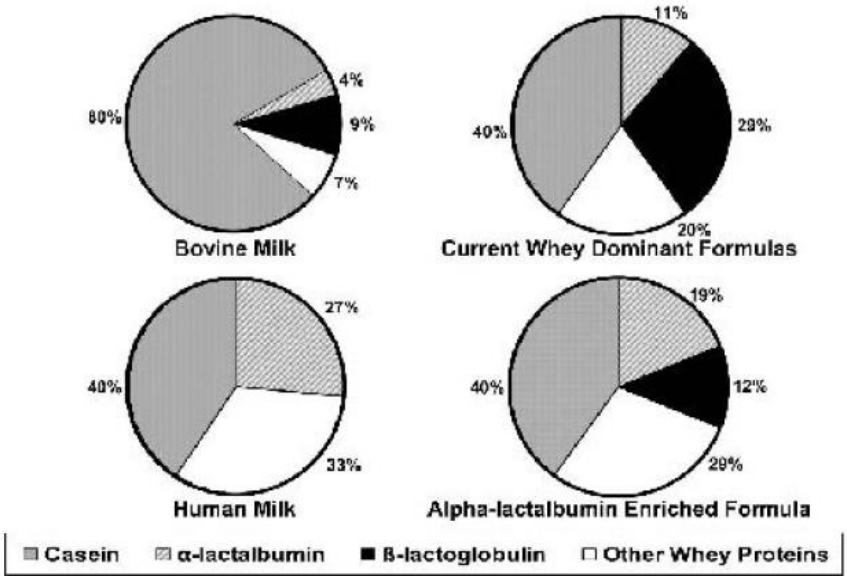

Figure 8. Protein Comparison of Human Milk, Bovine Milk and Study Formulas. From Rudolf and Kunz (1997) and Jackson et al. (2004).

Table 15 provides the amino acid content of human milk (derived from a literature average) compared to the study formulas. Importantly, proportions of both cysteine and tryptophan in the EF were similar to human milk and higher than the proportions found in the CF.

Table 15. Reference Values for the Amino Acid Content of Human Milk and Study Formulas (Lien et al. 2004).

	Percent of amino acids		
Amino acid	HM*	Experimental	Control
Essential			
Arginine	4.0	3.6	3.7
Cystine	1.8	1.9	1.5
Histidine	2.6	2.5	2.5
Isoleucine	5.3	5.7	5.8
Leucine	10.0	9.9	9.4
Lysine	6.8	7.5	7.5
Methionine	1.6	2.2	2.4
Phenylalanine	4.2	4.5	4.1
Threonine	4.7	5.4	5.4
Tryptophan	1.8	1.8	1.5
Tyrosine	4.5	4.1	3.6
Valine	5.7	6.0	5.8
Nonessential			
Alanine	3.9	3.5	4.2
Aspartic	9.3	9.0	9.0
Glutamic	17.8	17.2	18.6
Glycine	2.4	2.1	2.0
Proline	8.8	7.8	7.8
Serine	4.8	5.4	5.1
Total	100.0	100.0	100.0

* Literature average.
HM = human milk.

A total of 193 healthy term infants <14 days of age were recruited. Anthropometrics were measured at 4, 8, and 12 weeks of age. Adverse events were evaluated as a primary safety measure; these events were defined as any pathology or unintended change in anatomic, metabolic, or

physiologic functioning while consuming a study formula. Unintended changes included physical signs, reported symptoms, or laboratory data. These events were recorded during scheduled visits at weeks 4, 8 and 12, and by telephone contacts at weeks 2, 6 and 10. Secondary safety end points were protein status measurements (serum albumin, creatinine and blood urea nitrogen), and the acceptability and tolerance of study formula. Serum mineral concentrations (calcium, phosphorus, and magnesium) were determined as markers of metabolic safety.

Table 16 provides data relevant to the infants at birth. Both groups were in the normal range of gestational age as well as birth weight, length, and head circumference. Figure 9 outlines patient disposition and both this figure and Table 16 provide information concerning the ability of infants to successfully complete the trial on their assigned formula. Completion rates were higher in the EF group (73.5%) than in the CF group (65.2%) and discontinuations due to adverse events were lower in the EF group (15.3%) than the CF group (21.1 %). More infants fed CF than EF discontinued due to spitting up (8 vs 4), irritability (7 vs 3) and flatulence (7 vs 4), while more infants receiving the EF than CF discontinued due to constipation (6 vs 3). In spite of these discontinuance profiles, there were no statistically significant differences in adverse event profiles of the infants who discontinued the study and infants who remained in the study until its completion. Most of the adverse events were mild and resolved without treatment. Less than 50% of either group had adverse events that were reported to be related to formula consumption (42.9% in the EF group, 46.3% in the CF group). The most common formula-related events were flatulence (EF: 18.4%, CF: 20.0%) and constipation (EF: 22.4%, CF: 15.8%). Three infants in the EF group had one serious adverse event each while 2 infants in the CF group had a total of 4 serious adverse events. None of the serious adverse events was reported to be due to the formula consumed.

Table 16. Enrollment Demographics and Disposition (Lien et al. 2004).

	Experimental		Control	
	Mean	[SD]	Mean	[SD]
Enrolled	98		95	
Completed n (%)	72 (73.5%)		62 (65.3%)	
Gestational age (wk)	39.4	[1.1]	39.3	[1.0]
Male	50 (51.0)		51 (53.7)	
Birth weight (g)	3,419.7	[367.7]	3,336.6	[431.1]
Birth length (cm)	51.2	[2.1]	50.7	[2.2]
Birth head circumference (cm)	34.3	[1.4]	34.4	[1.5]
Study disposition				
Discontinued study*	26 (26.5%)		33 (34.7%)	
(Reason for study discontinuation)				
Failed to return	5 (5.1%)		4 (4.2%)	
Adverse event†	15 (15.3%)		20 (21.1%)	
Spitting up	4		8	
Vomiting	4		4	
Constipation	6		3	
Irritability	3		7	
Flatulence	4		7	
Abdominal pain	3		1	
Diarrhea	3		1	
MD/family request	19 (19.4%)		23 (24.2%)	
Protocol violation	8 (8.2%)		4 (4.2%)	
Other	2 (2.0%)		3 (3.2%)	

* More than one reason could be listed for discontinuation.
† More than one adverse event could be listed for discontinuation.

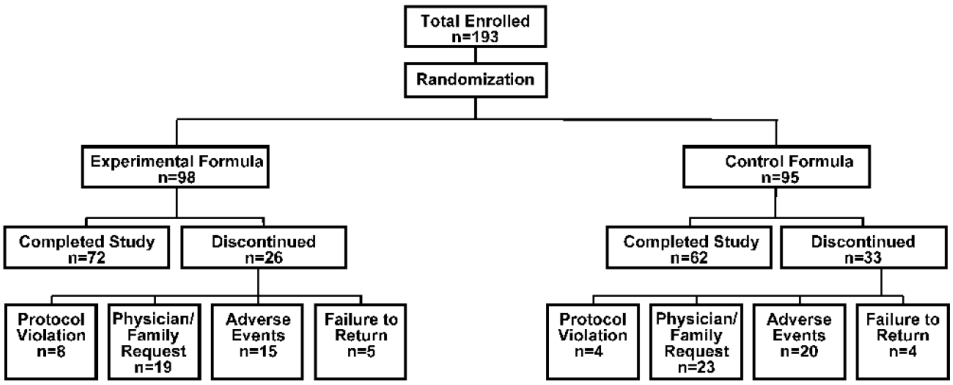

Figure 9. Trial Profile of Infants Who Completed and Who Discontinued the Study (Lien et al. 2004).

Although weight was marginally but statistically significantly higher in the EF group at baseline, no other significant differences were found between groups in weight, length and head circumference at any time point during the trial (Table 17). Z-score data (weight-for age, length-for-age, and weight-for-length) did not differ significantly between groups. These data reflect growth dynamics that are similar between the study groups and demonstrate that the ALA-enriched formula supported normal growth. Biochemical parameters are presented in Table 18. Although serum albumin concentrations did not differ between groups, both blood urea nitrogen and creatinine were lower at 12 weeks in the EF group than in controls; although these differences were statistically significant, they were of no clinical significance. Due to its long half-life of approximately 2 weeks (Bessler 2005), serum albumin is a useful marker of protein status in this 12 week trial. The serum albumin data demonstrated that the ALA-enriched formula protein system supported normal protein status (providing adequate amounts of all essential amino acids), while markers of total protein intake and disposal of unnecessary nitrogen (reflected by blood urea nitrogen and creatinine concentrations) were lower in the EF group than in the CF group. Serum calcium, phosphorus, and magnesium concentrations did not differ between groups, demonstrating adequate absorption and metabolism of these secondary safety markers.

Table 17. Mean Weight, Length and Head Circumference: Comparison of EF and CF (Lien et al. 2004).

	Experimental			Control		
Variable	n	Mean	SD	n	Mean	SD
Weight (g)						
Baseline	98	3,536.4	423.0	95	3,458.5	486.9*
Week 4	89	4,573.9	518.1	85	4,482.7	660.2
Week 8	77	5,490.5	610.9	72	5,351.5	735.1
Week 12	72	6,229.7	654.9	63	6,141.9	810.5
Length (cm)						
Baseline	98	51.5	1.8	95	51.3	2.3
Week 4	89	55.1	2.1	85	54.9	2.5
Week 8	77	58.3	2.1	72	57.8	2.7
Week 12	72	61.2	2.1	63	61.0	2.4
Head circumference (cm)						
Baseline	98	35.4	1.4	95	35.5	1.4
Week 4	89	37.8	1.2	85	37.7	1.3
Week 8	77	39.2	1.3	71	39.3	1.3
Week 12	72	40.7	1.2	63	40.9	1.3

* P value = 0.0423.

Table 18. Biochemical Measures at Baseline and Week 12 in Infants Receiving EF and CF (Lien et al. 2004).

	Experimental			Control			
	n	Mean	SD	n	Mean	SD	*P* value*
Albumin (g/dL)							
Baseline	85	3.6	0.3	86	3.5	0.3	0.1045
Week 12	68	4.1	0.3	71	4.1	0.3	0.6171
BUN (mg/dL)							
Baseline	88	6.1	2.7	85	6.2	2.3	0.5368
Week 12	70	8.2	1.9	71	9.3	1.8	0.0016
Creatinine (mg/dL)							
Baseline	84	0.34	0.13	80	0.32	0.13	0.4754
Week 12	67	0.20	0.07	71	0.24	0.07	0.0054
Calcium (mg/dL)							
Baseline	85	10.1	0.5	82	10.1	0.6	0.6249
Week 12	70	10.2	0.4	71	10.2	0.4	0.4119
Phosphorus (mg/dL)							
Baseline	77	7.4	0.8	65	7.4	0.7	0.9883
Week 12	68	6.7	0.5	68	6.8	0.4	0.2657
Magnesium (mEq/dL)							
Baseline	88	1.8	0.2	85	1.8	0.2	0.5772
Week 12	70	1.9	0.1	71	1.8	0.1	0.6545

* *P* values are between formula groups at each time period.

The acceptability and tolerance of the formulas was assessed every second week during the study period. As demonstrated in Figure 10, after the first 2 weeks of the study, the EF formula had higher ratings than the CF group. At week 8 this difference reached a statistically significant difference (*post hoc* analysis).

In conclusion, this study demonstrated the safety of an ALA enriched formula; the formula supported comparable growth to a standard, commercial formula. Protein status, using serum albumin as a marker, was comparable between formula groups. The ALA-enriched formula had improved tolerance compared to standard formula.

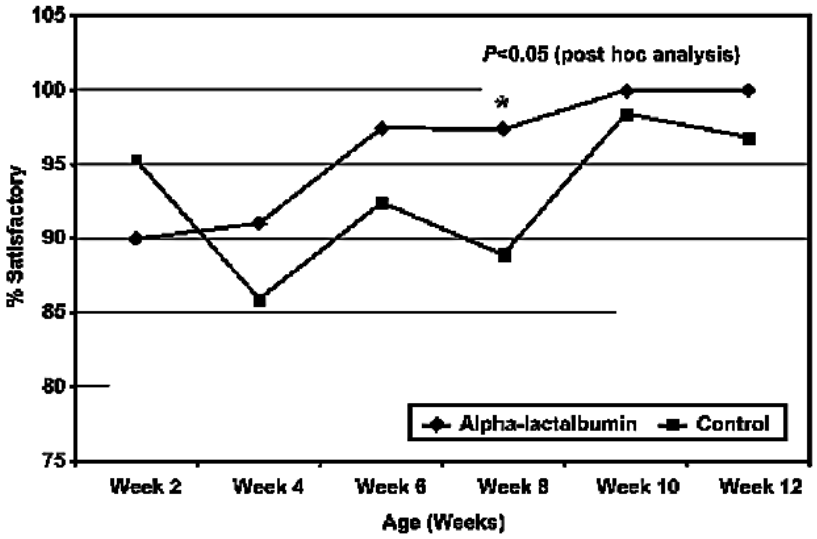

Figure 10. Formula Acceptability and Tolerance (Lien et al. 2004).

Davis et al. (2008) reported the results of a prospective, randomized, double-blind, placebo-controlled, multicenter study comparing plasma amino acids and GI tolerance in infants receiving formulas similar to those of Lien et al. (2004), including identical amino acid profiles. A breast-fed group was also included in this study. The study was designed to evaluate the effect of lowering the formula protein content on growth, serum biochemical parameters, and plasma amino acids. The total protein was 15 g/L (1.3 g/L ALA) in the standard formula (labelled SF in this study while it was labelled Control Formula in the previous study) and 14 g/L (2.2 g/L ALA) in the EF formula. A total of 128 healthy term infants <14 days of age were recruited to the study and were exclusively fed infant formula (CF and EF groups—n = 64/group) or human milk (HM group—n = 88).

This study was designed to evaluate both efficacy and safety. The primary efficacy end point was post-prandial plasma essential amino acid levels at week 8. Blood was drawn between 1.5 and 2.5 hours after the infant's last feeding. Secondary efficacy parameters involved markers of protein status (serum albumin, blood urea nitrogen, creatinine, and complete blood count with differential).

Primary safety parameters were growth and study events associated with the feeding modality. A study event was defined as any unintended change in pathology or in anatomic, metabolic, or physiologic functions temporally associated with consumption of formula or HM (guidelines of the International Conference on Harmonization and Good Clinical Practice). Study events were categorized as "any causality" (all study events regardless of causality) or "feeding-related." Feeding-related events were defined as those that, in the opinion of the study physician, were definitely, probably, or possibly related to study feeding. These events were recorded during scheduled visits at weeks 4 and 8, and by telephone contacts at weeks 2, 6, and 10. Growth parameters were determined at baseline and weeks 4 and 8; they included weight, length, weight-for-length ratio, and head circumference.

The enrollment information and discontinuations are reported in Table 19. Although differences in age at enrollment and gestational age occurred, these minor differences among groups would not be expected to influence the results of this study. Anthropometrics at birth were not significantly different among groups, and the groups were within normal ranges. Discontinuations due to study events (which included, but were not limited to, adverse events) differed among groups. In the SF group, 21.9% of infants discontinued due to study events, while only 10.9% of the infants in the EF group discontinued for this reason and none of the breast-fed infants left the study due to study events. These data suggest improved tolerance to the EF, and are considered in further detail below.

Table 19. Demographic Characteristics and Disposition of Enrolled Infants by Feeding Group (Davis et al. 2008).

	Standard	*Experimental*	*HM*
Enrolled	64	64	88
Completed (%)	43 (67.2)	49 (76.6)	74 (84.1)
Age at enrollment (days)	9.8 (3.8)[a]	9.3 (3.9)	10.1 (3.4)
Gestational age (week)	38.9 (1.0)	38.9 (1.2)	39.3 (1.0)[b]
Female (%)	34 (53.1)	35 (54.7)	49 (55.7)
Birth weight (g)	3342 (442.7)	3430 (444.9)	3521 (447.6)
Birth length (cm)	50.2 (2.4)	50.4 (2.5)	51.0 (2.4)
Birth head circumference (cm)	34.3 (1.61)	34.4 (1.5)	34.8 (1.7)
Study disposition (%)			
Discontinued study[c]	21 (32.8)	15 (23.4)	14 (15.9)
Reason for study discontinuation (%)			
Study event[d]	14 (21.9)	7 (10.9)	0 (0)
Protocol violation	2 (3.1)	2 (3.1)	8 (9.1)
MD/family request	3 (4.7)	3 (4.7)	3 (3.4)
Lost to follow-up	1 (1.6)	3 (4.7)	1 (1.1)
Other	1 (1.6)	0 (0)	2 (2.3)

Abbreviations: ANOVA, analysis of variance; HM, human milk.
[a]Mean (s.d.).
[b]Significant difference across groups (ANOVA, $P = 0.036$).
[c]More than one reason could be listed for discontinuation.
[d]More than one study event could be listed for discontinuation.

There were no significant differences among the groups in growth velocity from baseline to the end of the study (Table 20). Weight gain (g/day), length gain (cm/week), and head circumference gain (cm/week) were similar among the groups. These data demonstrate that a formula with modestly lower protein levels provides all essential amino acids required for normal growth while providing lower total protein intake, closer to human milk. This topic was more thoroughly explored in the third Wyeth study (Trabulsi et al. 2011), discussed below.

