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June 27, 2022

Via FedEx

Dr. Susan Carlson
Director, Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied
Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for Arla for Fractionated Whey Protein Concentrate containing 41% Alpha-Lactalbumin

Dear Dr. Carlson:

We respectfully submit the attached Generally Recognized As Safe (GRAS) notification on behalf of our client, Arla Foods Ingredients Group P/S (Arla) for fractionated whey protein concentrate containing 41% alpha-lactalbumin to be used in conventional foods as a source of protein. More detailed information regarding product identification, intended use levels, the manufacturing process, and safety of the ingredient is set forth in the attached GRAS Notification. This notice is a resubmission of GRAS Notification 909 and addresses FDA's feedback provided to Arla on GRAS Notification 909, as well as GRAS Notification 809 (concerning the use of the same substance in infant formula). The revised notification also addresses feedback provided to Arla by FDA in its August 19, 2021 correspondence.

Arla has determined that its fractionated whey protein concentrate containing 41% alpha-lactalbumin is GRAS for its intended uses based on scientific procedures in accordance with 21 CFR § 170.30(b). Therefore, the use of the fractionated whey protein concentrate containing 41% alpha-lactalbumin as described in this GRAS Notification is exempt from the requirement of premarket approval as set forth in the Federal Food, Drug, and Cosmetic Act.

The analytical data, published studies, and information that are the basis for this GRAS Notification are available for FDA review and will be sent to FDA upon request.

We look forward to the Agency's review of this submission and would be happy to provide Agency officials with any information they may need to complete their assessment. Thank you for your attention to this matter.

Cordially yours,

Natalie E. Rainer

Enclosure

Generally Recognized as Safe (GRAS) Determination for the Intended Use of Fractionated Whey Protein Concentrate Containing 41% Alpha-Lactalbumin in Conventional Foods

Prepared for:
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, Maryland 20740

Submitted by:
Keller and Heckman LLP
1001 G Street, N.W.
Suite 500 West
Washington, DC 20001

On behalf of our client:

Arla Foods Ingredients Group P/S
Sonderhoj 10-12
8260 DK- Viby J
Denmark

Date: June 27, 2022

GRAS Notice for Alpha-Lactalbumin

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Part 1: Signed Statements and Certification

1.1 GRAS Notice Submission

Keller and Heckman LLP submits the enclosed information on behalf of our client, Arla Foods Ingredients Group P/S ("Arla"), in accordance with the requirements of 21 C.F.R. Part 170, Subpart E.

1.2 Name and Address of Notifier

Arla Foods Ingredients Group P/S
Sonderhoj 10-12
8260 DK- Viby J
Denmark

All communications on this matter are to be sent to Counsel for the Notifier:

Natalie E. Rainer
Keller and Heckman LLP
Three Embarcadero Center, Suite 1420
San Francisco, California 94111
Telephone: (415) 948-2821
Facsimile: (415) 948-2828
Email: rainer@khlaw.com

1.3 Name of Notified Substance

The subject of this Generally Recognized as Safe (GRAS) notice is Lacprodan® ALPHA-10 brand alpha-lactalbumin, often abbreviated α-LAC or ALA. Lacprodan® ALPHA-10 can be denoted descriptively as fractionated whey protein concentrate containing 41% alpha-lactalbumin, although this is not suggested as necessarily an appropriate name for labeling purposes.

The intended addition to infant formula of fractionated whey protein concentrate containing 41% alpha-lactalbumin was the subject of GRAS Notice No. 809, submitted on August 17, 2018, and filed on October 9, 2018. FDA's closure letter, indicating that the agency had no questions regarding the GRAS conclusion, was dated April 6, 2019. The intended use of the substance in GRAS Notice 809 was as a source of protein in cow-milk-based infant formula at a use level of 2.5 g/L, while the intended use addressed in the current GRAS notice concerns the same product, manufactured in the same way and complying with the same specifications.

1.4 Intended Conditions of Use

As described in detail in Section 3.1, the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin is its addition to conventional foods as a source of protein.

1.5 Statutory Basis for GRAS Status

Arla Foods Ingredients' GRAS determination for the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin is based on scientific procedures in accordance with 21 C.F.R. § 170.30(b).

Determination of the safety and GRAS status of the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin has been made through this GRAS Notice, prepared by Keller and Heckman LLP.

1.6 Exclusion from Premarket Approval

The intended use of fractionated whey protein concentrate containing 41% 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.

1.7 Availability of Data and Information

The information for this GRAS conclusion including analytical data, published studies, and information that are the basis for this GRAS determination are available to FDA upon request as required by 21 C.F.R. § 170.225(c)(7)(ii)(A) or (B) by contacting Keller and Heckman LLP at the below address.

Natalie E. Rainer
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1.8 Freedom of Information Act Statement

Arla Foods Ingredients is not claiming any information in Parts 2 through 7 of this document as trade secret, confidential or financial information that is privileged or confidential. Thus, all information and data in this submission are not exempt from the Freedom of Information Act (FOIA), 5 U.S.C. Section 552.

1.9 Certification

I hereby certify that, 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 fractionated whey protein concentrate containing 41% alpha-lactalbumin.

Signature:

Natalie E. Rainer
Arla Foods Ingredients Group P/S
Date: June 27, 2022

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

2.1. Name of the GRAS Substance

The notified substance is Lacprodan® ALPHA-10 brand alpha-lactalbumin, often denominated α-lactalbumin. Lacprodan® ALPHA-10 is a brand-name product marketed by Arla Foods Ingredients that is descriptively denoted as fractionated whey protein concentrate containing 41% alpha-lactalbumin.

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

2.2.1. Source

Consistent with GRAS Notice 809, Lacprodan® ALPHA-10 is composed of sweet whey with a pH of 5.9 – 6.6 that is created in cheese production. The sweet whey used as raw material conforms to the European Union Food Hygienic Guidelines and EU Regulation 853/2004 which, in turn, allows for the use of only U.S.-approved pesticides and veterinary drugs. The starting material comes from processing plants that are 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.

The composition of the starting material, WPC (41% ALA) and ALA-Reduced Retentate, was evaluated in systematic analysis of 5 lots of each of the materials and is demonstrated in the below table. The analysis was conducted in-house and by a third-party laboratory 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 (Elgar *et al*. 2000).

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 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.

Table 1. 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.

2.2.2. Description

GRAS Notice 809 provides a relevant description of the fractionated whey protein concentrate containing 41% alpha-lactalbumin. It states:

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 and with reduced beta-lactoglobulin content. It also includes casein glycomacropeptide (CGMP). Regular whey has about 20% CGMP whereas fractionated WPC (41% ALA) has about 28%.

Consistent with GRAS Notice 809, the bovine milk and commercial whey protein concentrates have higher beta-lactoglobulin than alpha-lactalbumin; in cow's milk the beta-lactoglobulin is approximately 2-3 times higher than alpha-lactalbumin (*i.e.,* ~52% beta-lactoglobulin and ~19% alpha-lactalbumin). The Lacprodan® ALPHA-10 fraction has approximately 2-fold higher enrichment of alpha-lactalbumin, while the beta-lactoglobulin is reduced, as compared to what is found in a typical whey protein concentrate.

We note that this does not include the 249 different proteins that are present in smaller amounts and contribute to sum of the major proteins to reach 100%. The concentration of minor proteins in the WPC is influenced by factors such as the net charge and molecular size of the proteins. Thus, it is not possible to meaningfully determine the likely concentration of the minor proteins in the fractionated WPC.

In addition, the raw material and reduced retentate may also contribute small amounts of non-protein nitrogen, mainly consisting of minor peptides, urea, nucleotides, metabolites of nucleotides, creatine, creatinine, and a free amino acid pool (Wolfschoon-Pombo A *et al*. 1981). These two pools of protein and protein equivalents account for the remaining amount of proteins in the starting material and reduced retentate.

2.2.3. Manufacture

The manufacture of the Lacprodan® ALPHA-10 is identical to the manufacture of the Lacprodan® ALPHA-10 described in GRAS Notice 809, which provides:

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 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, which then 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, or reduced retentate fraction, from the same separation is a beta-lactoglobulin-enriched fraction. The lactose-enriched fraction and the β-LG-enriched fraction are then transferred to tanks for further processing for other applications.

The ALA fraction undergoes a purification process based on the principle of the difference in the molecular weight of proteins, involving a two-stage membrane process for obtaining concentrates enriched in ALA. The membrane process is selective and serves as a thin barrier between miscible fluids that allows for the preferential transport of feed components when a driving force, such as a pressure differential, is applied. Further, as noted in GRAS Notice 809:

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 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 of Lacprodan® ALPHA-10 is not less than 41.0 %. The production process is summarized in Figure 1.

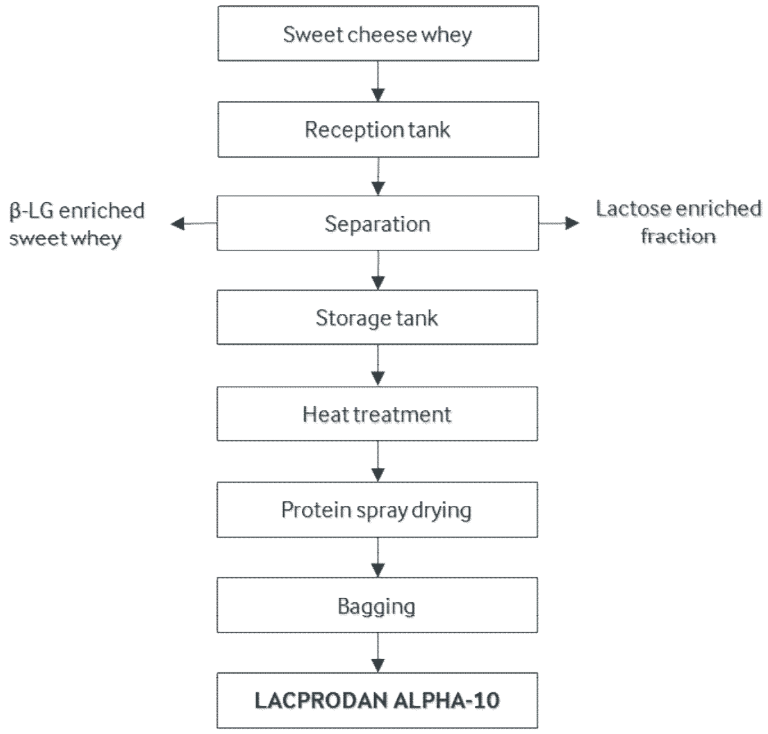

Figure 1. Process Flow Diagram of Lacprodan® ALPHA-10

2.2.4. Specifications

The specifications for the fractionated whey protein concentrate containing 41% alpha-lactalbumin are identical to those described for Lacprodan® ALPHA-10 in GRAS 809, which provides:

Food-grade specifications for Lacprodan® ALPHA-10 are displayed in Table 2, 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 2. 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	≤0.45	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	≤0.40	0.26	0.28	0.29	0.24	0.25	0.23
Calcium (%)	ICP	≤0.60	0.50	0.49	0.50	0.52	0.52	0.48
Potassium (%)	ICP	≤0.90	0.66	0.63	0.72	0.59	0.66	0.72
Heavy metals								
Arsenic[1] (mg/kg)	ICP-HRMS ISO 17294m:2016	<0.2	<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

[1] The reason for the different limits of quantification (LOQs) is that different versions of the same analysis method were used over time, due to development of the method (*i.e.*, ISO 17294m:2016). However, we note that all results are below the LOQ and within the set specification limit.

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
Enterobacteri-aceae (cfu/g)	ISO 21528-2	<10	<10	<10	<10	<10	<10	<10
Staphylococcus aureus (cfu/g)	ISO 6888-1	Absent/1g	Absent/1 g	Absent	Absent	Absent	Absent	Absent
Salmonella (cfu/g)	ISO 6579	Absent/25 g	Absent/25 g	Absent	Absent	Absent	Absent	Absent
Source: Arla Foods Ingredients								

2.3. Stability

The stability information is the same as that described for Lacprodan® ALPHA-10 in GRAS Notice 809, as provided here.

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 3) 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 3. 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				

2.4. Technical Effect

The intended technical effect of the addition of fractionated whey protein concentrate containing 41% alpha-lactalbumin to conventional foods is as a source of protein.

Part 3: Dietary Exposure

3.1. Intended Conditions of Use

Fractionated whey protein concentrate containing 41% alpha-lactalbumin is intended for addition as a source of protein to the food categories listed in Table 4 at the maximum addition levels also shown in the table. Since alpha-lactalbumin constitutes 41% by weight of the fractionated whey protein concentrate, the addition level of alpha-lactalbumin is as shown in the last column of the table.