Table 20. Growth Velocity and Formula Intake by Feeding Group (Davis et al. 2008).

Growth parameter	*Standard* n = *40*	*Experimental* n = *47*	*HM* n = *70*
Weight gain (g/day)	35.0 ± 8.0[a]	35.9 ± 9.5	34.5 ± 8.7
Length gain (cm/week)	0.92 ± 0.19	0.90 ± 0.21	0.88 ± 0.21
Head circumference gain (cm/week)	0.52 ± 0.12	0.50 ± 0.09	0.50 ± 0.12
Intake (volume) (ml/day, week 8)	1068 ± 342	984 ± 222	N/A
Protein intake (g/day, week 8)	16.0 ± 5.1	13.8 ± 3.1	N/A

Abbreviation: HM, human milk; N/A, not available.
[a]Mean ± s.d.

Serum concentrations of albumin and creatinine were similar among groups at baseline and week 8. Serum BUN at baseline and week 8 were within normal ranges for all study groups, although the human milk-fed infants had higher BUN at baseline than the formula groups and

lower BUN at week 8. These data are not surprising, since human colostrum contains higher protein levels than the formulas used in this study and HM protein levels decrease dramatically during the first few weeks of lactation to levels lower than the study formulas. No significant differences in hematocrit or hemoglobin concentrations were found among groups at any time point.

Table 21 presents post-prandial plasma essential amino acid concentrations at week 8. All amino acid concentrations were within normal ranges for healthy infants of similar age (Lepage et al. 1997), although 4 of these amino acids were significantly higher in the EF group than the SF group: cystine, lysine, tryptophan and tyrosine. These data demonstrated that feeding a lower protein ALA-enriched formula (with an amino acid profile closer to human milk) results in plasma essential amino acid concentrations at least as great as a standard formula.

Table 21. Post-Prandial Plasma Essential Amino Acids at Week 8 (Davis et al. 2008).

Amino acid	*Standard* n = *40*	*Experimental* n = *47*	*HM* n = *70*
Arginine	78.2 (22.2)[a]	84.6 (18.3)	90.3 (25.8)
Cysteine	28.3 (11.9)[b,c]	36.0 (10.8)[b,c]	34.0 (13.4)
Histidine	76.3 (15.2)	79.9 (20.0)	67.8 (21.7)
Isoleucine	70.0 (21.5)	75.8 (15.7)	61.9 (19.2)
Leucine	115.1 (28.0)	126.2 (26.3)	113.3 (37.3)
Lysine	195.2 (39.1)[b]	214.1 (41.8)[b]	166.0 (39.0)
Methionine	34.4 (7.7)	34.7 (8.4)	26.2 (9.0)
Phenylalanine	46.8 (10.1)	52.5 (20.7)	40.6 (19.7)
Threonine	182.7 (50.1)	193.2 (52.9)	119.7 (37.5)
Tryptophan	55.7 (10.9)[b]	62.9 (19.1)[b]	56.8 (15.6)
Tyrosine	78.6 (20.4)[b,c]	95.8 (20.8)[b,c]	79.1 (21.9)
Valine	182.3 (35.4)	197.6 (39.3)	154.0 (43.9)

Abbreviation: HM, human milk.
[a]Mean (s.d.) (μmol/l).
[b]Statistically significant difference (Student's *t*-test) between standard and experimental formula at a traditional significance level ($P<0.05$).
[c]Statistically significant difference (Student's *t*-test) between standard and experimental formula after adjustment for multiplicity ($P<0.004$).

The most common adverse events were constipation, gastroesophageal reflux, regurgitation, abdominal pain, vomiting, diarrhea, and eructation. Figure 11 illustrates the incidence of GI study events, either "any causality" or "feeding–related." Although the incidence of "any causality" GI events was higher in the SF group than the EF or the HM-fed group, the differences were not statistically significant. However, when "feeding-related" events were considered, the SF group had a significantly higher rate of events than the HM group. For both "any causality" and "feeding-related" events, the EF and HM groups were similar. Figure 12 shows the incidence of study withdrawals due to GI study events. Significant differences were found for withdrawals due to "any causality" and "feeding-related" GI study events (SF>HM).

The authors observed that:

> "The unique finding in the present study was that the cumulative GI event profile of EF was similar to that of the HM profile after study day 18. Additionally, the timing of GI study events suggests that the SF was not as well tolerated when compared to HM. GI effects usually present soon after the introduction of a new feeding, and to our knowledge, a detailed time course of study events has never before been reported. Of potential clinical importance is that the incidence of constipation and regurgitation in the EF group was

similar to HM-fed infants, and lower than that of the SF group. The improved GI profile observed in the present study may be attributed to a formula matrix that is closer to HM. This outcome complements the findings of the α-lactalbumin growth and safety study where Lien et al. (2004) found improved tolerance in infants fed with α-lactalbumin enriched formula, as demonstrated by superior acceptability and tolerance (% satisfactory) ratings in EF versus SF after week 2, and a significantly higher rating at week 8 (EF: 97% and SF: 89%)" (Davis et al. 2008).

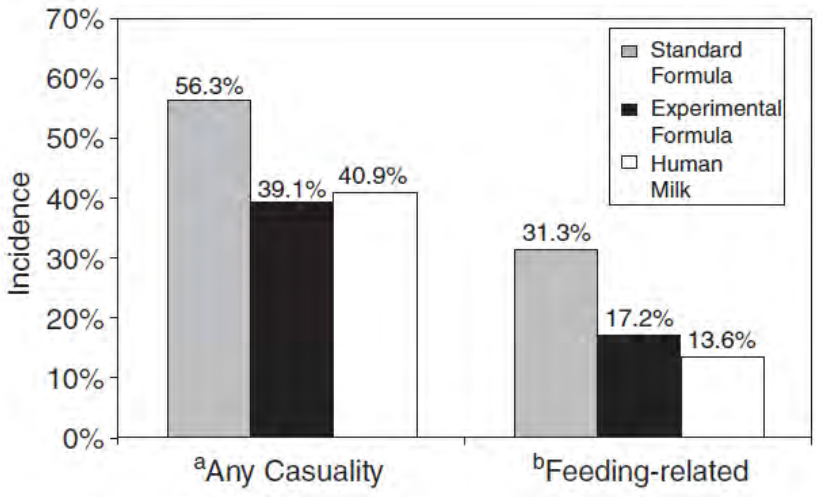

Figure 11. Incidence of GI Study Events Among All Feeding Groups (Davis et al. 2008)[1].

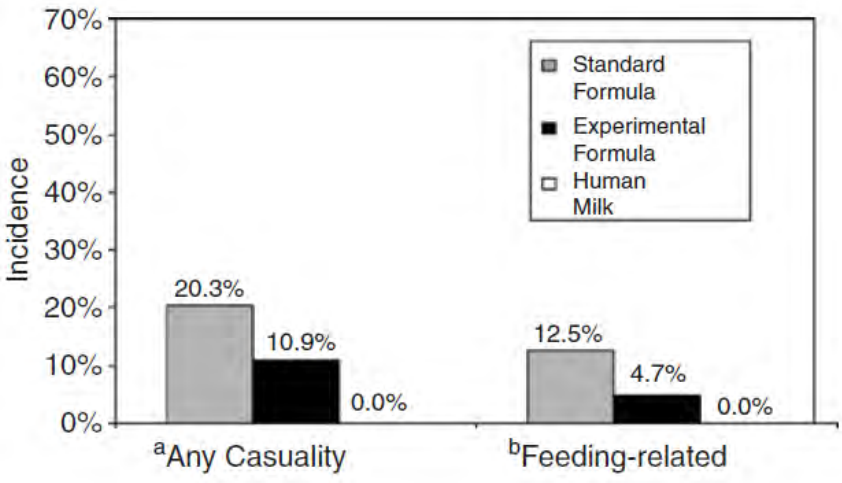

Figure 12. Incidence of Study Withdrawals Due to GI Events (Davis et al. 2008)[2].

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[1] [a]The incidence of GI study events of any causality was not significantly different across feeding groups (ANOVA, $p < 0.092$). [b]The incidence of feeding-related GI study events was significantly different across feeding groups (ANOVA, $p < 0.025$). The incidence of feeding-related GI study events was significantly higher in the SF versus HM group (Fisher's exact test, $p < 0.015$).

[2] [a]The incidence of study withdrawals due to GI study events of any causality was significantly different across groups (ANOVA, $p < 0.001$). [b]The incidence of study withdrawals due to feeding-related GI study events was significantly different across groups (ANOVA, $p < 0.001$). The incidence of study withdrawals due to feeding-related study events was significantly higher in the SF versus HM group (Fisher's exact test, $p < 0.001$).

The authors concluded: "This study of a lower protein infant formula with increased α-lactalbumin concentration represents a major improvement in the infant formula protein matrix to more closely match the protein composition and GI tolerance profile of HM."

The first 2 Wyeth studies (Lien et al. 2004 and Davis et al. 2008) compared the feeding of an ALA-enriched formula with a standard formula, involving a substantially higher ALA concentration but only a marginally lower protein concentration. However, human milk contains substantially less protein than standard formula (10-12 vs. 14-15 g/L), and data from Koletzko et al. (2009) indicate that feeding formulas with lower protein leads to a weight gain pattern closer to breast-fed infants than infants fed higher protein formulas, a difference that may have long term effects on obesity. The next study by Wyeth (Trabulsi et al. 2011) was designed to address this hypothesis.

Trabulsi et al. (2011) reported a large prospective, randomized, double-blind, placebo-controlled growth study (comparing an ALA-enriched formula similar to that evaluated in previous studies, termed standard formula, SF, to a lower protein ALA-enriched formula, termed experimental formula, EF. The protein concentrations were 14.1 g/L and 12.8 g/L, respectively. Both formulas employed an ALA-enriched whey isolate and small amounts of added L-tyrosine and L-tryptophan. The total ALA contents in these formulas were similar to human milk levels at 2.2 and 2.3 g/L, respectively. The formulas contained essential amino acid and ALA concentrations similar to human milk (Table 22). A breast-fed group was also included.

Table 22. Composition of Study Formulas (Trabulsi et al. 2011).

	SF	EF	Recommendations[b]			
			Codex alimentarus		EU commission	
			Min	Max	Min	Max
Energy, kcal/l	672	666	600	700	600	700
Protein: energy ratio, g protein per 100 kcal	2.1	1.9	1.8	3.0	1.8	3.0
Protein, g/l	14.1	12.8	See above		See above	
Histidine, mg/100 kcal	51	45	40		41	
Isoleucine	113	103	90		92	
Leucine	205	186	166		169	
Lysine	182	170	113		114	
Threonine	122	110	77		77	
Tryptophan	36	37	32		33	
Methionine + cysteine[c]	84	76	61		62	
Tyrosine + phenylalanine[d]	176	170	159		156	
α-Lactalbumin, g/l	2.2	2.3	No recommendations		No recommendations	
Whey:casein	60:40	66:34	No recommendations		No recommendations	
Carbohydrate, g/100 kcal	10.8	10.8	9.0	14.0	9.0	14.0
Fat, g/100 kcal	5.4	5.4	4.4	6.0	4.4	6.0

Abbreviations: EF, experimental formula; EU, European union; Min, minimum; Max, maximum; SF, standard formula.
[a]Nutrient composition analyzed by Covance Laboratories, Madison, WI, USA; total protein calculated as total nitrogen × 6.25.
[b]Codex and EU recommendations for infant formula composition are based on a compilation of published literature values on human milk composition.
[c]The concentration of methionine and cystine/cysteine may be added together if the ratio between methionine and cystine is not greater than 2.
[d]The concentration of tyrosine and phenylalanine may be added together if the ratio between tyrosine:phenylalanine is not greater than 2.

A total of 224 healthy term formula-fed infants aged 5-14 days with weight, length, and head circumference >5th and <95th percentile for age were recruited. Table 23 provides demographics of the subjects at birth, and the data demonstrated that the group means fell within the normal range of World Health Organization anthropometrics for healthy term infants (WHO 2006). Infants received study formulas for 120 days. Each group contained 112 infants at baseline. Anthropometrics were measured at baseline and on Days 30, 60, 90, and 120. Two hours post prandial blood samples were collected at baseline as well as on Days 60 and 120.

Table 23. Subject Demographics (Trabulsi et al. 2011).

	SF (n = 112)	*EF* (n = 112)	*HM* (n = 112)
Gestational age (weeks)	38.5 (0.9)	38.6 (0.8)	38.6 (0.9)
Birth weight (kg)	3.14 (0.42)[b]	3.17 (0.43)[c]	3.00 (0.40)
Birth length (cm)	49.4 (2.0)	49.5 (2.0)	49.4 (1.7)
Age at enrollment (days)	9.5 (3.0)	9.5 (2.7)	9.8 (3.0)
Gender (% male)	50	50	50
Race (% Asian)	100	100	100

Abbreviations: HM, human milk; EF, experimental formula; SF, standard formula; values presented are means (s.d.).
[a]Intent-to-treat (ITT)population.
[b]Significant difference between SF vs HM, $P<0.05$ (*P*-value based on two-sample *t*-test).
[c]Significant difference between EF vs HM, $P<0.05$ (*P*-value based on two-sample *t*-test).
Differences observed for both ITT population (data shown in table) and efficacy analyzable population (data not shown).

Adverse events (termed "study events") were defined in a manner identical to that of Davis et al. (2008). Symptoms related to the digestive system and GI tolerance were of particular interest and included hard stools, constipation, difficulty having a bowel movement, acute diarrhea, chronic diarrhea, spitting up, regurgitation, vomiting, gastroesophageal reflux disease, colic and crying/ neonatal abnormal crying. Investigators were provided with standard definitions of these symptoms to ensure consistency.

A high proportion of the infants enrolled in the study completed the protocol (96%). Discontinuations due to study events were low and were not significantly different among the groups (high protein formula: 2.7%, low protein formula: 2.7%, HM-fed group: 0%). Feeding-related study events were relatively uncommon and not significantly different among groups (high protein formula: 11.6%, low protein formula: 6.3%, HM-fed group: 4.5%).

Mean weight and length gain over the 120 day study did not differ between formula groups. The higher protein group gained significantly more weight than the HM group, while the lower protein group gained significantly greater length (Table 24). These differences were also reflected in Z-score analysis (data not shown). Both weight-for-age and length-for-age Z-scores increased throughout the study. In addition, both formula groups had significantly greater head circumference z-scores than the HM group at Day 120 of the study.

Table 24. Growth Velocity by Feeding Group (Trabulsi et al. 2011).

	SF (n = 108)	*EF* (n = 103)	*HM* (n = 110)
Weight gain, g/day	28.1 (5.4)[b]	27.8 (5.3)	26.6 (5.4)
Length gain, cm/month	3.21 (0.33)	3.22 (0.35)[c]	3.12 (0.32)
Head circumference, cm/month	1.60 (0.20)	1.61 (0.22)[c]	1.55 (0.18)

Abbreviations: HM, human milk; EF, experimental formula; SF, standard formula; values presented are means (s.d.).
[a]Efficacy analyzable population; growth velocity = rate of change from baseline to day 120.
[b]Significant difference between SF vs HM group, $P<0.05$ (*P*-value based on two-sample *t*-test).
[c]Significant difference between EF vs HM group, $P<0.05$ (*P*-value based on two-sample *t*-test).

Mean serum albumin, total protein, BUN, and creatinine concentrations were within normal ranges for all groups at baseline, Day 60, and Day 120 (Table 25). The only significant differences occurred in the BUN. SF was higher than the breast-fed group at Day 60 and both formula groups were higher than the breast-fed group at Day 120. These data suggest that the lower protein formula yielded an improvement in the requirement to dispose of excess protein compared to the standard formula, but further protein lowering is required to match breast-fed infants. Serum insulin and glucose levels were not significantly different among groups at Day 60. The essential amino acid concentrations did not differ between the 2 formula groups, although some of the amino acids were higher in the formula groups than the breast-fed group (data not shown). The mean concentrations of all amino acids for all groups were within normal ranges.

Table 25. Serum Biochemistries (Trabulsi et al. 2011).

	Units	*SF (n = 108)*	*EF (n = 103)*	*HM (n = 110)*
Albumin				
Baseline	g/l	40.0 (3.0)	40.9 (2.5)	40.5 (2.8)
Day 60		42.7 (2.2)	42.6 (2.2)	42.3 (2.3)
Day 120		44.5 (2.2)	45.0 (2.3)[b]	44.0 (2.1)
Total protein				
Baseline	g/l	61.4 (4.6)	61.9 (4.4)	61.3 (4.5)
Day 60		61.4 (4.3)	60.8 (6.3)	61.2 (3.6)
Day 120		63.8 (4.0)	64.1 (4.2)	63.9 (4.2)
BUN				
Baseline	mg per 100ml	8.4 (2.9)	8.4 (2.3)	8.8 (2.8)
Day 60		6.7 (1.3)[c]	5.7 (1.4)	5.3 (1.8)[c]
Day 120		6.9 (1.8)[c]	6.4 (1.8)[b]	4.8 (1.4)[b,c]
Creatinine				
Baseline	µmol/l	42.5 (9.5)	41.7 (9.3)	41.1 (8.1)
Day 60		26.8 (3.3)	26.5 (3.5)	27.1 (2.2)
Day 120		27.3 (3.2)	26.9 (3.6)	27.3 (2.3)
Insulin				
Day 60	µU/ml	6.48 (4.18)	7.35 (4.83)	6.58 (4.47)
Glucose				
Day 60	mg per 100ml	67.9 (10.1)	68.1 (10.2)	70.2 (8.84)

Abbreviations: BUN, blood urea nitrogen; HM, human milk; EF, experimental formula; SF, standard formula; values presented are means (s.d.).
[a]Efficacy analyzable population.
[b]Significant difference between EF vs HM, $P<0.05$ (P-value based on two-sample t-test).
[c]Significant difference between SF vs HM, $P<0.05$ (P-value based on two-sample t-test).