Table 4. Intended Use of Fractionated Whey Protein Concentrate Containing 41% Alpha-Lactalbumin (in addition to Infant-Formula Use Described in GRAS Notice 809)

Food/Beverage Category	NHANES Description	Serving Size[1]	Max Whey Protein Concentrate Addition (g)	Max Whey Protein Concentrate Addition (%)	Max Alpha-Lactalbumin Addition (g)
Protein-enriched baked products; nutrition bars	All nutrition or meal replace-ment bars	40 g	12 g	30%	4.9 g
RTD milk-based nutritional beverages and meal replacements	All nutritional beverages	240 ml	12 g	5%	4.9 g
Protein enriched milks, sport drinks	RTD flavored milk and all sport drinks	240-360 ml	10 g	4%	4.1 g
Protein-enriched water or fruit-juice based beverages	Enhanced/fortified waters and fruit juice drinks	240-360 ml	10 g	4%	4.1 g
Yogurt	All yogurts, including regular, Greek, and non-dairy yogurts	170 g	8.5 g	5%	3.5 g
Fermented milk drinks, drinkable yogurts	Buttermilk, kefir, liquid yogurt	240 g	12 g	5%	4.9 g
Other nutritional beverages & powders	Non-reconstituted protein and nutritional powders	Amount to make 240 ml	12 g	5%	4.9 g

1. Serving sizes correspond to values in Table 2 – Reference Amounts Customarily Consumed per Eating Occasion: General Food Supply as cited in *Federal Register* Vol 81, No. 103, Friday, May 27, 2016, pp 34000-47. Available at: https://www.govinfo.gov/content/pkg/FR-2016-05-27/pdf/2016-11865.pdf.

The fractionated whey protein concentrate is intended to be used as a protein source that will be substituted to replace other dairy-based protein sources containing alpha-lactalbumin. As the use of the fractionated whey protein is substitutional with regard to other alpha-lactalbumin ingredients on the market, intake of these products will not be additive to the intake of WPC containing 41% alpha-lactalbumin. Bovine milk contains about 32 g protein/L (Haug *et al*.

2007), of which about 3.5% is alphalactalbumin (Layman *et al*. 2018). Thus, the alpha-lactalbumin content of bovine milk is about 1.12 g/L.

According to USDA's Agricultural Research Service (Sebastian *et al*. 2010), "the average intake of fluid milk was slightly more than ¾ cup for individuals 2 years of age and over." The age group with the highest level of fluid milk consumption in the U.S. is children aged 2-11 years, about 80% of whom consume fluid milk with an average daily intake of about 1-3/8 cup, equivalent to 325 ml. The alpha-lactalbumin content of this daily milk intake is about 364 mg. Since the mean intake of alpha-lactalbumin from WPC containing 41% alpha-lactalbumin is 3.08 g/day (41% of 7.5 g/day) for toddlers aged 1-3 years and 4.59 g/day (41% of 11.2 g/day) for those aged 4+ years, milk consumption adds about 11.8% to toddlers' intake of alphalactalbumin and about 7.9% to the alpha-lactalbumin intake of those aged 4 years and older.

3.2. Estimated Daily Exposure

The estimated daily intakes (EDI) of fractionated whey protein concentrate and alpha-lactalbumin from the intended use in foods and beverages were calculated by E^{x}ponent® based on food consumption records collected in the *What We Eat in America* component of the NHANES conducted in 2013-2014 and 2015-2016 (WWEIA/NHANES 2013-2016). Two-day average intakes of all respondents aged 1-3 years and 4+ years were estimated both per capita and per user and expressed per person and on a bodyweight basis.

Overall, 41% of NHANES respondents over 4 years of age and 57% of those aged 1-3 years consumed one or more of the intended-use food categories on at least one of the two survey days. The mean and 90th percentile intakes of fractionated whey protein concentrate containing 41% alpha-lactalbumin by those aged 4+ years were 11.2 and 31.7 g/day, respectively, equivalent to 0.18 and 0.403 g/kg bw/ day. Among toddlers aged 1-3 years, mean and 90th percentile intakes of WPC were 7.5 and 15.8 g/day, respectively, equivalent to 0.56 and 1.09 g/kg bw/day.

Part 4: Self-limiting Levels of Use

There is no meaningful technological limitation to the concentration of fractionated whey protein concentrate containing 41% alpha-lactalbumin in foods.

Part 5: Experience Based on Common Use in Food

The conclusion that the intended use of fractionated whey protein concentrate containing 41% 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

The pharmacokinetics narrative for the fractionated whey protein concentrate containing 41% alpha-lactalbumin is the same as in GRAS notice 809, which we provide below:

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

The relevant animal studies are the same studies as are described in GRAS notice 809, which we summarize below.

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 5.

Table 5. 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 2). 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.

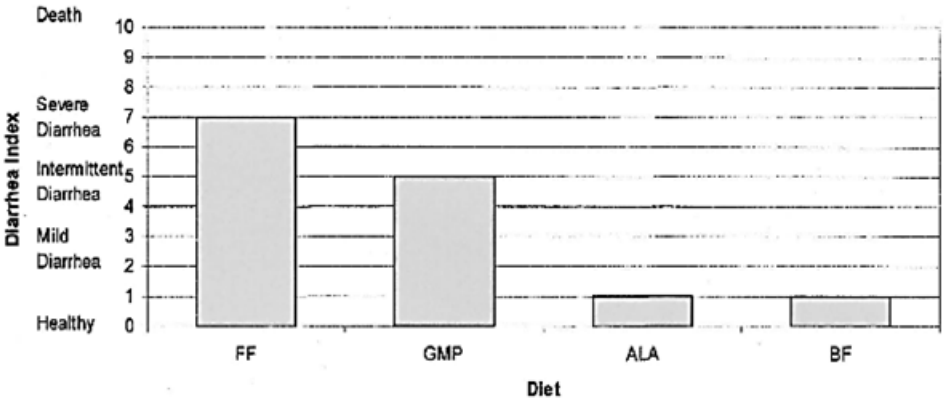

Figure 2. Diarrhea Scores Following EPEC Administration (Bruck *et al*. 2003)

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).

6.2.3. Toxicity Studies

Zhi *et al*. (2011) reported on a subchronic study of the toxicity of powdered milk containing transgenic human ALA in Wistar rats. Weanling rats were divided into 7 groups assigned to receive 15, 30, or 60% dietary concentrations of transgenic human ALA milk powder, 15, 30, or 6% dietary concentrations of non-transgenic human ALA milk powder, or basic animal feed for 90 days. Hematological and biochemical parameters were measured on days 45 and 90 and organs were examined after day 90. No adverse effects were reported on feed intake, body weight, absolute or relative organ weights, hematology (WBC, RBC, HGB, PLT, LY, MO, GR), serum biochemistry (ALT, AST, TP, ALB, BUN, CRE, TG, CHO, GLU, ALP, LDH), or histopathology. The NOAEL was the highest dietary concentration tested, 60%.

6.3. Human Studies

6.3.1. Studies in Infants

The published literature regarding alpha-lactalbumin ingestion by infants is summarized in Tables 6 and 7.

Table 6. Studies in Infants Using Alpha-Lactalbumin Produced by Arla Foods Ingredients

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related 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, 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	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).	4-6 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.
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)	4-6 months	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. 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

Table 6. Studies in Infants Using Alpha-Lactalbumin Produced by Arla Foods Ingredients

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
					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."
Dupont *et al*. (2010)	Prospective, randomized, double-blind, placebo-controlled, multi-center study 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)	30 days	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. Side effects were classified 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, "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 authors' primary conclusion was that "an α-lactalbumin-enriched and probiotic-supplemented formula proved

Table 6. Studies in Infants Using Alpha-Lactalbumin Produced by Arla Foods Ingredients

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
					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 iron absorption at 5.5 months	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 $FeSO_4$.	4-6 months	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."

Table 7. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Lien *et al*. (2004)	Prospective, randomized, double-blind, placebo-controlled study to assess growth and safety of formula with added ALA	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	12 weeks	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

Table 7. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
					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.
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	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	8 weeks	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

Table 7. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
					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."
Trabulsi *et al*. (2011)	Prospective, randomized, double-blind, placebo-controlled growth study comparing ALA-enriched formulas with different total protein levels	224 healthy term formula-fed infants aged 5-14 days with weight, length, and head circumference >5th and	Standard formula with 14.1 g protein/L and experimental formula with 12.8 g protein/L, both ALA-enriched with added L-	120 days	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.

Table 7. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
		<95th percentile for age, n = 112 for each formula, + HM group	tyrosine and L-tryptophan		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

Table 7. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
					supported age-appropriate growth; weight gain with EF was intermediate between SF and HM groups and resulted in growth similar to HM-fed infants."
Wernimont *et al*. (2015)	Prospective, randomized, double-blind, placebo-controlled, multicenter trial testing effect of ALA-enriched formula w or w/o oligofructose	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	8 weeks	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 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	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	6 weeks	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."

Table 7. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
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	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	48 days	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."
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	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*	6 months	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."
Raiha *et al*. (2002)	Prospective, randomized, double-blind, placebo-controlled multicenter trial comparing growth of infants receiving	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 &	4 months	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.

Table 7. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
	formulas with different total protein and ALA		70% modified sweet whey enriched in ALA and reduced in GMP. (ALA and GMP levels were not 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."
Fleddermann *et al*. (2014a) Fleddermann *et al*. (2018)	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 4-year follow-up	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 187 children from the BeMIM study, 65 from the IF group, 59 from the CF group, and 63 from the breastfed group	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	90-120 days	Weight gain (g/day) did not differ significantly between the 2 formula-fed groups. 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."

Table 7. Studies in Infants of Alpha-Lactalbumin Other Than Arla Foods Ingredients Products

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Vivatvakin *et al*. (2020)	Prospective, randomized, double-blind, parallel-group multi-center trial	259 infants age 30-90 days with feeding intoler-ance issues	ALA-enriched whey-predominant intact protein with full lactose or partially hydrolyzed 100% whey formula with reduced lactose	14 days	No difference between groups in daily duration of fussiness-crying (primary endpoint) or in gassiness, spitting up, or vomiting. The incidences of parent-reported and physician-confirmed AEs were similar between the 2 groups. Six infants experienced SAEs. One SAE led to the discontinuation of the study product. No SAE was considered study-product related. Both formulas were safe and supported age-appropriate infant growth.

6.3.2. Studies in Adults

The published literature regarding alpha-lactalbumin ingestion by adults is summarized in Table 8. Although most studies had purported efficacy as their primary endpoints, the study reports were examined for reports of side effects, adverse events, and study dropouts, and the discussion of study findings in Table 8 is limited to these findings. In addition, and as provided in GRAS Notice 809, a number of acute studies in adult humans have evaluated the effects of ALA consumption on central nervous system (CNS) function. We provide the analysis from GRAS Notice 809 below.

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 stressprone 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, placebocontrolled 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.

Table 8. Studies of Alpha-Lactalbumin in Adults

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Chungchun-lam *et al*. 2017	Prospective, randomized, single-blind crossover study of the relative effects of various protein isolates	20 apparently healthy women aged 24.2±0.8 years, with normal bodyweight	~50 g total whey protein, α-lactalbumin, or beta-lactoglobulin	Single dose	Energy intake at the test meal and total energy intakes (preload+test meal) did not differ among the three preload meals. No adverse effects were reported of any of the protein isolates.
Hursel *et al*. 2010	Prospective, randomized, double-blind crossover trial to differentiate the effects on energy expenditure and satiety of total whey protein and α-lactalbumin	35 apparently healthy university students, 17M and 18F aged 20.9±1.9 years	Breakfast yogurt that was unenriched, enriched with whey, or enriched with whey with increased α-lactalbumin and depleted in caseinomacro-peptide at 14% or 27% of the energy	Single dose	The authors reported that "No adverse events occurred, and no subjects reported any feelings of discomfort after consuming the yoghurt drinks."
Jaatinen *et al*. 2014	Prospective, randomized, double-blind, placebo-controlled trial on the ability of yogurt supple-mented with α-lactalbumin, casein tripeptides, and B vitamins to reduce anxiety	67 adults with high anxiety scores, 7M and 60F aged 18-63 years (mean age = 39.3±3.2 years)	125 g yogurt twice a day for 4 weeks, providing daily doses of 17 g α-lactalbumin, 4.2 g casein tri-peptides, 1.2 mg vitamin B_6, 121 µg folacin, and 2.6 µg vitamin B_{12}	4 weeks	No adverse effects were reported.

Table 8. Studies of Alpha-Lactalbumin in Adults

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Lagana *et al*. 2018	Prospective, randomized, double-blind, placebo-controlled trial of α-lactalbumin in treatment of iron deficiency anemia in pregnant women	50 pregnant women aged 26.6±6.1 years, between 19 and 21 gestational weeks, suffering from iron-deficiency anemia	30 mg micro-nized dispersible ferric pyrophos-phate plus 300 mg α-lactalbumin or 80 mg ferrous gluconate for 30 days	30 days	The authors reported that "the cumulative side effects rate was 24% in the ferrous-gluconate group [primarily constipation, darkened stools, diarrhea, loss of appetite, nausea, stomach cramps, and vomiting], whereas the ferric-pyrophosphate/α -lactalbumin group did not show any significant side effect." They concluded that, "overall, ferric-pyrophosphate/α-lactalbumin is more effective and safe than ferrous gluconate for the treatment of iron-deficiency anemia in pregnant women."
Mariotti *et al*. 2015	Prospective, randomized, double-blind crossover trial of the effects of dietary protein on postprandial lipemia	10 apparently healthy but overweight men aged 34±9 years	Meals with 233 g high-fat milk cream, 45 g sucrose, and protein isolates of 54 g casein, 55 g whey, or 49 g α-lactalbumin-enriched whey	Single dose	While the high-fat meal produced adverse postprandial effects, no adverse effects were associated with the protein supplements. The authors reached no conclusion regarding whey proteins, but concluded that, "In healthy overweight men, casein has specific physical interactions with fat that affect postprandial triacylglycerol, leading to the formation of fewer chylomicrons or an increase in chylomicron clearance."