In this study, feeding a lower protein formula (lower than standard commercial formula) resulted in normal growth, serum markers of protein status, and plasma amino acids compared to both a standard formula and a breast-fed group. Weight gain in infants receiving the low protein formula was between the standard formula group and breast-fed infants while weight-for-age and weight-for-length Z-scores were similar to infants in the breast-fed group. The incidence of adverse events was relatively low and not significantly different among groups.

The authors concluded that, "α-Lactalbumin-enriched formula containing12.8 g/l protein was safe and supported age-appropriate growth; weight gain with EF was intermediate between SF and HM groups and resulted in growth similar to HM-fed infants."

In a prospective, randomized, double-blind, placebo-controlled, multicenter trial, Wernimont et al. (2015) evaluated an ALA-enriched formula (2.2 g ALA/L) with or without added

oligofructose (OF) in healthy term infants. Formulas were fed for 8 weeks and a HM reference group was included. Infants completing the study were: 28 in the control formula (CF) group without OF, 25 in the experimental formula (EF) group with OF, and 31 in the HM group. The total proportions of infants who discontinued the study were similar among the study groups, and the total incidence of adverse events (AEs) was also similar among groups. However, some differences were noted concerning withdrawals due to feeding related GI AEs, including 4 infants in the CF group, 12 infants in the EF group, and one infant in the HM group. None of the events which led to withdrawals was considered serious.

The infants in the EF group had higher bifidobacteria levels and softer stools than the CF group at study completion. The results of this study demonstrate that infants receiving an ALA-enriched formula have excellent formula tolerance. These results contribute to the body of data that demonstrate the safety of formulas enriched in ALA.

A final Wyeth study (Hays et al. 2016) was a prospective observational cohort study evaluating the health-related quality of life in Chinese infants receiving an ALA-enriched formula with increased sn-2 palmitate and OF (formula group), a group receiving a mix of the study formula and HM (mix group), and an exclusively HM-fed group for 42 days. Formula assignment (formula alone or mix) was chosen by parents rather than by randomization. This was a relatively large study with the following numbers completing the study: formula, n = 140; mix, n = 151; HM, n = 136. Formula tolerance was excellent with only 10 infants discontinued in the formula group, 11 in the mix group, and 11 in the HM group. Health-related quality of life was assessed using a validated questionnaire, and was high for all groups. Several parameters had significant differences with the formula group being marginally lower in categories such as temperament and mood, general health perception, and parent-focused concerns than the other groups. The safety of the formula is reflected in the authors' conclusion that "[health-related quality of life] was high in this population of healthy infants, with only a few small differences in [health-related quality of life] concept scores observed between breastfed, formula-fed and mixed-fed infants."

The same group carried out a similar prospective observational cohort study 2 years later using the same 3 feeding conditions (Mao et al. 2018). Healthy term infants aged 35-49 days were assigned to a feeding group based on parental decision prior to enrollment—n = 150 exclusively formula fed, n = 163 mix, and n = 147 breastfed. Infants remained on their assigned feed for 48 days. The incidence of either hard or watery stools was low, and did not differ among groups, and all groups had similar rates of growth. The majority of infants (81.5%) who participated in the study did not manifest any AEs. The percentages of subjects with any AEs in the breastfed, formula-fed, and mixed-fed groups throughout the entire study period (~48 days) plus a 4-week poststudy followup after the last clinical visit were 22%, 16%, and 18%, respectively. A total of 4 serious AEs (resulting in hospitalization) was reported: one report of bronchopneumonia in each group and one report of pneumonia in the breastfed group. Only one AE led to study discontinuation (in the formula-fed group; due to umbilical hernia). None of the serious AEs was considered related to infants' feeding. The authors concluded that the ALA-enriched formula "was well tolerated based on both parent questionnaire and physician-reported GI study events."

An ALA-enriched and symbiotic-supplemented (both prebiotics and probiotics added) formula was evaluated for safety, tolerance and prevention of atopic dermatitis in a 6-months prospective, randomized, double-blind, placebo-controlled, multicenter study (Roze et al. 2012). The control formula contained 15 g protein/L; the ALA content was not reported. The experimental formula (ALA+syn) contained 14 g protein/L and 3 g ALA/L as well as FOS and GOS (prebiotics) and *Lactobacillus rhamnosus* and *Bifidobacterium longum* (probiotics). Ninety-seven healthy term neonates were enrolled and randomized to receive experimental formula (n = 48) or control formula (n = 49). The primary outcome was weight at 6 months. This parameter and all other anthropometrics and z-scores were not significantly different between groups. At one month,

infants receiving the ALA-syn formula exhibited less crying or agitation than infants in the control group, and at 6 months the incidence of atopic dermatitis was lower in the ALA-syn group than controls. Nine infants withdrew from the experimental group and 4 from the control group. The authors concluded:

> "In the double-blind, multicentre, randomised trial reported here, the experimental α-lactalbumin-enriched and symbiotic- supplemented infant formula ensured the same growth as a standard formula in terms of weight and height gain. This finding confirms the nutritional adequacy of the protein profile of the experimental formula(15). In addition, in this unselected population, the a-lactalbumin-enriched and symbiotic-supplemented formula was better tolerated at 1 month of age, and had a protective effect against the occurrence of mild atopic dermatitis at 6 months of age" (Roze et al. 2012).

Raiha et al. (2002) reported a prospective, randomized, double-blind, placebo-controlled multicenter trial comparing growth of 85 healthy term infants receiving either standard formula (SF) with 2.2 g protein/100 kcal (n = 29) or one of 2 test formulas with 1.8 g protein/100 kcal (TF1 based on 70% acid whey [n = 27] and TF2 based on 70% modified sweet whey enriched in ALA and reduced in GMP [n = 29]) from birth to age 4 months. (ALA and GMP levels were not reported.) A breast-fed reference group (n = 28) was included. Anthropometrics (weight and length) were obtained at birth and at 30, 60, 90, and 120 days while blood was taken for biochemical assessment at 30, 60, and 120 days.

The primary outcome of the study, growth from 30 to 120 days, showed no differences between the 3 formula-fed groups for length and weight gains. Furthermore, the formula-fed groups did not differ significantly with the breast-fed group for weight and length gains. No adverse events were reported. The authors suggested that simple protein reduction in infant formulas risks disturbing amino acid profiles—especially reduction in tryptophan, but that use of a protein fraction high in ALA (and thus in tryptophan) "might be a safer means of providing this amino acid in sufficient quantity without leading to an imbalance in plasma amino acid profiles of the infant." The authors further stated, "We conclude that an improved whey predominant formula with a protein/energy ratio of 1.8 g/100 kcal provides adequate intakes of protein from birth to 4 months without signs of compensatory increased food and energy intakes and that such formulas can be considered safe."

The effects of an infant formula's protein source, macronutrient composition, and content of long-chain polyunsaturated fatty acids (LC-PUFA) on growth were evaluated in a prospective, randomized, double-blind, placebo-controlled trial (BeMIM Study; Fleddermann et al. 2014a). Growth of infants fed one of 2 formulas provided by HiPP GmbH from the first month of life to Day 120 was compared. Both formulas had 60:40 whey:casein ratios and provided 67 kcal/100 mL, but the intervention formula (IF) had 1.89 g protein/100 kcal vs. 2.2 g protein/100 kcal in the control formula (CF), a higher content of ALA than the CF, and 14.2 mg LC-PUFA/100 mL vs. none in the CF. The IF was also supplemented with free L-phenylalanine and L-tryptophan, resulting in an IF content of 73 vs. 63 mg Phe/100 mL and 25 vs. 24 mg Trp/100 mL in the CF. A total of 213 apparently healthy term infants aged <29 days were enrolled, 107 assigned to receive the IF and 106 the CF; a reference group of 185 breastfed infants was also enrolled. Anthropometric measures (weight, length, and head circumference) were taken at enrollment and at 30, 60, 90, and 120 days of life. Parents completed questionnaires on formula consumption and acceptance, consistency and color of stool, and occurrence of colic, flatulence, regurgitation, and vomiting.

Forty formula-fed infants were withdrawn from the study—34 by parental refusal, 4 due to illness or medical treatment, and 2 through loss of contact—while 93 of the 185 breastfed infants

were lost—76 by parental refusal, 3 due to illness or medication, and 14 through loss of contact. Weight gain (g/day) did not differ significantly between the 2 formula-fed groups—a mean of 30.2±6.3 g/day in the IF group and 28.3±6.5 g/day in the CF group; mean daily weight gain in the breastfed reference group was significantly less (26.7±6.4 g/day) than in the IF group. Daily gain in length was significantly greater among IF infants (0.11±0.02 cm/day) than either CF or breastfed infants (both 0.10±0.02 cm/day). Mean daily gains in head circumference were similar in all groups of infants.

Formula consumption and energy intake were similar in the IF and CF groups at 30 and 60 days of age, but significantly higher in the CF group at 90 and 120 days. Protein intake was significantly higher in CF infants than IF infants at all time points; data for energy and protein intakes are shown in Figure 13.

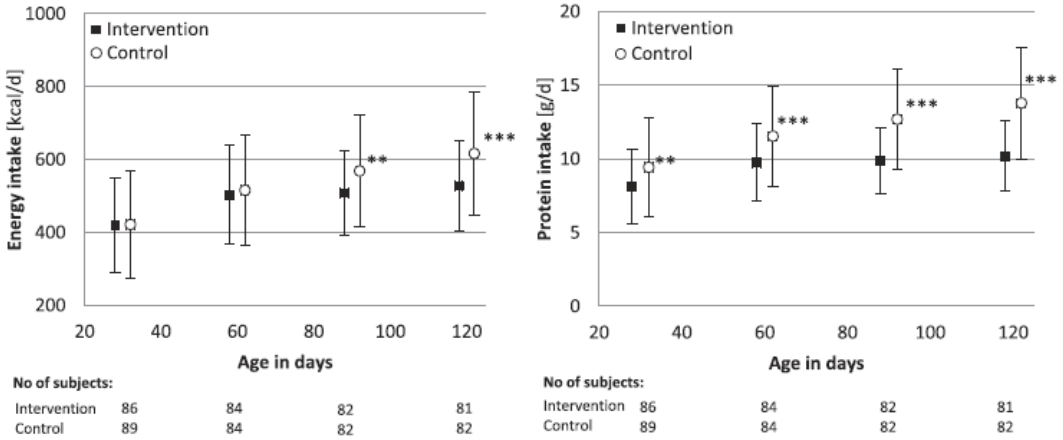


Figure 13. Mean (±SD) daily energy and protein intake at 30, 60, 90 and 120 days of age in intervention and control group of the intention-to-treat population. **p<0.01, ***p<0.001. Significantly different between groups (Fleddermann et al. 2014a).

There were no significant differences between the IF and CF groups for albumin, urea, or creatinine. Compared with the breastfed group, urea and creatinine were significantly higher and glucose was significantly lower in both formula groups. All analyzed amino-acid and fatty-acid concentrations were within the normal ranges for healthy infants, although Phe, Tyr, and Trp were significantly higher in IF infants while Ala, Asn, Asp, His, Ile, Leu, Met, Orn, Pro, Thr, and Val were significantly higher in CF infants. With regard to adverse events, the authors reported:

> "Both formulae were well-accepted and no differences were reported for acceptance as well as consistency and color of stool, colic, flatulence, regurgitation and vomiting. The total number of adverse events (adverse event plus serious adverse event) was 21 in 88 IF infants, 41 in 92 CF infants and 45 in 185 BF infants. Thus, a significantly higher rate of adverse events was observed in CF infants compared to IF ($p<0.003$) and BF infants ($p<0.001$), while IF and BF infants did not differ. The types of adverse events were similarly distributed between formula groups (49% respiratory tract infections, 24% skin infection/eczema, 10% gastrointestinal problems, 4% urinary tract infections, and 13% others). The number of serious adverse events was 12 in the formula groups (IF=9, CF=3) and 4 in the reference group, with one serious adverse event in each formula group considered potentially associated with the study formula (IF: vomiting, blood in stool, reflux and CF: vomiting, blood in stool)" Fleddermann et al. (2014a).

The authors reported that, "All infants accepted IF well and for all parameters studied no negative effects were found," and that, "This randomized, controlled, double-blind intervention study demonstrated that the growth of infants fed a modified infant formula with reduced protein with ALA and LC-PUFA is similar and within normal ranges for formula fed infants." They

concluded that, “an ALA-containing infant formula with a protein/energy ratio of 1.89 g/100 kcal and improved LC-PUFA status provides an adequate intake during the first months of life [and] can be considered safe. This modified infant formula is appropriate for term infants as evidenced by growth velocities, acceptance and tolerance.”

Fleddermann et al. (2018) were able to enroll 187 children from the BeMIM study for a 4-year follow-up at which anthropometric and body-fat measures were taken and blood was sampled for analysis of plasma metabolites. The children included 65 from the IF group, 59 from the CF group, and 63 from the breastfed group. There were no significant differences in weight, standing height, head circumference, or percent body-fat among the groups at 4 years of age, nor did the groups differ significantly in the incidence of serious illnesses.

Associations between plasma metabolites measured during infancy and anthropometric measures at 4 years were examined. Three acyl-carnitine esters (C8:1, C12:0, and C14:0) were significantly higher in IF infants compared to CF infants, while infants in the CF group had significantly higher levels of acyl-carnitine C3:0. No association was found between the infant metabolome or short-term infant growth and growth to age 4 years. The authors concluded that:

> “In contrast to previous evidence indicating that early protein intake and weight gain have an impact on later obesity risk, results of the current study do not indicate long-term effects of early diet and growth on anthropometry at 4 years of age. The 4-year follow-up of the BeMIM study showed that the administration of a new formula with a lower protein content, a higher content of alpha-lactalbumin enriched whey and containing LC-PUFA does not produce measurable growth differences in children compared with children receiving a standard protein content formula during the first 4 months of life” (Fleddermann et al. 2018).

Table 26. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Heine et al. (1996)	Prospective, randomized, double-blind, cross-over study of the effect of ALA enrichment of infant formulas on serum tryptophan levels of infants; 2 weeks for each formula	20 heathy term infants under 3 months of age	Control formula (whey: casein 60:40, protein 18 g/L, tryptophan 1.66 g/16 g N); Tryptophan intermediate formula (whey:casein 59:41, protein 13.4 g/L, tryptophan 1.88 g/16 gN); High tryptophan formula (whey:casein 75:25, protein 13.4 g/L, tryptophan 2.21 g/16 g N). ALA levels were not reported.	Blood was drawn prior to the introduction of the experimental formulas and at 2 and 4 weeks of experimental formula feeding. Infants in the TRP Plus group had plasma tryptophan concentrations similar to the breast-fed infants, while those fed formulas with lower tryptophan did not. These results demonstrated that formula tryptophan is bioavailable and plasma levels depend on the amount of ALA provided. No anthropometric data were provided concerning infants at study entry or following consumption of either formula. The authors concluded that, “The supplementation of ALA resulting in a higher TRP supply to low-protein diets is a further step towards the production of infant formulas more closely adapted to human breast milk.”

Table 26. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Lien et al. (2004)	Prospective, randomized, double-blind, placebo-controlled study to assess growth and safety of formula with added ALA over 12 weeks	193 healthy term infants <14 days of age	Control formula with 15.1 g protein and 1.2 g ALA/L and Experimental formula with 14.4 g protein and 2.2 g ALA/L	Adverse events, evaluated as a primary safety measure, were recorded during scheduled visits at weeks 4, 8 and 12, and by telephone contacts at weeks 2, 6 and 10. Secondary safety end points were protein status and the acceptability and tolerance of study formula. Completion rates were 73.5% in the EF group and 65.2% in the CF group; discontinuations due to adverse events were lower in the EF group (15.3%) than the CF group (21.1 %). More infants fed CF than EF discontinued due to spitting up (8 vs 4), irritability (7 vs 3) and flatulence (7 vs 4), while more infants receiving the EF than CF discontinued due to constipation (6 vs 3). Most of the adverse events were mild and resolved without treatment. Less than 50% of either group had adverse events that were reported to be related to formula consumption (42.9% in the EF group, 46.3% in the CF group). The most common formula-related events were flatulence (EF: 18.4%, CF: 20.0%) and constipation (EF: 22.4%, CF: 15.8%). Each of 3 infants in the EF group had one serious adverse event while 2 infants in the CF group had a total of 4 serious adverse events. None of the serious adverse events was reported to be due to the formula consumed. No significant differences were found between groups in weight, length, and head circumference during the trial, showing that the ALA-enriched formula supported normal growth. Serum albumin concentrations did not differ between groups, evidence that the ALA-enriched formula supported normal protein status. Blood urea nitrogen and creatinine, markers of total protein intake and disposal of unnecessary nitrogen, were lower at 12 weeks in the EF group than in controls. Serum calcium, phosphorus, and magnesium concentrations did not differ between groups, demonstrating adequate absorption and metabolism of these secondary safety markers. The acceptability and tolerance of the formulas was assessed every second week during the study period; the EF had higher ratings than the CF. This study demonstrates the safety of an ALA enriched formula; the formula supported comparable growth to a standard, commercial formula. Protein status was comparable between formula groups. The ALA-enriched formula had improved tolerance compared to standard formula.

Table 26. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Davis et al. (2008)	Prospective, randomized, double-blind, placebo-controlled, multicenter study comparing plasma amino acids and GI tolerance in infants receiving formulas differing in total protein and ALA content over 8 weeks	128 healthy term infants <14 days of age (64 to consume each formula) and 88 healthy term infants in the human milk reference group	Standard formula with 15.1 g protein and 1.2 g ALA/L and Experimental formula with 14.4 g protein and 2.2 g ALA/L	The primary efficacy end point was post-prandial plasma essential amino acid levels at week 8. Secondary efficacy parameters were markers of protein status. Primary safety parameters were study events associated with the feeding modality and growth. Study events were categorized as “any causality” (all study events regardless of causality) or “feeding-related.” Events were recorded during scheduled visits at weeks 4 and 8, and by telephone contacts at weeks 2, 6, and 10. Growth parameters were determined at baseline and weeks 4 and 8. In the SF group, 21.9% of infants discontinued due to study events, vs. only 10.9% of the infants in the EF group. There were no significant differences among the groups in growth velocity .Serum albumin and creatinine were similar among groups at baseline and week 8. No differences in hematocrit or hemoglobin were reported. At week 8, all amino acids were within normal ranges in all groups. The most common adverse events were constipation, gastro-esophageal reflux, regurgitation, abdominal pain, vomiting, diarrhea, and eructation. For both “any causality” or “feeding-related” events, the SF group was higher than the EF or HM group, which did not differ from each other. The authors observed that: “The unique finding in the present study was that the cumulative GI event profile of EF was similar to that of the HM profile after study day 18. GI effects usually present soon after the introduction of a new feeding, and to our knowledge, a detailed time course of study events has never before been reported. Of potential clinical importance is that the incidence of constipation and regurgitation in the EF group was similar to HM-fed infants, and lower than that of the SF group. The improved GI profile observed in the present study may be attributed to a formula matrix that is closer to HM. The authors concluded: “This study of a lower protein infant formula with increased α-lactalbumin concentration represents a major improvement in the infant formula protein matrix to more closely match the protein composition and GI tolerance profile of HM.”

Table 26. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Trabulsi et al. (2011)	Prospective, randomized, double-blind, placebo-controlled growth study comparing ALA-enriched formulas with different total protein levels over 120 days	224 healthy term formula-fed infants aged 5-14 days with weight, length, and head circumference >5th and <95th percentile for age, n = 112 for each formula, + HM group	Standard formula with 14.1 g protein/L and experimental formula with 12.8 g protein/L, both ALA-enriched with added L-tyrosine and L-tryptophan	Anthropometrics were measured at baseline and on Days 30, 60, 90, and 120. 2 hours post prandial blood samples were collected at base line as well as on Days 60 and 120. Symptoms related to the digestive system and GI tolerance were of particular interest and included hard stools, constipation, difficulty having a bowel movement, acute diarrhea, chronic diarrhea, spitting up, regurgitation, vomiting, gastroesophageal reflux disease, colic and crying/neonatal abnormal crying. A high proportion of the infants enrolled in the study completed the protocol (96%). Discontinuations due to study events were low and were not significantly different among the groups (high protein formula: 2.7%, low protein formula: 2.7%, HM-fed group: 0%). Feeding-related study events were uncommon and not significantly different among groups (high protein formula: 11.6%, low protein formula: 6.3%, HM-fed group: 4.5%). Mean weight and length gain over the 120 day study did not differ between formula groups. Mean serum albumin, total protein, BUN, and creatinine concentrations were within normal ranges for all groups at baseline, Day 60, and Day 120. Serum insulin and glucose levels were not significantly different among groups at Day 60. The essential amino acid concentrations did not differ between the 2 formula groups, although some of the amino acids were higher in the formula groups than the breast-fed group Feeding a lower protein formula resulted in normal growth, serum markers of protein status, and plasma amino acids compared to both a standard formula and a breast-fed group. Weight gain in infants receiving the low protein formula was between the standard formula group and breast-fed infants while weight-for-age and weight-for-length Z-scores were similar to infants in the breast-fed group. Adverse events were relatively low and not significantly different among groups. The authors concluded that, “α-Lactalbumin-enriched formula containing12.8 g/l protein was safe and supported age-appropriate growth; weight gain with EF was intermediate between SF and HM groups and resulted in growth similar to HM-fed infants.”

Table 26. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Wernimont et al. (2015)	Prospective, randomized, double-blind, placebo-controlled, multicenter trial testing effect of oligofructose fed for 8 weeks in ALA-enriched formula	Healthy term infants: 28 in the control formula (CF) group without OF, 25 in the experimental formula (EF) group with OF, and 31 in the HM group	ALA-enriched formula (2.2 g ALA/L) with or without added OF	The total proportions of infants who discontinued the study were similar among the study groups, and the total incidence of AEs was also similar among groups. However, some differences were noted concerning withdraws due to feeding related GI AEs, which included 4 infants in the CF group, 12 infants in the EF group, and one infant in the HM group. None of the events which led to withdraws was considered serious. The infants in the EF group had higher bifidobacteria levels and softer stools than the CF group at study completion. The results of this study demonstrate that infants receiving an ALA-enriched formula have excellent formula tolerance.
Hays et al. (2016)	Prospective observational cohort study evaluating the quality of life in infants receiving an ALA-enriched formula with increased sn-2 palmitate and OF for 42 days	Healthy term infants: formula, n = 140; mix, n = 151; HM, n = 136	ALA-enriched formula with increased sn-2 palmitate and OF (formula group), a group receiving a mix of the study formula and HM (mix group), and an exclusively HM-fed group	Formula tolerance was excellent with only 10 infants discontinued in the formula group, 11 in the mix group, and 11 in the HM group. Health-related quality of life was assessed using a validated questionnaire, and was high for all groups. Several parameters had significant differences with the formula group being marginally lower in categories such as temperament and mood, general health perception, and parent-focused concerns than the other groups. The safety of the formula is reflected in the authors' conclusion that "[health-related quality of life] was high in this population of healthy infants, with only a few small differences in [health-related quality of life] concept scores observed between breastfed, formula-fed and mixed-fed infants."
Mao et al. (2018)	Prospective observational cohort study evaluating the quality of life in infants receiving an ALA-enriched formula with increased sn-2 palmitate and OF for 48 days	Healthy term infants: formula, n = 150; mix, n = 163; HM, n = 147	ALA-enriched formula with increased sn-2 palmitate and OF (formula group), a group receiving a mix of the study formula and HM (mix group), and an exclusively HM-fed group	The incidence of either hard or watery stools was low, and did not differ between groups, and all groups had similar rates of growth. The majority of infants (81.5%) who participated in the study did not manifest any AEs. The percentages of subjects with any AEs in the breastfed, formula-fed, and mixed-fed groups throughout the entire study period (~48 days) plus a 4-week poststudy followup after the last clinical visit were 22%, 16%, and 18%, respectively The authors concluded that the ALA-enriched formula "was well tolerated based on both parent questionnaire and physician-reported GI study events."

Table 26. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Roze et al. (2012)	Prospective, randomized, double-blind, placebo-controlled, multicenter study of an ALA-enriched and symbiotic-supplemented formula for safety, tolerance and efficacy over 6 months	97 healthy term neonates; experimental formula (n = 48), control formula (n = 49)	Control formula contained 15 g protein/L and the experimental formula had 14 g protein/L and 3 g ALA/L as well as FOS & GOS and *Lactobacillus rhamnosus* and *Bifidobacterium longum*	The primary outcome was weight at 6 months. This parameter and all other anthropometrics and z-scores were not significantly different between groups. At one month, infants receiving the ALA-syn formula exhibited less crying or agitation than infants in the control group, and at 6 months the incidence of atopic dermatitis was lower in the ALA-syn group than controls. 9 infants withdrew from the experimental group and 4 from the control group. The authors concluded: "In the double-blind, multicentre, randomised trial reported here, the experimental α-lactalbumin-enriched and symbiotic- supplemented infant formula ensured the same growth as a standard formula in terms of weight and height gain. This finding confirms the nutritional adequacy of the protein profile of the experimental formula. In addition, in this unselected population, the a-lactalbumin-enriched and symbiotic- supplemented formula was better tolerated at 1 month of age, and had a protective effect against the occurrence of mild atopic dermatitis at 6 months of age."
Raiha et al. (2002)	Prospective, randomized, double-blind, placebo-controlled multicenter trial comparing growth of infants receiving formulas with different total protein and ALA for 4 months	85 healthy term neonates; a breast-fed reference group (n = 28) was included.	SF with 2.2 g protein/100 kcal; TF1 with 1.8 g protein/100 kcal & 70% acid whey; TF2 with 1.8 g protein/100 kcal & 70% modified sweet whey enriched in ALA and reduced in GMP. (ALA and GMP levels were not reported.)	Anthropometrics (weight and length) were obtained at birth and at 30, 60, 90, and 120 days while blood was taken for biochemical assessment at 30, 60, and 120 days. The primary outcome of the study, growth from 30 to 120 days, showed no differences between the 3 formula-fed groups for length and weight gains. Furthermore, the formula-fed groups did not differ significantly with the breast-fed group for weight and length gains. No adverse events were reported. The authors stated, "We conclude that an improved whey predominant formula with a protein/energy ratio of 1.8 g/100 kcal provides adequate intakes of protein from birth to 4 months without signs of compensatory increased food and energy intakes and that such formulas can be considered safe."

Table 26. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
Fleddermann et al. (2014a)	Prospective, randomized, double-blind, placebo-controlled trial of the effects of protein source, macronutrient composition, and content of LC-PUFA on growth of healthy term infants	213 apparently healthy term infants aged <29 days, 107 in the IF group and 106 in the CF group; and a reference group of 185 breastfed infants	Both formulas had 60:40 whey:casein ratios and provided 67 kcal/100 mL, but the IF had 1.89 g protein/100 kcal vs. 2.2 g protein/ 100 kcal in the CF, a higher content of ALA than the CF, and 14.2 mg LC-PUFA/100 mL vs. none in the CF. The IF was supplemented with Phe and Trp	Weight gain (g/day) did not differ significantly between the 2 formula-fed groups; mean daily weight gain in the breastfed reference group was significantly less than in the IF group. Daily gain in length was significantly greater among IF infants than either CF or breastfed infants. Mean gains in head circumference were similar in all groups. Formula consumption and energy intake were similar in the IF and CF groups at 30 and 60 days of age, but higher in the CF group at 90 and 120 days. Protein intake was higher in CF infants than IF infants at all time points. All analyzed amino-acid and fatty-acid concentrations were within the normal ranges for healthy infants. The authors reported that both formulae were well-accepted and no differences were reported for acceptance as well as consistency and color of stool, colic, flatulence, regurgitation and vomiting. A signif-icantly higher rate of adverse events was observed in CF infants compared to IF and BF infants, while IF and BF infants did not differ. One serious adverse event was reported in each formula group considered potentially associated with the study formula (IF: vomiting, blood in stool, reflux and CF: vomiting, blood in stool). All infants accepted IF well and for all parameters studied no negative effects were found. “This randomized, controlled, double-blind intervention study demon-strated that the growth of infants fed a modified infant formula with reduced protein with ALA and LC-PUFA is similar and within normal ranges for formula fed infants.”
Fleddermann et al. (2018)	4-year follow-up	187 children from the BeMIM study, 65 from the IF group, 59 from the CF group, and 63 from the breastfed group		There were no significant differences in weight, standing height, head circumference, or percent body-fat among the groups at 4 years of age, nor did the groups differ significantly in the incidence of serious illnesses. No association was found between the infant metabolome or short-term infant growth and growth to age 4 years. The authors concluded that, “Results of the current study do not indicate long-term effects of early diet and growth on anthropometry at 4 years of age. The 4-year follow-up of the BeMIM study showed that the administration of a new formula with a lower protein content, a higher content of alpha-lactalbumin enriched whey and containing LC-PUFA does not produce measurable growth differences in children compared with children receiving a standard protein content formula during the first 4 months of life.”

6.8. Safety Assessment and GRAS Determination

This section presents an assessment that demonstrates that the intended use of Lacprodan® ALPHA-10 alpha-lactalbumin is safe and is GRAS based on scientific procedures.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of the intended use of alpha-lactalbumin is demonstrated. Safety is established by demonstrating a reasonable certainty that the exposure of consumers to alpha-lactalbumin under its intended conditions of use is not harmful. In the second step, the intended use of Lacprodan® ALPHA-10 alpha-lactalbumin is determined to be GRAS by demonstrating that the safety of this product under its intended conditions of use is generally recognized among qualified scientific experts and is based on publicly available and accepted information.

The regulatory framework for establishing whether the intended use of a substance (or organism) is GRAS, in accordance with Section 201(s) of the Federal Food Drug and Cosmetic Act, is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under §170.30(c). This GRAS determination employs scientific procedures established under §170.30(b).

A scientific procedures GRAS determination requires the same quantity and quality of scientific evidence as is needed to obtain approval of the substance as a food additive. In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components:

1. Data and information relied upon to establish the scientific element of safety must be generally available; and
2. There must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific-procedures GRAS determination are applied below in an analysis of whether the intended use of alpha-lactalbumin is safe and is GRAS.

6.8.1. Evidence of Safety

If there is no other alpha-lactalbumin source in an infant formula, the amount of Lacprodan® ALPHA-10 needed to provide the target level of 2.5 g alpha-lactalbumin/L formula is 2.5/.3321, or 7.5 g/L. For infant formulas, a typical protein range of 100% to 110% is used to support label claims. Thus, the maximum intended addition level of Lacprodan® ALPHA-10 is 1.1x7.5 g, or 8.3 g/L.

The calculations above are for formulations that use Lacprodan® ALPHA-10 as the only alpha-lactalbumin source. Typically, most infant formula manufacturers strive for whey dominant infant formulas with whey:casein ratios ranging from 60:40 to 80:20. In formulas containing some level of alpha-lactalbumin prior to addition of Lacprodan® ALPHA-10 (i.e., most infant formulas containing added whey), the addition level of Lacprodan® ALPHA-10 needed to achieve a total alpha-lactalbumin content level of 2.5 g/L throughout the product shelf-life will be less than 8.3 g/L.

Bovine alpha-lactalbumin as part of cow's milk has been consumed by humans for thousands of years at varying doses without any serious safety concerns. Infants who consume casein dominant milk based formulations have been exposed to around 1.2g/L of bovine alpha-lactalbumin for the past 100 years.

Demineralized whey fractions have been employed as components of infant formulas since the early 1960s. Whey concentrates require a major fractionation of cow's milk with removal of the casein fraction (80-82 percent of total cow's milk protein). Such formulas have an extensive history of safe use, and whey-dominant formulas with about 1.3g/L of bovine alpha-lactalbumin are currently the most common type of infant formulas in use today in the United States.

Lacprodan® ALPHA-10 alpha-lactalbumin is a whey fraction produced by standard dairy technology that has been commercialized since the 1990s. This fraction meets the compositional requirements of CFR 21.184.1979c (whey protein concentrate), but has a modified protein profile with an enrichment of alpha-lactalbumin and a reduction of beta-lactoglobulin (a protein not produced by the human mammary gland). Infant formulas containing up to 2.5g/L of bovine lactalbumin using Lacprodan® ALPHA-10 as one of the protein ingredients have been clinically evaluated (Sanstrom et al. 2008, Dupont et al. 2010) and are being consumed by infants worldwide in Europe, Asia, Australia, and South America.

The safety of infant formulas enriched in alpha-lactalbumin has been demonstrated in numerous clinical studies (see Section 6.3.2). Two major studies (Lien et al. 2004 and Trabulsi et al. 2011, both discussed in detail in Section 6.3.2.2) powered specifically to evaluate growth of infants consuming alpha-lactalbumin enriched formulas over 3 months (Lien et al. 2004) or 4 months (Trabulsi et al. 2011) are available in the published literature.