Table 8. Studies of Alpha-Lactalbumin in Adults

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Markus *et al*. 2000	Prospective, randomized, double-blind crossover study of the ability of α-lactalbumin to help high- and low-stress-vulnerable adults cope with stress	29 stress-vul-nerable (10M and 19F aged 17-34 years, mean age = 20.5±3.1 years) and 29 stress-invulnerable subjects (9M and 20F aged 17-34 years, mean age = 20.9±3.2 years)	Drinks with 40 g whey protein rich in α-lactalbumin or 31 g casein	Single dose	All changes were regarded as beneficial and no adverse effects were reported.
Markus *et al*. 2002	Prospective, randomized, double-blind crossover study on α-lactalbumin to ameliorate loss of cognitive performance under pressure	23 stress-vulnerable subjects (10M and 13F aged 17-33 years, mean age = 20±2 years) and 29 relatively stress-invul-nerable sub-jects (13M and 16F aged 17-33 years, mean age = 21±3 years)	Drinks with 40 g whey protein rich in α-lactalbumin or 31 g casein	Single dose	The authors reported no adverse effects.

Table 8. Studies of Alpha-Lactalbumin in Adults

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Markus *et al*. 2005	Prospective, randomized, double-blind crossover study on the effect of consumption of a whey protein rich in α-lactal-bumin during the evening on alertness and cognitive function the following morning	28 apparently healthy university students [14M and 14F] with mean age = 22±2.5 years	Drinks with 40 g whey protein rich in α-lactalbumin or 31 g casein	Single dose	No adverse effects were reported.
Merens *et al*. 2005	Prospective, randomized, double-blind crossover study of effects of α-lactalbumin on mood following a stressful test	23 subjects (2M and 21F aged 30.0±9.7 years) suffering from depression and 20 healthy subjects (3M and 17F aged 27.0±10.1 years)	Drinks with 40 g whey protein rich in α-lactalbumin or 31 g casein	Single dose	The authors reported that "α-lactalbumin had no side-effects."
Nieuwen-huizen *et al*. 2009	Prospective, randomized, single-blind crossover study of the effect of α-lactalbumin on satiety	24 apparently healthy adults, 11M and 13F, aged 19-37 years (mean age = 21±0.8 years)	10%-protein breakfasts with α-lactalbumin, gelatin, or gelatin +tryptophan	Single dose	No adverse effects were reported.

Table 8. Studies of Alpha-Lactalbumin in Adults

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Ong *et al*. 2017	Prospective, randomized, double-blind crossover pilot study of the effect of α-lactalbumin intake on sleep quality and duration	10 apparently healthy males aged 26.9±5.3 years	20 g of either α-lactalbumin or sodium casein-ate one hour before bedtime	2 days	No adverse effects attributable to α-lactalbumin were reported.
Pujos-Guillot *et al*. 2018	Prospective, randomized, double-blind crossover study on metabolomic effects of α-lactalbumin	10 apparently healthy but overweight men aged 21-50 years (mean age = 34±9 years)	Meals with 233 g high-fat milk cream, 45 g sucrose, and protein isolates of 54 g casein, 55 g whey, or 49 g α-lactalbumin-enriched whey	Single dose	No adverse effects were reported and the authors concluded that, "our study has revealed that protein type regulates the oxidative pathways of fatty acids and amino acids after a high-fat meal."
Qin *et al.* 2017	Prospective, randomized, double-blind crossover study comparing the effects of α-lactalbumin and total whey protein on muscle damage, muscle pain, and mood states after prolonged strenuous exercise	12 apparently healthy male endurance runners aged 30.4±2.8 years	Whey isolate or α-lactalbumin consumed at a rate of 0.34 g/kg bw/hour	Single dose	No adverse effects were reported.

Table 8. Studies of Alpha-Lactalbumin in Adults

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Qin *et al*. 2019	Prospective, randomized, double-blind crossover trial of α-lactalbumin or whey protein isolate before a 21-kilometer run	11 apparently healthy male endurance runners aged 31±2 years	α-lactalbumin or whey protein isolate	Single dose	No adverse effects attributable to α-lactalbumin or whey isolate were reported, and the authors concluded that, "compared with the pre-exercise ingestion of whey protein, that of α-lactalbumin led to superior results during similar levels of endurance exercise."
Sashihara *et al*. 2013	Prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. gasseri*, with or without added α-lactalbumin, on athletes under-going strenuous exercise	Male university students: placebo group, n = 14, age = 20.2±1.1 years; *L. gasseri* group, n = 15, age = 19.8±0.9 years; *L. gasseri* + α-lactalbumin, n = 15, age = 19.1±0.9 years	*L. gasseri* with 900 mg α-lactalbumin	4 weeks	No subject withdrew from the study and all completed the trial successfully. The authors reported that, "No adverse effects due to the treatment were observed throughout the study. No significant changes in all of the blood or blood biochemical examination results obtained from the pre-exercise blood samples were observed during the study period in any of the subjects."
Schmitt *et al*. 2005	Prospective, randomized, double-blind crossover trial of the effect of α-lactalbumin on memory	16 women aged 18-45 years (mean age = 29±2 years) suffering from premenstrual symptoms	Drinks with 40 g whey protein rich in α-lactalbumin or 31 g casein	Single dose	No adverse effects were reported, and the authors concluded that "the cognitive effect of acute premenstrual administration of α-lactal-bumin… was modest."

Table 8. Studies of Alpha-Lactalbumin in Adults

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Scrutton *et al*. 2007	Prospective, randomized, double-blind, placebo-controlled study of α-lactalbumin and anxiety and cognitive function	28 apparently healthy women aged 19-45 years (mean age = 26.8±6.6 years)	Drinks with 40 g whey protein rich in α-lactalbumin or 31 g casein	Single dose	Women receiving α-lactalbumin experienced significantly more nausea than those receiving casein, an effect the authors suggested might reflect increased brain serotonin activity.
Veldhorst *et al*. 2009	Prospective, randomized, single-blind crossover satiety study	24 apparently healthy subjects, 10M and 14F, aged 18-45 years (mean age = 25±2 years),	Breakfast with α-lactalbumin, gelatin, casein, soy, whey, glycomacro-peptide-depleted whey, or gelatin+ tryptophan providing either 10% or 25% of the energy	Single dose	No adverse effects were reported.
Verschoor *et al*. 2010	Prospective, randomized, double-blind crossover trial of α-lactalbumin effects on mood, stress, and appetite	13 high- and 12 low-trait anxiety university students; high-anxiety group--2M and 11F aged 21.5±5.7 years; low-anxiety group--1M and 11F aged 21.0±1.7 years	Lunch containing either 40 g α-lactalbumin or 40 g casein on 2 consecutive test days	Single dose	All effects were regarded as beneficial, and no adverse effects were reported.