6.8.2. Conclusion of the Expert Panel

The intended use of Lacprodan® ALPHA-10 alpha-lactalbumin has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was shown by reviewing the extensive published reports of studies of infants consuming alpha-lactalbumin enriched formulas, and concluding that the expected exposure to alpha-lactalbumin is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the intended use of alpha-lactalbumin has been made through the deliberations of an Expert Panel consisting of Ronald E. Kleinman, M.D., Berthold V. Koletzko, M.D., Ph.D., and John A. Thomas, Ph.D., who reviewed a monograph prepared by Eric L. Lien, Ph.D., and James T. Heimbach, Ph.D., as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food ingredients intended for consumption by infants. They independently critically reviewed and evaluated the publicly available information and the potential human exposure to alpha-lactalbumin anticipated to result from its intended use, and individually and collectively determined that no evidence exists in the available information on Lacprodan® ALPHA-10 alpha-lactalbumin that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of Lacprodan® ALPHA-10 alpha-lactalbumin.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion regarding the safety of the substance under its intended conditions of use. Therefore, the intended use of Lacprodan® ALPHA-10 alpha-lactalbumin is GRAS by scientific procedures.

6.9. Statement Regarding Information Inconsistent with GRAS

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with our conclusion of GRAS status of the intended use of Lacprodan® ALPHA-10 alpha-lactalbumin.

6.10. Statement of the Expert Panel

We, the undersigned members of the Expert Panel, are qualified by scientific education and experience to evaluate the safety of substances intended for addition to foods, including infant formulas. We have individually and collectively critically evaluated the publicly available information on Lacprodan® ALPHA-10 alpha-lactalbumin summarized in a monograph, *Generally Recognized As Safe (GRAS) Determination for the Intended Use of Lacprodan® ALPHA-10 Alpha-Lactalbumin* (July 2018), prepared by Eric L. Lien, Ph.D., and James T. Heimbach, Ph.D., and other material deemed appropriate or necessary.

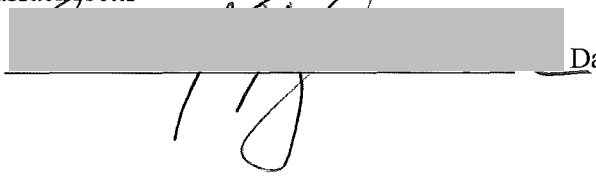
We have individually and collectively determined that no evidence exists in the available information on Lacprodan® ALPHA-10 alpha-lactalbumin that demonstrates, or suggests reasonable grounds to suspect, a hazard to infants under the intended conditions of use of Lacprodan® ALPHA-10 alpha-lactalbumin.

We unanimously conclude that the intended addition of Lacprodan® ALPHA-10 alpha-lactalbumin, produced consistent with current good manufacturing practice (cGMP) and meeting the food-grade specifications presented in the monograph, to cow-milk-based infant formula at a level not exceeding 8.3 g/L is safe and is GRAS by scientific procedures.

It is our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions.

Ronald E. Kleinman, M.D.
Professor of Pediatrics
Harvard Medical School
Boston, Massachusetts

Date

Signature:  Date: July 31, 2018

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

Signature: _____ Date: _____

John A. Thomas, Ph.D.
Adjunct Professor
Indiana University School of Medicine
Indianapolis, Indiana

Signature: _____ Date: _____

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Date

Signature: _____ Date: _____

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

Signature: _____ Date: 4 August 2018

John A. Thomas, Ph.D.
Adjunct Professor
Indiana University School of Medicine
Indianapolis, Indiana

Signature: _____ Date: _____

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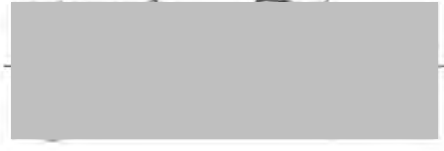
Signature:  Date: July 31, 2018

Part 7: List of Supporting Data and Information

Acharya K.R., J.S. Ren, D.I. Stuart, D.C. Phillips, R.E. Fenna. 1991. Crystal structure of human alpha-lactalbumin at 1.7 A resolution. *J Mol Biol* 221:571–581.

Andersson Y., M.-L. Hammarstrom, B. Lonnerdal, G. Graverholt, H. Falt, O. Hernell. 2009. Formula feeding skews immune cell composition toward adaptive immunity compared to breastfeeding. *J Immunol* 183:4322–4328.

Bessler S. 2005. Nutritional assessment. In P. Samour, K. King (Eds.), *Handbook of Pediatric Nutrition*. 3rd ed., Jones and Bartlett, Sudbury, pp. 1–10.

Booij L., W. Merens, C.R. Markus, A.J.W. Van der Does. 2006. Diet rich in alpha-lactalbumin improves memory in unmedicated recovered depressed patients and matched controls. *J Psychopharmacol* 20:526–535.

Brew K. 2013. α-lactalbumin. In P. McSweeney, P. Fox (Eds.), *Advanced Dairy Chemistry*, 4th ed, Springer US, Boston, MA, pp. 261–273.

Brodbeck U., W.L. Denton, N. Tanahashi, K.E. Ebner. 1967. The isolation and identification of the B protein of lactose synthetase as alpha-lactalbumin. *J Biol Chem* 242:1391–1397.

Bruck W.M., M. Redgrave, K.M. Tuohy, B. Lonnerdal, G. Graverholt, O. Hernell, G.R. Gibson. 2006. Effects of bovine alpha-lactalbumin and casein glycomacropeptide-enriched infant formulae on faecal microbiota in healthy term infants. *J Pediatr Gastroenterol Nutr* 43:673–679.

Bruck W.M., S.L. Kelleher, G.R. Gibson, K.E. Nielsen, D.E.W. Chatterton, B. Lonnerdal. 2003. rRNA probes used to quantify the effects of glycomacropeptide and alpha-lactalbumin supplementation on the predominant groups of intestinal bacteria of infant rhesus monkeys challenged with enteropathogenic *Escherichia coli*. *J Pediatr Gastroenterol Nutr* 37:273–280.

Chandra N., K. Brew, K.R. Acharya. 1998. Structural evidence for the presence of a secondary calcium binding site in human alpha-lactalbumin. *Biochemistry* 37:4767–4772.

Chowanadisai W., S.L. Kelleher, J.F. Nemeth, S. Yachetti, C.F. Kuhlman, J.G. Jackson, A.M. Davis, E.L. Lien, B. Lonnerdal. 2005. Detection of a single nucleotide polymorphism in the human alpha-lactalbumin gene: implications for human milk proteins. *J Nutr Biochem* 16:272–278.

Davies M.S., L.F. West, M.B. Davis, S. Povey, R.K. Craig. 1987. The gene for human alpha-lactalbumin is assigned to chromosome 12q13. *Ann Hum Genet* 51:183–188.

Davis A.M., B.J. Harris, E.L. Lien, K. Pramuk, J. Trabulsi. 2008. α-lactalbumin-rich infant formula fed to healthy term infants in a multicenter study: plasma essential amino acids and gastrointestinal tolerance. *Eur J Clin Nutr* 62:1294-1301.

Dupont C., M. Rivero, C. Grillon, N. Belaroussi, A. Kalindjian, V. Marin. 2010. α-lactalbumin-enriched and probiotic-supplemented infant formula in infants with colic: growth and gastrointestinal tolerance. *Eur J Clin Nutr* 64:765–767.

Fitzgerald J. and E. Swaisgood. 1989. Binding of ions and hydrophobic probes to α-lactalbumin and κ-casein as determined by analytical affinity chromatography. *Arch Biochem Biophys* 268:239–248.

Fleddermann M., H. Demmelmair, V. Grote, T. Nikolic, B. Trisic, B. Koletzko. 2014a. Infant formula composition affects energetic efficiency for growth: the BeMIM study, a randomized controlled trial. *Clin Nutr* 33:588-595.

Fleddermann M., H. Demmelmair, B. Koletzko. 2014b. Energetic efficiency of infant formulae: a review. *Ann Nutr Metab* 64:276-283.

Fleddermann M., H. Demmelmair, C. Hellmuth, V. Grote, B. Trisic, T. Nikolic, B. Koletzko. 2018. Association of infant formula composition and anthropometry at 4 years: follow-up of a randomized controlled trial (BeMIM study). *PLoS One* 13:e0199859.

Fomon S. and E. Bell. 1993. Energy. In S. Fomon (Ed.), *Nutrition of Normal Infants*. Mosby Year Book, Inc., St. Louis, pp. 103–120.

Fushiki T., N. Yamamoto, I. Naeshiro, K. Iwai. 1986. Digestion of α-lactalbumin in rat gastrointestinal tract. *Agric Biol Chem* 50:95–100.

Gene ID. 2017. 3906, *LALBA lactalbumin alpha* [Homo sapiens (human)].

Hays N.P., M. Mao, L. Zhang, J. Ge, R. Northington, M. Yao, S. Volger. 2016. Infant feeding and health-related quality of life in healthy Chinese infants: results from a prospective, observational cohort study. *Health Qual Life Outcomes* 14:116.

Heine W., M. Radke, K.D. Wutzke, E. Peters, G. Kundt. 1996. α-Lactalbumin-enriched low-protein infant formulas: a comparison to breast milk feeding. *Acta Pediatr* 85:1024–1028.

Heine W.E., P.D. Klein, P.J. Reeds. 1991. The importance of alpha-lactalbumin in infant nutrition. *J Nutr* 121:277–283.

Hiraoka Y., T. Segawa, K. Kuwajima, S. Sugai, N. Murai. 1980 Alpha-lactalbumin: a calcium metalloprotein. *Biochem Biophys Res Commun* 95:1098–1104.

Institute of Medicine (IOM). 2004. *Infant formula: evaluating the safety of new ingredients*. National Academies Press, Washington DC.

Jackson J.G., D.B. Janszen, B. Lonnerdal, E.L. Lien, K.P. Pramuk, C.F. Kuhlman. 2004. A multinational study of α-lactalbumin concentrations in human milk. *J Nutr Biochem* 15:1517-1521.

Jakobsson I., T. Lindberg, B. Benediktsson. 1982. *In vitro* digestion of cow's milk proteins by duodenal juice from infants with various gastrointestinal disorders. *J Pediatr Gastroenterol Nutr* 1:183–191.

Kelleher S.L., D. Chatterton, K. Nielsen, B. Lonnerdal. 2003. Glycomacropeptide and alpha-lactalbumin supplementation of infant formula affects growth and nutritional status in infant rhesus monkeys. *Am J Clin Nutr* 77:1261–1268.

Khatra B.S., D.G. Herries, K. Brew. 1974. Some kinetic properties of human-milk galactosyl transferase. *Eur J Biochem* 44:537–560.

Klee W.A. and C.B. Klee. 1972. The interaction of -lactalbumin and the A protein of lactose synthetase. *J Biol Chem* 247:2336–2344.

Koletzko B., R. von Kries, R. Closa, J. Escribano, S. Scaglioni, M. Giovannini, J. Beyer, H. Demmelmair, D. Gruszfeld, A. Dobrzanska, A. Sengier, J.P. Langhendries, M.F. Rolland Cachera, V. Grote, European Childhood Obesity Trial Study Group. 2009. Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. *Am J Clin Nutr* 89:1836–1845.

Kunz C. and B. Lonnerdal. 1992. Re-evaluation of the whey protein/casein ratio of human milk. *Acta Paediatr* 81:107–112.

Lepage, N. McDonald, L. Dallaire, M. Lambert. 1997. Age-specific distribution of plasma amino acid concentrations in a healthy pediatric population. *Clin Chem* 43:2397–2402.

Lien E.L. 2003. Infant formulas with increased concentrations of alpha-lactalbumin. *Am J Clin Nutr* 77:1555S–1558S.

Lien E.L., A.M. Davis, A.R. Euler. 2004. Growth and safety in term infants fed reduced-protein formula with added bovine alpha-lactalbumin. *J Pediatr Gastroenterol Nutr* 38:170–176.

Lindberg T., S. Engberg, I. Jakobsson, B. Lonnerdal. 1997. Digestion of proteins in human milk, human milk fortifier, and preterm formula in infant rhesus monkeys. *J Pediatr Gastroenterol Nutr* 24:537–543.

Lonnerdal B. 2003. Nutritional and physiologic significance of human milk proteins. *Am J Clin Nutr* 77:1537S–1543S.

Lonnerdal B. and E.L. Lien. 2003. Nutritional and physiologic significance of alpha-lactalbumin in infants. *Nutr Rev* 61:295–305.

Lonnerdal B. and C. Glazier. 1985. Calcium binding by alpha-lactalbumin in human milk and bovine milk. *J Nutr* 115:1209–1216.

Lonnerdal B., P. Erdmann, S.K. Thakkar, J. Sauser, F. Destaillats. 2017. Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: a developmental perspective. *J Nutr Biochem* 41:1–11.

Mahe S., B. Messing, F. Thuillier, D. Tome. 1991. Digestion of bovine milk proteins in patients with a high jejunostomy. *Am J Clin Nutr* 54:534–538.

Mao M., L. Zhang, J. Ge, J. Yan, R. Northington, M. Yao, J Nowacki, N.P. Hays. 2018. Infant feeding regimens and gastrointestinal tolerance: a multicenter, prospective, observational cohort study in China. *Global Pediatr Health* 5:1-12.

Markus C.R., B. Olivier, G.E.M. Panhuysen, J. Van Der Gugten, M.S. Alles, A. Tuiten, H.G.M. Westenberg, D. Fekkes, H.F. Koppeschaar, E.E.H.F. De Haan. 2000. The bovine protein α-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. *Am J Clin Nutr* 71:1536–1544.

Markus C.R., B. Olivier, E.H.F. de Haan. 2002. Whey protein rich in alpha-lactalbumin increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects. *Am J Clin Nutr* 75:1051–1056.

Markus C.R., L.M. Jonkman, J.H.C.M. Lammers, N.E.P. Deutz, M.H. Messer, N. Rigtering. 2005. Evening intake of alpha-lactalbumin increases plasma tryptophan availability and improves morning alertness and brain measures of attention. *Am J Clin Nutr* 81:1026–1033.

Matsumoto H., Y. Shimokawa, Y. Ushida, T. Toida, H. Hayasawa. 2001. New biological function of bovine α-lactalbumin: protective effect against ethanol- and stress-induced gastric mucosal injury in rats. *Biosci Biotechnol Biochem* 65:1104-1111.

McManaman J.L. and M.C. Neville. 2003. Mammary physiology and milk secretion. *Adv Drug Deliv Rev* 55:629–641.

Merens W., L. Booij, R. Markus, F.G. Zitman, W. Onkenhout, A.J.W. Van der Does. 2005. The effects of a diet enriched with alpha-lactalbumin on mood and cortisol response in unmedicated recovered depressed subjects and controls. *Br J Nutr* 94:415–422.

Minet-Ringuet J., P.M. Le Ruyet, D. Tome, P.C. Even. 2004. A tryptophan-rich protein diet efficiently restores sleep after food deprivation in the rat. *Behav Brain Res* 152:335–340.

Nieuwenhuizen A.G., A. Hochstenbach-Waelen, M.A.B. Veldhorst, K.R. Westerterp, M.P.K.J. Engelen, R.-J.M. Brummer, N.E.P. Deutz, M.S. Westerterp-Plantenga. 2009. Acute effects of breakfasts containing alpha-lactalbumin, or gelatin with or without added tryptophan, on hunger, "satiety" hormones and amino acid profiles. *Br J Nutr* 101:1859–1866.

Pantako O.T. and J. Amiot. 2001. The effects of α-lactalbumin and whey protein concentrate on α-amino acids, calcium\nand phosphorus levels in blood and gastrointestinal tract of rats. *Reprod Nutr Dev* 41:227–238.

Raiha N.C.R., A. Fazzolari-Nesci, C. Cajozzo, G. Puccio, A. Monestier, G. Moro, I. Minoli, E. Haschke-Becher, C. Bachmann, M.V. Hof, A.-L.C. Fassler, F. Haschke. 2002. Whey predominant, whey modified infant formula with protein/energy ratio of 1.8 g/100 kcal: adequate and safe for term infants from birth to four months. *J Pediatr Gastroenterol Nutr* 35:275-281.

Rao K.R. and K. Brew. 1989. Calcium regulates folding and disulfide-bond formation in α-lactalbumin. *Biochem Biophys Res Commun* 163:1390–1396.

Ren J., D.I. Stuart, K.R. Acharya. 1993. Alpha-lactalbumin possesses a distinct zinc binding site. *J Biol Chem* 268:19292–19298.

Roze J-C., S. Barbarot, M.-J. Butel, N. Kapel, A.-J. Waligora-Dupriet, I. De Montgolfier, M. Leblanc, N. Godon, P. Soulaines, D. Darmaun, M. Rivero, C. Dupont. 2012. An α-lactalbumin-enriched and symbiotic-supplemented v. a standard infant formula: a multicentre, double-blind, randomised trial. *Br J Nutr* 107:1616–1622.

Rudolff S. and C. Kunz. 1997. Protein and non-protein nitrogen components in human milk, bovine milk, and infant formula: quantitative and qualitative aspects of infant nutrition. *J Pediatr Gastroenterol Nutr* 24:328–344.

Samour P. and K. King. 2005. Biochemical evaluation of nutritional status. In P. Samour, K. King (Eds.), *Handbook of Pediatric Nutrition*. 3rd ed, Jones and Bartlett Publishers, Boston, MA, pp. 639–649.

Sanchez M.P., A. Govignon-Gion, M. Ferrand, M. Gele, D. Pourchet, Y. Amigues, S. Fritz, M. Boussaha, A. Capitan, D. Rocha, G. Miranda, P. Martin, M. Brochard, D. Boichard. 2016. Whole-genome scan to detect quantitative trait loci associated with milk protein composition in 3 French dairy cattle breeds. *J Dairy Sci* 99:8203–8215.

Sandstrom O., B. Lonnerdal, G. Graverholt, O. Hernell. 2008. Effects of alpha-lactalbumin-enriched formula containing different concentrations of glycomacropeptide on infant nutrition. *Am J Clin Nutr* 87:921–928.

Savino F., E. Palumeri, E. Castagno, F. Cresi, P. Dalmasso, F. Cavallo, R. Oggero. 2006. Reduction of crying episodes owing to infantile colic: a randomized controlled study on the efficacy of a new infant formula. *Eur J Clin Nutr* 60:1304–1310.

Schmitt J.A.J., B.L. Jorissen, L. Dye, C.R. Markus, N.E.P. Deutz, W.J. Riedel. 2005. Memory function in women with premenstrual complaints and the effect of serotonergic stimulation by acute administration of an alpha-lactalbumin protein. *J Psychopharmacol* 19:375–384.

Scientific Committee on Food. 2003. *Report on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae*. SCF/CS/NUT/IF/65 Final, 18 May.

Scrutton H., A. Carbonnier, P.J. Cowen, C.J. Harmer. 2007. Effects of alpha-lactalbumin on emotional processing in healthy women. *J Psychopharmacol* 21:519–524.

Stacey A., A. Schnieke, M. Kerr, A. Scott, C. McKee, I. Cottingham, B. Binas, C. Wilde, A. Colman. 1995. Lactation is disrupted by alpha-lactalbumin deficiency and can be restored by human alpha-lactalbumin gene replacement in mice. *Proc Natl Acad Sci* 92:2835–2839.

Szymlek-Gay E.A., B. Lonnerdal, S.A. Abrams, A.S. Kvistgaard, M. Domellof, O. Hernell. 2012. α-Lactalbumin and casein-glycomacropeptide do not affect iron absorption from formula in healthy term infants. *J Nutr* 142:1226–1231.

Trabulsi J., R. Capeding, J. Lebumfacil, K. Ramanujam, P. Feng, S. McSweeney, B. Harris, P. DeRusso. 2011. Effect of an α-lactalbumin-enriched infant formula with lower protein on growth. *Eur J Clin Nutr* 65:167–174.

Ushida Y., Y. Shimokawa, H. Matsumoto, T. Toida, H. Hayasawa. 2003. Effects of bovine alpha-lactalbumin on gastric defense mechanisms in naive rats. *Biosci Biotechnol Biochem* 67:577–583.

Veldhorst M.A.B., A.G. Nieuwenhuizen, A. Hochstenbach-Waelen, K.R. Westerterp, M.P.K.J. Engelen, R.-J.M. Brummer, N.E.P. Deutz, M.S. Westerterp-Plantenga. 2009. A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. *Clin Nutr* 28:147–155.

Vincent D., A. Elkins, A., M.R. Condina, V. Ezernieks, S. Rochfort. 2016. Quantitation and identification of intact major milk proteins for high-throughput LC-ESI-Q-TOF MS analyses. *PLoS One* 11:e0163471

Wada Y., B.S. Phinney, D. Weber, B. Lonnerdal. 2017. *In vivo* digestomics of milk proteins in human milk and infant formula using a suckling rat pup model. *Peptides* 88:18–31.

Wernimont S., R. Northington, M.J. Kullen, M. Yao, J. Bettler. 2015. Effect of an α-lactalbumin-enriched infant formula supplemented with oligofructose on fecal microbiota, stool characteristics, and hydration status: a randomized, double-blind, controlled trial. *Clin Pediatr (Phila)* 54:359–370.

World Health Organization (WHO). 2006. WHO Child Growth Standards.

Wyeth Nutrition. 2018. History of infant formula. Available on-line at https://www.wyethnutrition.com.hk/en/aboutus_history.aspx.

Zeisel S.H. 1986. Dietary influences on neurotransmission. *Adv Pediatr* 33:23–47.

Zhou J.P. and C.H. Dong. 2013. Association between a polymorphism of the ??-lactalbumin gene and milk production traits in Chinese Holstein cows. *Genet Mol Res* 12:3375–3382.

From: Jim Heimbach
To: Morissette, Rachel
Subject: RE: clarification requested on GRAS notice for alpha-lactalbumin
Date: Thursday, September 27, 2018 7:13:02 AM
Attachments: image007.png

Dear Dr. Morissette—

I'm sorry that we failed to make the intended use fully clear. The intended use is in non-exempt formula targeted to full-term infants.

Regards,
Jim H.

James T. Heimbach, Ph.D., F.A.C.N.
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From: Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
Sent: Wednesday, September 26, 2018 1:50 PM
To: jh@jheimbach.com
Subject: clarification requested on GRAS notice for alpha-lactalbumin

Dear Dr. Heimbach,

My name is Dr. Rachel Morissette and I am the Consumer Safety Officer assigned to your recent GRAS notice submission for alpha-lactalbumin. Before I can issue your filing letter, I need to clarify the intended use of your ingredient. You specified in the notice that alpha-lactalbumin is intended for cow's-milk-based infant formulas, but can you also please clarify the type of infant formula and the intended infant population for this ingredient (e.g. non-exempt infant formula for term infants, exempt infant formula for pre-term infants, etc.).

Thank you for your attention to this matter.

Best regards,

Rachel Morissette, Ph.D.
Consumer Safety Officer

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FDA U.S. FOOD & DRUG ADMINISTRATION

From:	Jim Heimbach
To:	Morissette, Rachel; jheimbach@va.metrocast.com
Cc:	Kal Ramanujam; ericlien@comcast.net
Subject:	RE: clarification requested on GRAS notice for alpha-lactalbumin
Date:	Thursday, December 20, 2018 2:03:53 PM
Attachments:	image007.png image024.png Response to Questions on GRN 809.pdf

Dear Rachel—

As we agreed last week, I am providing the attached response to FDA's questions. It provides complete answers to only a couple of "easy" questions and promises complete responses to the more difficult ones by mid-January. As you recognized, our ability to provide full responses in a timely manner is challenged by the Christmas holidays, but we trust that mid-January will be acceptable.

You will note in the attached letter that there are two issues regarding which we suggest the possibility of a face to face meeting with FDA. The first is with respect to minor proteins, for which FDA's question raises issues of analytical capability for which discussion might be helpful. The second is with regard to production methods, which are highly confidential and for which we are reluctant to provide written descriptions that might potentially be FOIA-able. On the other hand, we are perfectly willing to meet with FDA and walk through the production methods orally.

Best wishes for a Merry Christmas!
Jim

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From: Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
Sent: Tuesday, December 11, 2018 9:23 AM
To: Jim Heimbach; jheimbach@va.metrocast.com
Subject: RE: clarification requested on GRAS notice for alpha-lactalbumin

Hi Jim,

I think go ahead and send what you can within the 10 business days. Do you have an idea when the rest of the responses would be ready? I wouldn't want to go too much past the second week of January. Merry Christmas and Happy New Year to you as well.

Best,

Rachel

Rachel Morissette, Ph.D.
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FDA U.S. FOOD & DRUG ADMINISTRATION

From: Jim Heimbach <jh@jheimbach.com>
Sent: Tuesday, December 11, 2018 9:02 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; jheimbach@va.metrocast.com
Subject: RE: clarification requested on GRAS notice for alpha-lactalbumin

Rachel—

Based on my first response from my contacts at Arla, in which they pointed out that some of their key people are on extended holiday right now (the joys of being in Europe where 6-8 weeks of vacation is usual), we may need to request an extension. With that supposition, would you prefer that we address what we can within 10 day and send you those responses, with the others to follow in January, or should we hold off on everything until we can submit a single complete response?

Regards, and best wishes for a Merry Christmas!
Jim

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From: Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
Sent: Tuesday, December 11, 2018 8:51 AM
To: Jim Heimbach; jheimbach@va.metrocast.com
Subject: RE: clarification requested on GRAS notice for alpha-lactalbumin

Ok, thanks for letting me know. I understand the holidays may affect this. If there's an issue, let me know and we can work something out with extending the deadline for a short period.

Best,

Rachel

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FDA U.S. FOOD & DRUG ADMINISTRATION

From: Jim Heimbach <jh@jheimbach.com>
Sent: Monday, December 10, 2018 4:52 PM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; jheimbach@va.metrocast.com
Subject: RE: clarification requested on GRAS notice for alpha-lactalbumin

Rachel—

Received! With regard to the 10-day response time, I think that will be feasible, but I need to touch base with the other members of my team regarding their availability. I'll confirm ASAP.

Merry Christmas!
Jim

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From: Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
Sent: Monday, December 10, 2018 3:51 PM
To: Jim Heimbach; jheimbach@va.metrocast.com
Subject: RE: clarification requested on GRAS notice for alpha-lactalbumin

Dear Dr. Heimbach,

Please see attached our questions for GRN 000809. Please confirm receipt of this email.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Acting Supervisory Consumer Safety Officer

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JHeimbach LLC

December 20, 2018

Rachel Morissette, Ph.D.
Consumer Safety Officer
FDA Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review

Dear Rachel:

I am writing in response to your correspondence of December 10, in which you shared with us a number of questions developed by the FDA review team regarding GRAS Notice GRN 809.

Arla Foods Ingredients (the notifier) has formed study teams to address the questions. It will take some time to prepare responses to all of your questions regarding processing and composition, and, unfortunately, we are just entering upon the Christmas holiday season. Nevertheless, Arla anticipates having detailed responses ready to share with you by mid-January.

In what follows, I will address your questions individually.

General Comments

Q3. Please provide the date through which an updated literature search was conducted.

My last download of a research paper was on July 10, 2018, and so this constitutes the closing date of the literature search.

Chemistry

Q1a. What portion of the total protein in infant formula will be provided by the fractionated WPC (41% ALA) ingredient? Please provide an estimated range for the replacement level (for standard whey or casein) based on use levels of total protein and the casein:whey ratio in infant formula.

Arla will calculate the contribution from a regular whey formula and from Alpha whey formula (including the contribution from all protein sources in a typical infant formula), to get 2.5g/ L Alpha and also meet essential amino acid requirements. The results of these calculations will be provided to FDA by mid-January.

Q1b. On page 17 of the notice, Arla states that the intended technical effect is "to bring the level of whey protein, including ALA, in cow-milk-based infant formula up to a level approximating that of the whey protein and ALA concentration in human milk." Is the use limited to primarily whey-based formulas (where it would replace whey) or is it intended as a partial replacement for casein in milk-based infant formulas? When

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responding to this question, please use current protein levels used in infant formulas. Although mentioned on page 19 of the notice, we did not evaluate reduced protein levels during our review.

The intended use of Lacprodan Alpha-10 is in whey-dominant formulas. A complete response to FDA's question will be forthcoming in January.

Q2a. A comparison of the protein composition of fractionated WPC (41% ALA) to that of standard whey is missing in the notice. We note that the general identity and levels of individual whey proteins, including β-lactoglobulin, α-lactalbumin, immunoglobulins (IgG, IgA, IgM), serum albumin, lactoferrin, glycomacropeptide, and other proteins (e.g., lactoperoxidase, insulin-like growth factor-I, and transforming growth factor-β2 (TGF-β2)) are characterized in the literature.1 Please characterize the composition of the fractionated WPC (41% ALA) ingredient relative to the WPC starting material. Please provide this information in a table or provide a citation to a reference providing this information.

We will provide a response to this query in January. We have data on the major proteins in both the starting material and the Alpha fraction, but some minor proteins have not been analyzed and it is not clear that validated methods exist to quantitatively assay the minor proteins. As a result, some of the requested data are not currently available and it will be necessary to identify an appropriate contract laboratory to sponsor such analyses. Inferences regarding possibly altered levels of minor proteins can be made based on precise analyses of changes in the levels of major proteins, and we will plan to provide to FDA the results of such inferences. Arla would very much like to meet face to face with FDA reviewers at a time of your convenience to discuss exactly what analyses are needed.

Q2b. Arla provides a comparison of the amino acid composition of bovine ALA and human ALA; however, ALA is only a portion of the fractionated WPC (41% ALA) ingredient. Please provide a comparison of the amino acid composition of Arla's fractionated WPC (41% ALA) ingredient with that of standard whey protein that is used as an ingredient in infant formula. We note that the latter is available in several published reviews.

We will provide this information in January

Q2c. Please discuss if certain components of whey other than ALA and β-lactoglobulin are concentrated in the WPC (41% ALA) ingredient in terms of amounts provided per liter or per 100 kcal of infant formula in the estimates of exposure provided to support the GRAS conclusion.

We will provide data in January regarding anticipated exposure to all identified components of whey to the extent possible, based on protein profiling information on the Lacprodan Alpha-10 ingredient..

Q3a. Please provide a general description of the processes used in the method of manufacture. While we expect that the process includes membrane filtration and ion exchange separation processes, a general description (or reference to a publication describing the method) was not provided in the notice. Please provide this description, as well as any food contact materials (e.g., filtration membranes, ion exchange resins) used in the method of manufacture. Please provide a statement that the materials used are safe and suitable for their intended use and are either used in accordance with a cited regulation or effective food contact notification.

The process to enrich Lacprodan Alpha-10 whey is proprietary. FDA's question requests a "general description," which we will be happy to provide. We do, however, wish to avoid too high a degree of specificity, which would imperil some extremely confidential information. For example, we can state that any filtration membranes and ion exchange resins are approved food-grade products, but we would prefer not to identify the manufacturer or the precise characterization.

In response to Question 2a, we suggested the possibility of our coming to FDA to discuss analytical needs; similarly, we would be happy to meet with FDA and provide detailed information regarding processing methods and materials orally so as not to produce a potentially FOI-able record.

Q3 b. What other whey proteins are concentrated in Fraction 1 with ALA? Other than β-lactoglobulin, what other whey proteins are removed by the method of manufacture? Please address removal of these components in the process description.

This question will be addressed in January along with the response to Question 2a.

Q4a. The arsenic specification of <0.5 mg/kg is higher than batch analyses provided in the no-tice (0.01-0.1 mg/kg) and higher than limits we have seen for similar ingredients. Please consider reducing this specification.

We will reduce the arsenic specification at least to <0.2 mg/kg, or lower if feasible. Our January response will include a final specification.

Q4b. Is there a specification limit for β-lactoglobulin?

There is currently no such specification. Arla used to have a specification for β-lactoglobulin, but discontinued specifying limits for a variety of reasons. We will provide our reasoning in our detailed response in January.

Q5a. Please briefly address the estimated contribution of fractionated WPC (41% ALA) to total protein in term infant formula based on the range of intended uses indicated in response to Question 1a.

Q5b. If fractionated WPC (41% ALA) is used in infant formulas that contain additional whey, Arla notes that the level of use of fractionated WPC (41% ALA) will be reduced to achieve a set maximum level of ALA. However, exposure to other whey proteins are not addressed. Please address the estimated total intake of other whey proteins from use of whey (background intake), as well as the intended use of fractionated WPC (41% ALA). Other proteins include those that are concentrated with ALA in the fractionated WPC (41% ALA) ingredient. For minor proteins or unknown proteins, it may be possible to group them together as NMT x% of total ingredient.

The response to these questions will require the data that Arla will generate in response to Q2a, and will be available in January.

<u>*Toxicology*</u>

Q1. On page 33 of the notice, Arla discusses the publication by Andersson, et al. (2009) that observed changes in the CD3+ and NK cell populations in the formula-fed groups, including the ALA group. However, the publication also states that it is not clear whether the statistical differences in the studied parameters between the formula-fed (FF) groups and the breast-fed (BF) group are of clinical significance. Additionally, the authors did not find any differences between FF and BF infants with respect to fever episodes, number of days with fever, and episodes of airway infections. A discussion of this study conclusion from Andersson, et al. was not included in the notice to emphasize the safety of fractionated WPC (41% ALA). Please consider including this discussion in your safety narrative.

While the clinical findings from the RCT were not addressed in the GRAS notice in conjunction with the discussion of Andersson et al. (2009), they were reported earlier in the discussion of Sandstrom et al. (2008) on pages 29-32 of the GRAS notice.

The reason is that the findings of a single randomized controlled trial were reported in three separate publications—Bruck et al. (2006), Sandstrom et al. (2008), and Andersson et al. (2009), each publication dealing with one aspect of the findings. Andersson et al. (2009) focused on ALA's effect on immune cell composition and adaptive immunity, while Bruck et al. (2006) focused on the effect on fecal microbiota and Sandstrom et al. (2008) discussed effects on infant growth, nutrition, and morbidity. The brief mention of clinical aspects in Andersson et al. (2009) simply cited Sandstrom et al. (2008) rather than provide extensive discussion. For this reason, our GRAS notice discussed all of the clinical findings in the context of the Sandstrom et al. (2008) publication.

We believe that this response satisfies FDA's concern with full reporting of the findings of the RCT, but please let us know if this response is not satisfactory.

Regards, and best wishes for a Merry Christmas,

James T. Heimbach, Ph.D., F.A.C.N.

From:	Jim Heimbach
To:	Morissette, Rachel
Cc:	Kal Ramanujam
Subject:	Response to FDA Questions on GRN 809
Date:	Friday, February 01, 2019 3:34:37 PM
Attachments:	Morissette 20190201.pdf Appendix.xlsx Kamau et al 2010.pdf

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Dear Rachel—

As promised. Not by a whole lot, perhaps, but by close of business this week.