6.4. Safety Assessment and GRAS Determination

The safety assessment and GRAS determination for Lacprodan® ALPHA-10 entail two steps. In the first step, the safety of the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin is demonstrated. Safety is established by demonstrating a reasonable certainty that the exposure of consumers to fractionated whey protein concentrate containing 41% alpha-lactalbumin under its intended conditions of use is not harmful. In the second step, the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin is determined to be GRAS by demonstrating that the safety of this substance 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 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 fractionated whey protein concentrate containing 41% alpha-lactalbumin is safe and is GRAS.

6.4.1. Evidence of Safety

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 (Fox and McSweeney 1998). Infants who consume regular cow's milk or casein dominant milk based formulations have been exposed to around 1.2 g/L of bovine alpha-lactalbumin for the past 100 years (Jackson *et al*. 2004); the alpha-lactalbumin intake of infants consuming whey-dominant infant formula is higher, about 1.8 g/L.

On April 29, 2019, FDA stated that it had no questions at that time regarding Arla's conclusion that fractionated whey protein concentrate containing 41% alpha-lactalbumin is GRAS under the intended conditions of use described in GRAS Notice 809. The conditions of

use addressed in that notice were as an ingredient in cow's milk-based non-exempt infant formula for term infants at a maximum level of 8.3 g/L, resulting in a 90th percentile intake of 1.72 g/kg bw/day. By comparison, the 90th percentile intake of fractionated whey protein concentrate containing 41% alpha-lactalbumin by consumers of target foods in the current GRAS determination is only 0.43 g/ kg-bw/day, just ¼ the amount consumed by infants from its use in formula, albeit these foods are intended for adults and older children with different daily quantitative protein requirements and tolerances per unit of bodyweight compared to infants.

The fact that use of fractionated whey protein concentrate containing 41% alpha-lactalbumin is GRAS for use at up to 8.3 g/L in infant formula supports the ample additional corroborating evidence that it is also safe for addition to conventional foods at the concentrations intended. This corroborating evidence is provided by the 19 additional published studies discussed in this document. These studies enrolled 567 individuals, 466 of whom ingested alpha-lactalbumin with no reports of adverse effects associated with the intervention.

Part 7: List of Supporting Data and Information

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March 7, 2023

Via Email (CFSAN-OFASDFI-FILINGTEAM@fda.hhs.gov)

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Re: Arla Foods Ingredients Group P/S – Amendment to GRAS Notice for Fractionated Whey Protein Concentrate Containing 41% Alpha-Lactalbumin

Dear Dr. Deng:

This amendment responds to your email request for clarification dated February 28, 2023. Arla Foods Ingredients Group P/S confirms that the subject of this GRAS Notice (fractionated whey protein concentrate containing 41% alpha-lactalbumin) is not intended for use in food products that are under the jurisdiction of the U.S. Department of Agriculture.

* * *

We hope that this clarification is helpful. Please let us know if you need further information.

Sincerely yours,

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July 19, 2023

Via Electronic Mail

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Re: Response to FDA's Questions for GRAS Notice No. 001100

Dear Dr. Deng:

On behalf of Arla Food Ingredients P/S (Arla), we are writing in response to FDA's questions regarding GRAS Notice No. 001100 for fractionated whey-derived protein concentrate containing ≥41% alpha-lactalbumin (fractionated WPC (41% ALA)). For ease of reference, we reproduce each question below, followed by Arla's response.

1. **On p. 6, Arla notes that the "sweet whey used as a raw material conforms to the European Union Food Hygienic Guidelines and EU Regulation 853/2004 which, in turn, allows for the use of only U.S.-approved pesticides and veterinary drugs." We note that the cited reference does not include this information, and we are not aware of such a requirement. Please clarify or revise this statement, and provide references, as appropriate, to support the revised statement. Alternatively, the notifier can provide a statement that the cow's milk is produced using only U.S. approved pesticides (e.g., for feed) and veterinary drugs for dairy cows, in accordance with U.S. regulations for use and tolerances for residues of these materials (40 CFR Part 180 and 21 CFR Part 556).**

Arla confirms that the cow's milk is produced using only U.S. approved pesticides for feed and veterinary drugs for dairy cows, in accordance with U.S. regulations for use and tolerances for residues of these materials (40 CFR Part 180 and 21 CFR Part 556).

2. **Please confirm that the fluid whey starting material is obtained as a byproduct of cheesemaking from fluid milk that is:**

a. produced in accordance with current good agricultural practices, and

b. pasteurized and produced in accordance with the requirements of the Pasteurized Milk Ordinance (PMO, 2019[1]; 21 CFR 1240.61)

Arla confirms that the fluid whey starting material used in GRN 1100 is produced in accordance with good agricultural practices and the 2019 Grade "A" Pasteurized Milk Ordinance (21 CFR 1240.61).

3. Arla briefly describes a high-performance liquid chromatography (HPLC)-mass spectrometry method for analysis of major proteins in fractionated WPC (41% ALA). Please confirm that the method of analysis has been validated for that particular purpose.

Arla confirms that they have developed an in-house HPLC method for determination of major proteins in Alpha-10 and that this method has been validated and is fit for purpose.

4. Please state the upper level of ALA as a % of protein. Given that Arla identifies the subject of GRN 001100 as "Lacprodan Alpha-10", which is distinct from Arla's "Lacprodan Alpha-20"containing ≥57% alpha-lactalbumin,[2] it is our understanding that the upper level of the ingredient in GRN 001100 would be below this level.

There is no upper level of ALA for Lacprodan Alpha-10. Importantly, however, Lacprodan Alpha-20 has been discontinued and is no longer in production. Therefore, there is no risk of protein level overlap between the two ingredients.

5. In the February 1, 2019 amendment to GRN 000809, Arla noted that its fractionation method was consistent with the membrane separation approach described in Kamau et al (2010).[3] In GRN 001100, Arla describes the method as a two-stage membrane process.

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1 Available from the National Conference on Interstate Milk Shipments website: https://ncims.org/about/2017-procedures-constitution-bylaws/

2 https://www.arlafoodsingredients.com/health-foods/our-ingredients/alpha-lactalbumin/.

3 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.

a. Please provide a general description of the membranes used in the method of manufacturing process (e.g., ultrafiltration or microfiltration membranes).

The membranes provide two levels of ultrafiltration. The first level of ultrafiltration removes non-protein components, and the second level is a partial removal of water (i.e., further concentration of the product prior to spray-drying). The membranes comply with the requirements of 21 CFR 177.2910 ("Ultra-filtration membranes").

b. Please provide a statement that all filtration materials complying with applicable U.S. regulations, are GRAS for their intended use or are the subject of an effective food contact notification and are suitable for their intended use.

Arla confirms that all filtration materials complying with applicable U.S. regulations, are GRAS for their intended use or are the subject of an effective food contact notification and are suitable for their intended use.

6. The specification for arsenic is <0.2 mg/kg and the results of the batch analyses range from <0.1 mg/kg to <0.001 mg/kg, with the most recent batch analyses below 0.01 mg/kg. We request that specifications for ingredients be as low as possible, as highlighted in FDA's Closer to Zero[4] approach, and reflect the results of the batch analyses for an ingredient produced in accordance with current good manufacturing practices. Please lower the specifications for arsenic to reflect the results of the batch analyses.

Arla agrees to lower the specification for arsenic from 0.2 mg/kg to ≤0.1 mg/kg. Arla is not able to lower the specification any further due to the LOD for the analysis being only 10x lower (0.01). Additional data regarding arsenic levels are provided in the response to Question 7 below.

7. In Table 2 (pp. 10-11) summarizing food-grade specifications, please provide additional information about the methods used to support the specifications:

a. Please clarify the years associated with the cited ISO methods, where not provided in Table 2.

[4] https://www.fda.gov/food/environmental-contaminants-food/closer-zero-reducing-childhood-exposure-contaminants-foods#:~:text=Closer%20to%20Zero%20uses%20a,of%20a%20contaminant%20is%20unavoidable.

b. For the analyses of sodium, phosphorous, calcium, and potassium, does ICP refer to ICP-MS? Please provide a complete citation and confirm that the method(s) are validated for their intended use.

The data submitted with GRN 1100 contained data from "historical" batches going back several years prior to submission. Therefore, Arla is unable to answer these questions. That said, **Table 1** below contains batch data from five recent production batches, and includes the correct and complete methods of analysis. **Table 2** provides data for heavy metal parameters, but these data are not from the same five batches as the physiochemical and microbiological parameters. The data provided in Tables 1 and 2 directly respond to Questions 7a and 7b.

Table 1: Physiochemical and microbiological batch analysis data

Parameter	Spec	Method of analysis	Lacprodan® Alpha-10 batches				
			1	2	3	4	5
Protein (%)	81-87	ISO 8968-3:2007/IDF 20-3:2007	83	84	83.5	83.5	84
ALA % of protein	≥41.0	HPLC (in-house; validated)	54.8	51.2	49.1	52.1	58.3
Ash (%)	≤5.0	NMKL 173:2005	3.3	3.4	3.4	3.5	3.3
Moisture (%)	≤5.5	ISO 6731:2010(E)/IDF 21:2010(E)	4.7	4.4	4.5	4.6	4.5
Lactose (%)	≤10.0	DS/ISO 5765-2:2002, IDF 79-2:2002	8.5	8	8.5	8.5	8.5
Fat (%)	≤2.0	ISO 1736:2008, IDF 9:2008	0.1	0.1	0.5	0.1	0.1
Sodium (%)	≤0.45	AOAC 984.27, modified	0.37	0.39	0.35	0.32	0.37
Chloride (%)	≤0.20	ISO 5942:2006 / IDF 88:2006	0.07	0.05	0.05	0.05	0.07
Phosphorus (%)	≤0.40	AOAC 984.27, modified	0.22	0.22	0.22	0.21	0.20
Calcium (%)	≤0.60	AOAC 984.27, modified	0.47	0.45	0.45	0.47	0.48
Potassium (%)	≤0.90	AOAC 984.27, modified	0.55	0.59	0.58	0.55	0.53

TPC 30ºC (CFU/g)	≤10,000	ISO 4833-1:2013/A1:2022, modified	<100	20	300	20	200
Mold/yeast (CFU/g)	<10	ISO 6611:2004(E), IDF 94:2004(E), modified	<10	<10	<10	<10	<10
B. cereus (CFU/g)	<50	EN ISO 7932:2005E	<10	<10	<10	<10	<10
Enterobacteriaceae (in 10 x 10g)	<10	ISO 21528-1:2017	Absent	Absent	Absent	Absent	Absent
S. aureus (in 1g)	Absent	ISO 6888-3:2003	Absent	Absent	Absent	Absent	Absent
Salmonella (in 250g)	Absent	ISO 6579-1:2017	Absent	Absent	Absent	Absent	Absent

Table 2: Heavy metal batch analysis data

Parameter	**Spec**	**Method of analysis**	**Lacprodan® Alpha-10 batches**				
			A	**B**	**C**	**D**	**E**
Arsenic (ug/kg)	<0.1	ICP-HRMS ISO 17294m:2016	<10	<10	<10	<10	<10
Cadmium (ug/kg)	<0.05	ICP-HRMS ISO 17294m:2016	<0.6	<0.6	<0.6	1.5	2.3
Lead (ug/kg)	<0.05	ICP-HRMS ISO 17294m:2016	<3	<3	<3	7.5	8.3
Mercury (ug/kg)	<0.05	ICP-HRMS ISO 17294m:2016	<0.005	<0.005	<0.005	<0.005	<0.005

8. In the intended conditions of use (Table 4, p. 14), please clarify:

a. Are nutrition bars and meal replacement bars the only food included in the category of "protein-enriched baked products", or are foods such as breads and cakes also included?

The category "protein-enriched baked products" is also intended to cover protein bars, crackers that are usually used as snacks, and cookies.

b. The serving sizes cited in Table 4 (p. 14) appear to be for ages 4 years and above only. If the intended uses include infant and toddler foods, please clearly state these uses and indicate the intended use levels in g/100 g of these foods.

Arla confirms that the target population is for ages 4 years and above only.

c. The foods described as "fruit juice drinks" are typically water-based and do not contain protein. Please provide examples of foods that would fall within this category. Given that the intended uses are substitutional for other sources of milk protein, this category may be better described by a term other than "fruit juice drinks". Do the terms fruit-flavored smoothies and fruit-flavored protein drinks cover these uses?

The category "fruit-juice based beverages" is intended to cover both fruit-juice based beverages with protein and fruit-flavored water-based beverages, including fruit-flavored smoothies, shakes, and protein drinks.

9. Although Arla provides a summary of fluid milk consumption, other sources of milk protein are not discussed. The notice does not include an estimate of dietary exposure to ALA from the background intake of milk products and milk-based ingredients (e.g., ALA, milk protein concentrate, whey protein concentrate). Regarding the estimates of dietary exposure (pp. 14-15), we request that Arla clarify or correct the following:

a. Arla states that fractionated whey protein is intended to be used as a protein source that will replace other dairy-based protein sources containing ALA.

i. Please confirm that the use of fractionated WPC (41% ALA) will not result in an overall increase in the estimated consumption of total dietary protein.

Arla confirms that the use of Arla's ALA ingredient will not increase the total exposure to protein in the diet. ALA will be used as a replacement for other protein sources that could be used in the listed use categories.

ii. Clarify if the intended uses include only milk-based drinks (e.g., meal replacements, nutritional beverages) and bars, dairy foods (e.g., flavored milk, yogurt, yogurt drinks) and beverage powders.