Best wishes for a shut-down-free weekend.

Regards,
Jim

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JHeimbach LLC

February 1, 2019

Rachel Morissette, Ph.D.
Acting Supervisory Consumer Safety Officer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Dear Rachel:

This is a little bit later in the day than I had been hoping for, but I am fulfilling my promise of having our response to the FDA questions on GRN 809 back to you today. We hope that our answers to the questions are satisfactory, but we will be happy to provide any additional information needed.

In addition to our responses in this letter, I am including a spreadsheet that contains details of the calculations behind the tables and a copy of a published article by Kamau et al. (2010) that provides a general description of the production process used by Arla, although it differs in some details.

Sincerely,

James T. Heimbach, Ph.D., F.A.C.N.
President

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Responses to FDA Questions:

Chemistry:

Q1a. *What portion of the total protein in infant formula will be provided by the fractionated WPC (41% ALA) ingredient? Please provide an estimated range for the replacement level (for standard whey or casein) based on use levels of total protein and the casein:whey ratio in infant formula.*

As stated in the GRAS petition, as a business to business customer, we have no control over how a formula manufacturer will design its formulas. As shown below, the proportion of total protein that may be provided by fractionated WPC (41% ALA) will be between 25 and 42.8%, depending upon the formula, manufacturer's choices of the total protein (14 or 15 g/L) and the whey:casein ratio (from 40:60 to 80:20).

We used the following values in our calculations:

Skim Milk Powder (SMP):
Protein @ 35%
ALA @ 1.2% of protein

WPC80:
Protein @ 80%
ALA @ 16.6% of protein (13.28% of ingredient),

Fractionated (41% ALA) WPC/Alpha-10:
Protein @ 81%
ALA @ 41% of protein

The table below summarizes the percentage of total protein that would be replaced by the fractional WPC (41% ALA) in formulas with varying whey:casein ratios and providing either 14 or 15 g total protein/L.

Replacement Percentage: Total Protein by Fractionated WPC (41% ALA):

Whey:Casein Ratio	% Total Protein Replaced with Fractionated WPC (41% ALA)	
	For formula @14g protein	For formula @15g protein
40:60	25%	25%
50:50	38%	38%
60:40	40%	35%
70:30	32.5%	27.5%
80:20	42.8%	40.5%

In addition, we calculated the total whey (WPC 80 or equivalent intact proteins) that would be replaced from a typical whey dominant formula to accommodate fractionated WPC (41% ALA); the values are presented in the following table.

Replacement Percentage: Total Whey (WPC 80) Protein by Fractionated WPC (41% ALA)

Whey:Casein Ratio	% Regular WPC80 Replaced with Fractionated WPC (41% ALA)	
	For formula @14g protein	For formula @15g protein
40:60	100%	100%
50:50	100%	100%
60:40	80%	70%
70:30	52%	44%
80:20	100%	100%

The derivation of these values is presented in detail in the spreadsheet. The first part of this spreadsheet involves the use of regular WPC80 as the only whey ingredient. The second section of the table includes addition of fractionated WPC (41% ALA). Due to the confidential nature of the formulation, we have not provided the calculations for developmental formula (80:20 whey:casein), but used the same principles that we applied to other whey:casein ratios discussed in the table to arrive at the numbers.

To reach average human milk levels of ALA using various combinations of whey and casein, the replacement level of total whey or casein protein by fractionated WPC (41% ALA) needs to be in the range of 27.5% to 42.8%. Such addition would provide ALA levels in formulas that would reach 2.5 g/RL for declared content or approximately 7.5 g/L of fractionated WPC (41% ALA) covering our intended use levels. To account for analytical and manufacturing variabilities, we requested a 10% overage level of 8.3 g/L and the overage amount is not reflected in the above calculations. The 100% replacement of regular whey with fractionated whey (41% ALA) is still limited in reaching human milk average levels of 2.5 g/L (see spreadsheet) for whey:casein ratios 40:60 and 50:50, either at 14g total protein or at 15g total protein formulas. Using fractionated WPC (41% ALA) to bring alpha lactalbumin levels closer to human milk average of 2.5g/L is best suited for whey dominant formulas only.

Q1b. *On page 17 of the notice, Arla states that the intended technical effect is "to bring the level of whey protein, including ALA, in cow-milk-based infant formula up to a level approximating that of the whey protein and ALA concentration in human milk." Is the use limited to primarily whey-based formulas (where it would replace whey) or is it intended as a partial replacement for casein in milk-based infant formulas? When responding to this question, please use current protein levels used in infant formulas. Although mentioned on page 19 of the notice, we did not evaluate reduced protein levels during our review.*

Our ingredient addition is intended for use in whey dominant formulas where the whey % in the whey:casein ratio is at least 50%. Fractionated WPC (41% ALA) is not intended to be added to formulas that contain only milk (20:80 whey:casein) or formulas with 40:60 whey:casein ratios. Its use is limited to formulas that would normally have some WPC that could be replaced, either partially or totally. Approximation of average human milk levels of 2.5 g/RL can only be achieved by addition of fractionated WPC (41% ALA) to formulas with 50:50 or greater whey:casein ratios. The table in the spreadsheet contains these calculations.

Q2a. *A comparison of the protein composition of fractionated WPC (41% ALA) to that of standard whey is missing in the notice. We note that the general identity and levels of individual whey proteins, including β-lactoglobulin, α-lactalbumin, immunoglobulins (IgG, IgA, IgM), serum albumin, lactoferrin, glycomacropeptide, and other proteins (e.g., lactoperoxidase, insulin-like growth factor-I, and transforming growth factor-β2 (TGF-β2)) are characterized in the literature[1]. Please characterize the composition of the fractionated WPC (41% ALA) ingredient relative to the WPC starting material. Please provide this information in a table or provide a citation to a reference providing this information.*

To respond to this question, Arla conducted systematic analysis of 5 lots of each of the following materials:

- starting material (whey protein concentrate, comparable to other WPC),
- fractionated WPC (41% ALA), and
- ALA-reduced retentate fraction.

Analyses were conducted in-house and by a third-party laboratory for ALA, β-lactoglobulin, casein glycomacropeptide (CGMP), proteose peptones (PP8 and PP5), immunoglobulin G-1 (IgG-1), bovine serum albumin (BSA), and lactoferrin (LF). Our findings compare well with the published literature regarding the protein profile of a variety of whey products[2].

For the fractionated whey (41% ALA), we also used annotation in mass spec (in-house data) to detect peptides corresponding to major whey proteins including BSA, LF, and IgG-1.

Using both HPLC and mass spec, we were able to detect only four major proteins in our assays of WPC (41% ALA). The tables below show the average content of the major whey proteins and casein peptides in the three materials.

The major proteins--ALA, β-lactoglobulin and CGMP and the β-casein derived peptides PP8 slow and PP5 (measured by HPLC/UV)--account for essentially all the proteins in WPC (41% ALA) and the batch variation is very small for ALA, β-lactoglobulin, and CGMP, while the β-casein derived peptides PP8 and PP5 are found to be more variable. The high-molecular-weight proteins IgG-1, BSA, and LF were analyzed in fractionated WPC (41% ALA) by state-of-the-art Parallel Reaction Monitoring Mass Spectroscopy with a functional LOQ of 10 nM; none of the proteins was found in a detectible or quantifiable concentration. We have not assayed the minor components in our fractions (lactoperoxidase, TGF-b or IGF-1) as we achieve mass balance with other protein components. We do not have facilities to accurately quantitate these protein components

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[1] Vincent D., Elkins A., Condina, MR., Ezernieks V., and Rochfort S (2016). Quantitation and identification of intact major milk proteins by high throughput LC-ESI-Q-TOF MS Analyses. *PLOS ONE*, October 17, 2016:1-21.

[2] Elgar DF, Norris CS, Ayers JS, Pritchard M, Otter DE, and Palmano KP (2000). Simultaneous separation and quantitation of the major bovine whey proteins including proteose peptone and caseinomacropeptide by reversed-phase high-performance liquid chromatography on polystyrene-divinylbenzene. *J Chromatogr A* 878:183-186.

in our fractions and were also unable to find a suitable contract laboratory that would run validated assay targeted specifically for bovine proteins.

As can be seen from the composition of the starting material and the ALA-reduced retentate, most of the proteins other than ALA are present in the retentate at levels comparable to the starting material.

Composition of Starting Material, WPC (41% ALA), and ALA-Reduced Retentate

Material	g/100 g Protein (Mean±s.d.)						
	ALA	**β-lacto-globulin**	**CGMP**	**PP8/PP5**	**IgG-1**	**BSA**	**LF**
Starting Material (WPC)	17.8±0.1	47.1±0.3	19.4±0.6	2.1±0.2	3.0±0.1	N.A.	0.1±0.02
WPC (41% ALA)	48.3±0.7	19.2±0.7	28.4±0.4	4.9±0.9	<0.1 nM	<0.1 nM	N.D.
Reduced Retentate Fraction	8.6±0.7	53.6±1.1	16.0±0.4	2.5±0.4	5.8±0.6	2.5±0.3	0.7±0.1

(n=5 individual batches); N.A. = not analyzed; N.D. = not detected

The functional LOQ for IgG, BSA, and LF in WPC (41% ALA) was 10 nM.

Q2b. *Arla provides a comparison of the amino acid composition of bovine ALA and human ALA; however, ALA is only a portion of the fractionated WPC (41% ALA) ingredient. Please provide a comparison of the amino acid composition of Arla's fractionated WPC (41% ALA) ingredient with that of standard whey protein that is used as an ingredient in infant formula. We note that the latter is available in several published reviews.*

Comparison of Typical Whey Protein Concentrate (WPC 80) and Fractionated WPC (41% ALA)

Amino acid	g amino acid/100 g protein	
	WPC 80	Fractionated WPC (41% ALA)
Alanine	5.5	3.8
Arginine	2.7	1.6
Aspartic Acid (Asparagine)	11.3	14.1
Cysteine (Cystine)	2.4	3.2
Glutamic acid (Glutamine)	18.4	17.4
Glycine	2.0	**2.1**
Histidine*	**1.9**	**2.1**
Isoleucine	**6.6**	**7.1**
Leucine	**11.4**	**9.9**
Lysine	**9.9**	**10.1**
Methionine	**2.3**	**1.7**
Phenylalanine	**3.5**	**3.4**
Proline	6.6	5.8
Serine	5.7	5.6
Threonine	**7.5**	**7.9**
Tryptophan	**1.9**	**2.7**
Tyrosine	**3.2**	**3.2**
Valine	**6.6**	**5.7**
Total	109.4	107.4

*Essential amino acids in bold font

These are typical values based on multiple lots of production in our facility and are sent as part of specifications to infant formula customers.

Q2c. Please discuss if certain components of whey other than ALA and β-lactoglobulin are concentrated in the WPC (41% ALA) ingredient in terms of amounts provided per liter or per 100 kcal of infant formula in the estimates of exposure provided to support the GRAS conclusion.

The only major protein source in WPC (41% ALA) apart from ALA and β-lactoglobulin is casein glycomacropeptide (CGMP). CGMP is derived from κ-casein and cleaved during cheese pro-duction and migrates into the whey fraction during whey processing.

In regular whey there is about 20% CGMP, whereas in fractionated WPC (41% ALA) the amount of CGMP is about 28%, which will have a maximal exposure of 1.9 g/RL or 285 mg/100 Kcal.

In comparison, the consumption of CGMP from a whey-dominant formula (2.2 g protein/100 Kcal at a total protein content of 15 g/RL) is approximately 224 mg/100 Kcal.

Thus, on an average, CGMP exposure is about 25% greater at the maximal WPC (41% ALA) concentration of 8.3 g/RL specified in the petition. As can be seen in response to Q1a, for whey dominant formulas only a portion of standard WPC 80 is replaced by fractionated WPC (41% ALA). Therefore, in most formulas, the increase in CGMP intake would be less than the maximum projected level. Sandstrom et al. (2008)[3] studied the fraction with high (15% protein as CGMP, 294 mg/100 Kcal) and low CGMP (10% protein as CGMP, 196 mg/100 Kcal) content and found them both to be safe.

[3] Sandstrom O., Lonnerdal B, Graverholt G, and Hernell O. 2008. Effects of alpha-lactalbumin-enriched formula containing different concentrations of glycomacropeptide on infant nutrition. *Am J Clin Nutr* 87:921–928.

Q3a. Please provide a general description of the processes used in the method of manufacture. While we expect that the process includes membrane filtration and ion exchange separation processes, a general description (or reference to a publication describing the method) was not provided in the notice. Please provide this description, as well as any food contact materials (e.g., filtration membranes, ion exchange resins) used in the method of manufacture. Please provide a statement that the materials used are safe and suitable for their intended use and are either used in accordance with a cited regulation or effective food contact notification.

The process to enrich Lacprodan® Alpha-10 whey is proprietary. FDA's question requests a "general description," which we are happy to provide. We do, however, wish to avoid too high a degree of specificity, which would imperil some extremely confidential information.

The purification method used by Arla is based on the principle of the difference in the molecular weights of proteins. The enclosed published article by Kamau et al. (2010)[4] provides a general description of the two-stage membrane process for obtaining concentrates enriched in ALA, al-though none of the listed set-points in Kamau et al. (2010) for pH, salt concentration, or temperature are identical to those used in Arla's method for ALA purification. Arla has developed proprietary and highly confidential techniques for optimization with proper choice of buffer conditions, ultrafiltration membranes, and filtration velocity to maximize the overall selectivity of the membrane process.

In answer to Q3b, the membrane serves as a thin barrier between miscible fluids that allows for preferential transport of feed components when a driving force such as a pressure differential is applied.

All Arla production takes place under current Good Manufacturing Practice (cGMP). Production is compliant with EU Regulations No. 1935/2004, 2023/2006, and 10/2011 and amendments on food-contact materials, and all food-contact materials are compliant with FDA regulations. All materials are food-grade, safe and suitable for their intended use, and used in accordance with FDA regulations.

[4] Kamau SM, Cheison SC, Chen W, Liu X-M, and Lu R-R (2010). Alpha-lactalbumin: its production technologies and bioactive peptides. *Comp Rev Food Sci Food Safety* 9:197-212.

Q3b. What other whey proteins are concentrated in Fraction 1 with ALA? Other than β-lactoglobulin, what other whey proteins are removed by the method of manufacture? Please address removal of these components in the process description.

The first part of this question was addressed in the response to Q2a. Whey proteins concentrated include ALA, CGMP, and PP8/PP5, while β-lactoglobulin is partially removed. The membrane filtration process that results in the partial removal of β-lactoglobulin is described in response to Q3a.

Q4a. The arsenic specification of <0.5 mg/kg is higher than batch analyses provided in the no-tice (0.01-0.1 mg/kg) and higher than limits we have seen for similar ingredients. Please consider reducing this specification.

We will reduce the arsenic specification at least to <0.2 mg/kg. It is likely that infant-formula manufacturers would also note with disapproval the higher specification for arsenic, so we thank FDA for drawing our attention to it.

Q4b. Is there a specification limit for β-lactoglobulin?

β-lactoglobulin is the major whey protein in bovine milk, but is not present in human milk. There are several reasons why β-lactoglobulin is not included in our specifications. Of most importance is that the β-lactoglobulin content of whey-dominant formula is provided primarily by skim milk powder and regular WPC, and so quantifying the β-lactoglobulin in fractionated WPC (41% ALA) does not provide useful information to the infant formula manufacturer.

Q5a. Please briefly address the estimated contribution of fractionated WPC (41% ALA) to total protein in term infant formula based on the range of intended uses indicated in response to Question 1a.

It is possible that we are not properly understanding the question, but we believe that our response to Q1a encompassed this information; i.e., the protein contribution of WPC (41% ALA) to infant formula would range from 25% in formula with a 40:40 whey:casein ratio to 42.8% in formula with 14g protein/RL and an 80:20 whey:casein ratio. However, we also noted in response to Q1b that WPC (41% ALA) is not intended for addition to formulas with whey:casein ratios less than 50:50, and so the protein contribution of WPC (41% ALA) from its intended use would not fall below 35%.

Q5b. If fractionated WPC (41% ALA) is used in infant formulas that contain additional whey, Arla notes that the level of use of fractionated WPC (41% ALA) will be reduced to achieve a set maximum level of ALA. However, exposure to other whey proteins are not addressed. Please address the estimated total intake of other whey proteins from use of whey (background intake), as well as the intended use of fractionated WPC (41% ALA). Other proteins include those that are concentrated with ALA in the fractionated WPC (41% ALA) ingredient. For minor proteins or unknown proteins, it may be possible to group them together as NMT x% of total ingredient.

In response to Q2c and Q3b, we have addressed the typical composition of fractionated WPC (41% ALA). We are able to measure only CGMP and protease peptone PP8 (slow)/ PP5.

Toxicology

Q1. On page 33 of the notice, Arla discusses the publication by Andersson, et al. (2009) that observed changes in the CD3+ and NK cell populations in the formula-fed groups, including the ALA group. However, the publication also states that it is not clear whether the statistical differences in the studied parameters between the formula-fed (FF) groups and the breast-fed (BF) group are of clinical significance. Additionally, the authors did not find any differences between FF and BF infants with respect to fever episodes, number of days with fever, and episodes of airway infections. A discussion of this study conclusion from Andersson, et al. was not included in the notice to emphasize the safety of fractionated WPC (41% ALA). Please consider including this discussion in your safety narrative.

We appreciate FDA's suggestion.

While the clinical findings from the RCT were not addressed in the GRAS notice in conjunction with the discussion of Andersson et al. (2009), they were reported earlier in the discussion of Sandstrom et al. (2008) on pages 29-32 of the GRAS notice.

The reason is that Bruck et al. (2006), Sandstrom et al. (2008), and Andersson et al. (2009) all reported the findings of a single randomized controlled trial. Each publication addressed one aspect of the findings: Andersson et al. (2009) focused on ALA's effect on immune cell composition and adaptive immunity, while Bruck et al. (2006) focused on the effect on fecal microbiota and Sandstrom et al. (2008) discussed effects on infant growth, nutrition, and morbidity. The brief mention of clinical aspects in Andersson et al. (2009) simply cited Sandstrom et al. (2008) rather than provide extensive discussion. For this reason, our GRAS notice discussed all of the clinical findings in the context of the Sandstrom et al. (2008) publication.

Ratio in infant formula		% protein contributed by whey ingredient (100% whey protein) to formula	% protein contributed by milk ingredient (20% whey protein, 80% casein) to formula	Amount regular W	
				at 14 g pro/L (2.1 g pro/100 kcal)	
whey	casein			Per RL	Per 100g
70	30	62	38	10.85	8.68
60	40	50	50	8.75	7.00
50	50	38	62	6.65	5.32
40	60	25	75	4.38	3.50

Ratio in infant formula		% protein contributed by whey ingredient (100% whey protein) to formula	% protein contributed by milk ingredient (20% whey protein, 80% casein) to formula		

70	30	62	38	**5.16**	**4.13**
60	40	50	50	**1.75**	**1.4**
50	50	38	62	**0**	**0**
40	60	25	75	**0**	**0**

Infant formula (regular W					
VPC-80 added		Amount of ALA ir			
at 15 g pro/L (2.2 g pro/100 kcal)		at 14 g pro/L (2.1 g pro/100 kcal)			
		ALA contribution from WPC-80		ALA contribution from milk	
Per RL	Per 100g	Per RL	Per 100g	Per RL	Per 100g
11.63	9.30	1.44	1.15	0.0638	0.0511
9.38	7.50	1.16	0.93	0.0840	0.0672
7.13	5.70	0.883	0.71	0.104	0.083
4.69	3.75	0.581	0.46	0.126	0.1008

VPC-80 added		Amount of fractionated (41% ALA) WPC			
at 15 g pro/L (2.2 g pro/100 kcal)		at 14 g pro/L (2.1 g pro/100 kcal)		at 15 g pro/L (2.1 g pro/100 kcal)	
Per RL	Per 100g	Per RL	Per 100g	Per RL	Per 100g

6.47	5.18	5.62	4.50	5.09	4.072
2.81	2.25	6.91	5.528	6.48	5.184
0	0	6.57	5.256	7.04	5.632
0	0	4.32	3.456	4.63	3.704

see regular WPC-80 substitutions in rows AC and AD to the right

W

n infant formula from whey and milk ingredients (~18% alpha lactalbumin in whey protein)

		at 15 g pro/L (2.2 g pro/100 kcal)			
Total ALA		ALA contribution from WPC-80		contribution from milk	
Per RL	Per 100g	Per RL	Per 100g	Per RL	Per 100g
1.50	1.20	1.54	1.24	0.0684	0.0547
1.25	1.00	1.25	1.00	0.0900	0.0720
0.987	0.790	0.946	0.757	0.1116	0.0893
0.707	0.566	0.623	0.498	0.135	0.108

Infant formula (regular WPC-80 + fractionated WPC (41% ALA) WPC added as whe

Amount of A

		at 14 g pro/L (2.1 g pro/100 kcal)			
ALA contribution from WPC-80		ALA contribution from fractionated (41% ALA) WPC		ALA contribution from milk	
Per RL	Per 100g	Per RL	Per 100g	Per RL	Per 100g

0.685	0.548	1.87	1.49	0.0638	0.0511
0.232	0.186	2.29	1.84	0.0840	0.0672
0	0	2.18	1.75	0.104	0.0833
0	0	1.43	1.15	0.126	0.101

Total ALA	
Per RL	Per 100g
1.61	1.29
1.34	1.07
1.06	0.846
0.76	0.606

ey ingredients to reach 2.6 g ALA/L

ALA in infant formula from whey and milk ingredients (~18% alpha lactalbumin in whey prote

Total ALA		ALA contribution from WPC-80		ALA contribution (41% W
Per RL	Per 100g	Per RL	Per 100g	Per RL

2.62	2.09	0.859	0.687	1.69
2.61	2.09	0.373	0.299	2.15
2.29	1.83	0	0	2.34
1.56	1.25	0	0	1.54

in)				
at 15 g pro/L (2.2 g pro/100 kcal)				
from fractionated ALA) PC	ALA contribution from milk		Total ALA	
Per 100g	Per RL	Per 100g	Per RL	Per 100g

1.35	0.0684	0.0547	2.62	2.09
1.72	0.0900	0.0720	2.62	2.09
1.87	0.112	0.089	2.45	1.96
1.23	0.135	0.108	1.67	1.34

% of regular WPC-80 that is replaced by Fractionated (41% ALA) WPC for Formula @ 14% protein:	% of regular WPC-80 that is replaced by Fractionated (41% ALA) WPC for Formula @ 15% protein:

52	44
80	70
100	100
100	100

Sixteen pages have been removed in accordance with copyright laws. The removed reference is:

Kamau, S., Cheison, S., Chen, W., Liu, X., Lu, R. 2010. "Alpha-Lactalbumin: Its Production Technologies and Bioactive Peptides." *Comprehensive Reviews in Food Science and Food Safety*.

From:	Jim Heimbach
To:	Morissette, Rachel
Subject:	FW: clarification requested for GRN 000809
Date:	Friday, March 08, 2019 3:48:27 PM
Attachments:	image001.png Response to Further Questions on GRN 809.pdf

I got a message that this email did not go out. Sorry if this is redundant.

From: Jim Heimbach [mailto:jh@jheimbach.com]
Sent: Friday, March 8, 2019 3:02 PM
To: 'Morissette, Rachel'
Cc: 'Jim'
Subject: RE: clarification requested for GRN 000809

Rachel—

Here is our response to your chemist's questions.

Regards,
Jim

James T. Heimbach, Ph.D., F.A.C.N.
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From: Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
Sent: Monday, March 4, 2019 9:15 AM
To: Jim Heimbach
Cc: Jim
Subject: clarification requested for GRN 000809

Dear Jim,

Our chemist has some clarification questions on your responses to our questions for GRN 000809, which I've included below. Please provide your responses within 5 business days. Please let me know if you have questions.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

Please clarify the following:

1. On page 6 of your amendment (excerpted below), you provide means ± SD for the main protein components of the starting material (WPC), fractionated WPC (41% ALA), and reduced retentate fraction. However, the apparent sums of these values (highlighted) do not equal 100 for the starting material or retentate. Please discuss the significance of any differences in the sum of major proteins.

Composition of Starting Material, WPC (41% ALA), and ALA-Reduced Retentate

Material	**g/100 g Protein (Mean±s.d.)**							**Apparent sum (based on means)**
	ALA	**ß-lacto-globulin**	**CGMP**	**PP8/PP5**	**IgG-1**	**BSA**	**LF**	**Combined means ALA, ß-Lactoglobulin, CGMP,PP8/PP5, IgG-1, BSA, LF**
Starting Material (WPC)	17.8±0.1	47.1±0.3	19.4±0.6	2.1±0.2	3.0±0.1	N.A.	0.1±0.02	87.5 g/100 g protein
WPC (41% ALA)	48.3±0.7	19.2±0.7	28.4±0.4	4.9±0.9	<0.1 nM	<0.1 nM	N.D.	100.8 g/100 g protein
Reduced Retentate Fraction	8.6±0.7	53.6±1.1	16.0±0.4	2.5±0.4	5.8±0.6	2.5±0.3	0.7±0.1	89.7 g/100 g protein

(n=5 individual batches); N.A. = not analyzed; N.D. = not detected

2. Is the reduced retentate fraction the same as the "third fraction" noted in the text on page 13 of the original notice in the description of the method of manufacture?
3. In your description of the method of manufacture on page 12 of GRN 000809, you state that "raw milk is received at the cheese or casein production facility" and subsequently pasteurized. However, the CGMP composition of the fractionated WPC (41% ALA) appears to reflect only whey produced as a byproduct of cheesemaking. Please confirm that only whey from cheesemaking will be used as a starting material. Alternatively, if microfiltered whey from casein production is used as a starting material, the composition of the resulting product should also be characterized in the notice.
4. In the absence of data regarding levels of growth factors such as IGF-1 or TGF-ß in fractionated WPC (41% ALA), please comment on whether these components would be concentrated in the final ingredient based on the molecular weight cutoff of the ultrafiltration membrane used in your method of manufacture or the use of pasteurized milk for whey production from cheesemaking.

   (We note that effects of pasteurization and membrane processing on levels of TGF-ß1 and IGF-1 in ultrafiltered whey have been discussed in the published literature (Ollikainen et al., 2012; Akbache et al., 2009)).
5. Aside from the proteins mentioned in question 4, please comment on whether the method of manufacture of fractionated WPC (41% ALA) results in the concentration of other minor proteins (e.g., osteopontin) in whey above that of the WPC starting material.
6. You have referred to the use of ultrafiltration and the review by Kamau et al. (2010) to support the method of manufacture. Without giving confidential details, please clarify if precipitation/aggregation, enzyme treatment, or chromatography (ion exchange or gel filtration) are used in addition to ultrafiltration.

References:
Ollikainen et al. 2012. Int. Dairy J. 26:141-6.
Akbache et al. 2009. J. Membrane Sci. 326:435040.

JHeimbach LLC

March 8, 2019

Rachel Morissette, Ph.D.
Consumer Safety Officer
FDA Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review

Dear Dr. Morissette:

I am writing in response to your correspondence of March 4, in which you shared with us a number of additional questions developed by the FDA review team regarding GRAS Notice GRN 809.

1. *On page 6 of your amendment (excerpted below), you provide means ± SD for the main protein components of the starting material (WPC), fractionated WPC (41% ALA), and reduced retentate fraction. However, the apparent sums of these values (highlighted) do not equal 100 for the starting material or retentate. Please discuss the significance of any differences in the sum of major proteins.*

Answer:
We were focusing on quantification of proteins which are found in the GRAS-notified substance, fractionated WPC (41% ALA), and included concentrations in the starting material (WPC) and the reduced retentate fraction for completeness. Besides the more abundant proteins in WPC (α-lactalbumin, β-lactoglobulin, cGMP, BSA, IgG-1, and lactoferrin), we have identified by high resolution mass spectrometry 249 different proteins.

These 249 proteins are found in small amounts but contribute to the failure of the sum of the major proteins to reach 100%. Furthermore we expect the raw material and the reduced retentate to contain small amounts of non-protein nitrogen, mainly consisting of minor peptides, urea, nucleotides, metabolites of nucleotides, creatine, creatinine and free amino acids (Wolfschoon-Pombo & Klostermeyer, 1981). All in all we expect that these two pools of proteins and protein equivalents account for the missing 10% of the proteins in the starting material and the reduced retentate.

Reference:
Wolfschoon-Pombo A and Klostermeyer H. 1981. Die NPN Fraktion des Kuh Milch – I. Menge und Zusammensetzung. *Milschwissenschaft* **36:** 598-600.

2. *Is the reduced retentate fraction the same as the "third fraction" noted in the text on page 13 of the original notice in the description of the method of manufacture?*

Answer:
Yes. Sorry for the confusion.

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3. *In your description of the method of manufacture on page 12 of GRN 000809, you state that "raw milk is received at the cheese or casein production facility" and subsequently pasteurized. However, the CGMP composition of the fractionated WPC (41% ALA) appears to reflect only whey produced as a byproduct of cheesemaking. Please confirm that only whey from cheesemaking will be used as a starting material. Alternatively, if microfiltered whey from casein production is used as a starting material, the composition of the resulting product should also be characterized in the notice.*

Answer:
Whey obtained as a byproduct of cheesemaking is the only raw material for the production of Lacprodan Alpha-10, the notified GRAS substance. The mention of casein production facility was an error due to the use of whey from this source for another Arla product. Again, we apologize for the confusion.

4. *In the absence of data regarding levels of growth factors such as IGF-1 or TGF-β in fractionated WPC (41% ALA), please comment on whether these components would be concentrated in the final ingredient based on the molecular weight cutoff of the ultrafiltration membrane used in your method of manufacture or the use of pasteurized milk for whey production from cheesemaking.*

(We note that effects of pasteurization and membrane processing on levels of TGF-β1 and IGF-1 in ultrafiltered whey have been discussed in the published literature (Ollikainen et al., 2012; Akbache et al., 2009)).

Answer:
Thank you for the forwarded literature.

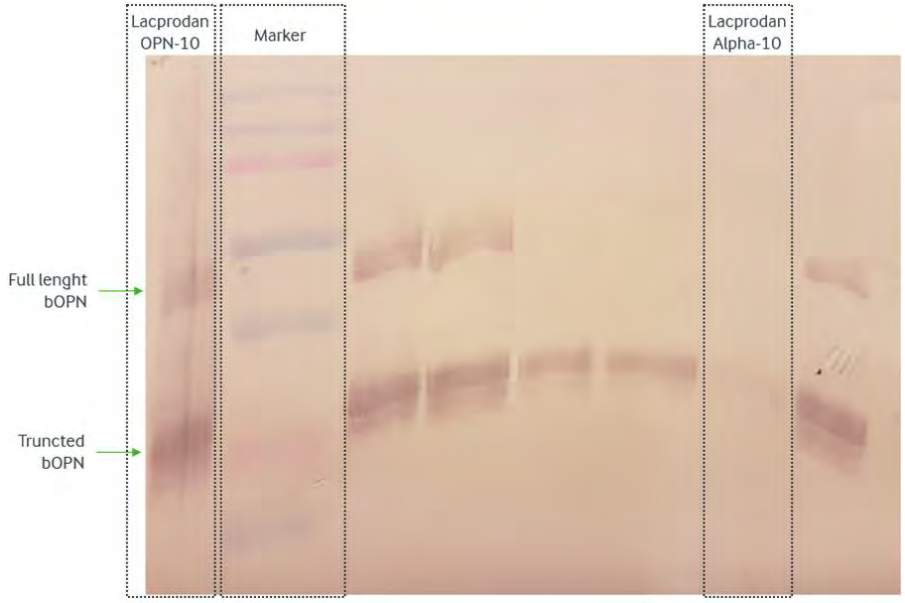
The manufacture of Lacprodan Alpha-10 utilizes an even tighter separation material than that employed by Ollikainen et al. (2012), which means that most TGF-β from the starting material (WPC) should be in the reduced retentate fraction rather than in the notified GRAS fraction, fractionated WPC (41% ALA).

Based on the smaller molecular weight of IGF-1 (7.5 kg/mol) and the measurements of IGF-1 after ultrafiltration in Akbache et al. (2009), IGF-1 would most likely pass through to the WPC (41% ALA fraction).

5. *Aside from the proteins mentioned in question 4, please comment on whether the method of manufacture of fractionated WPC (41% ALA) results in the concentration of other minor proteins (e.g., osteopontin) in whey above that of the WPC starting material.*

Answer:
The concentration of minor proteins in the WPC (41% ALA) fraction is influenced by factors such as net charge and molecular size of the proteins. Thus, it is not possible to comment meaningfully on the likely concentration of them in fractionated WPC (41% ALA).

Specifically for osteopontin, a western blot analysis (shown) was performed showing that almost none of the full-length or truncated osteopontin from the raw material remains in Lacprodan Alpha-10 (fractionated WPC (41% ALA)).



6. *You have referred to the use of ultrafiltration and the review by Kamau et al. (2010) to support the method of manufacture. Without giving confidential details, please clarify if precipitation/aggregation, enzyme treatment, or chromatography (ion exchange or gel filtration) are used in addition to ultrafiltration.*

Answer:
No, the manufacture of fractionated WPC (41% ALA) does not employ precipitation/aggregation, enzyme treatment, or chromatography (ion exchange or gel filtration).

Sincerely,

James T. Heimbach, Ph.D., F.A.C.N.

From:	Jim Heimbach
To:	Morissette, Rachel
Subject:	FW: alternate reference
Date:	Wednesday, April 03, 2019 3:39:21 PM
Attachments:	DePeters 1992, NPN composition bovine milk.pdf

Rachel--

Please find attached a reference in English instead of German. The authors in this paper reference their finding to the original German language reference provided.
Hope this will suffice.

Regards
Jim

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Eighteen pages have been removed in accordance with copyright laws. The removed reference is:

DePeters, E., Ferguson, J. 1992. "Nonprotein Nitrogen and Protein Distribution in the Milk of Cows." *Journal of Dairy Science*. Vol. 75, No. 11.