The intended uses include protein-enriched baked products (e.g., protein bars, crackers, cookies), nutrition bars, milk-based drinks (e.g., meal replacements, nutritional beverages), dairy foods (e.g., flavored milk, sports drinks, yogurt, yogurt drinks), fruit-juice based beverages (e.g., fruit-juice based beverages with protein, fruit-flavored water-based beverages), and beverage powders.

iii. Clarify if the uses of fractionated WPC (41% ALA) are intended to substitute or partially substitute for whey protein isolate and whey protein concentrate, or other ALA-containing ingredients (e.g., the subject of GRN 000763). We note that, except for new uses in yogurt and protein-enriched water or fruit juice beverages, and the increased level (30% vs. 25%) in meal replacement and nutrition bars, the intended uses seem partially substitutional for the intended uses listed in GRN 000763.

Arla confirms that Arla's ALA ingredient is intended to substitute or partially substitute for whey protein isolate and/or concentrate ingredients in the listed use categories and use levels.

iv. For the record, please provide a comparison (in table form) of the amino acid profile of fractionated WPC (41%) ALA with that of whey protein or other milk protein that the fractionated WPC (41%) ALA will replace in the diet.

Please find **Table 3** below which consists of amino acid distributions of whey and whey protein concentrate ingredients that are publicly available. As indicated by the data provided below, the amino acid distribution of ALA here is very comparable to the amino acid distributions found in other whey and whey concentrate products.

Table 3: Whey and whey protein concentrate amino acid distributions

Amino Acid	**ALA (3 Batch Average) g/100g**	**Whey[5]**	**WPC[6]**
Alanine	3.5	3.5	3.1
Arginine	1.3	2.3	1.6
Aspartic acid	11.8	8.4	14.1
Glutamic acid	15.3	13.3	17.4

[5] Banaszek *et al.* (2019) The effects of whey vs. pea protein on physical adaptations following 8-weeks of high-intensity functional training (HIFT): a pilot study. *Sports*. 7(12) doi:10.3390/sports7010012

[6] GRN 809 Fractionated whey protein concentrate containing 41% alpha-lactalbumin

Glycine	1.8	1.4	2.1
Histidine	1.8	1.6	2.1
Isoleucine	6.2	4.6	7.1
Leucine	8.5	8.8	9.9
Lysine	8.8	7.5	10.1
Phenylalanine	2.9	2.6	3.4
Proline	5.0	6.6	5.8
Serine	4.6	4.6	5.6
Threonine	6.9	4.5	7.9
Tyrosine	2.6	2.3	3.2
Valine	5.0	4.4	5.7
Cysteine	2.9	1.7	3.2
Methionine	1.5	1.6	1.7
Tryptophan	2.4	1.3	2.7

b. From the estimates of dietary intake of milk products (fluid milk, dairy foods, frozen dairy desserts) and fractionated WPC (41% ALA), please provide a cumulative estimate dietary exposure for ALA in the US diet. We note that, since the level of ALA is consistently above 41%, assuming a level of 41% ALA would underestimate dietary exposure to ALA, and it would be more appropriate to assume the average level of ALA in the ingredient or the upper level of ALA in the ingredient.

A conservative overestimation of exposure to protein from milk, including fluid milk, desserts, WPC, etc., can be derived from publicly available literature describing protein intakes based on NHANES 2011-2014 data.[7] Pikosky *et al.* report a mean dietary exposure to protein for adults (19+) of 83.6 g/day, with 2/3rds of this total coming from animal protein (which includes protein from all dairy sources). A conservative assumption that all animal protein is dairy protein would yield a total dietary exposure to dairy protein of 55.7 g/day. This is an extreme overestimation as this assumes a "worst case" exposure scenario where all animal protein consumed is dairy in origin. This exposure can be further refined to ALA exposure only by using the average ALA content of Arla's product of 53.1% (average from 5 batches reported above) and applying this to the conservative dairy protein exposure calculated above to give an upper bound ALA exposure in the diet of 29.6 g/day. This is an extremely conservative estimate (e.g., assumes all animal protein is dairy and all dairy contains ALA at the same level as Arla's concentrated ALA ingredient). A conservative 90th percentile exposure estimate can be made by doubling the mean exposure, or 59.2 g ALA/day.

[7] Pikosky *et al.* (2022) Association of dietary protein intake and grip strength among adults aged 19+ years: NHANES 2011-2014 analysis. *Front Nutr.* 9 doi.org/10.3389.fnut.2022.873512

10. For some human studies cited in Table 8, the amount of ALA consumed is mentioned but for many others the information is missing. Please provide the amount of ALA consumed in the studies where it is missing.

Table 4 revises, in part, GRN 1100's Table 8. "Studies of Alpha-Lactalbumin in Adults" and now lists the amount of ALA consumed for the following four studies: Nieuwen-huizen *et al*. 2009, Qin *et al.* 2017, Qin *et al*. 2019, and Veldhorst *et al*. 2009. This information, which was missing from GRN 1100's Table 8, is highlighted in green.

Table 4: Revised Portion of "Table 8. Studies of Alpha-Lactalbumin in Adults"

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Duration	Safety-Related Results
Nieuwen-huizen *et al*. 2009	Prospective, randomized, single-blind crossover study of the effect of α-lactalbumin on satiety	24 apparently healthy adults, 11M and 13F, aged 19-37 years (mean age = 21±0.8 years)	10%-protein breakfasts with α-lactalbumin, gelatin, or gelatin +tryptophan; approximate 13 g ALA exposure	Single dose	No adverse effects were reported.
Qin *et al.* 2017	Prospective, randomized, double-blind crossover study comparing the effects of α-lactalbumin and total whey protein on muscle damage, muscle pain, and mood states after prolonged strenuous exercise	12 apparently healthy male endurance runners aged 30.4±2.8 years	Whey isolate or α-lactalbumin consumed at a rate of 0.34 g/kg bw/hour; approximate 40g ALA exposure	Single dose	No adverse effects were reported.
Qin *et al*. 2019	Prospective, randomized, double-blind	11 apparently healthy	α-lactalbumin or whey	Single dose	No adverse effects attributable to α-

	crossover trial of α-lactalbumin or whey protein isolate before a 21-kilometer run	male endurance runners aged 31±2 years	protein isolate; approximate 1.3 g/kg ALA exposure		lactalbumin or whey isolate were reported, and the authors concluded that, "compared with the pre-exercise ingestion of whey protein, that of α-lactalbumin led to superior results during similar levels of endurance exercise."
Veldhorst *et al*. 2009	Prospective, randomized, single-blind crossover satiety study	24 apparently healthy subjects, 10M and 14F, aged 18-45 years (mean age = 25±2 years),	Breakfast with α-lactalbumin, gelatin, casein, soy, whey, glycomacro-peptide-depleted whey, or gelatin+ tryptophan providing either 10% or 25% of the energy; approximate 32g ALA for 25% diets	Single dose	No adverse effects were reported.

11. For the Zhi et al. (2011) subchronic animal study on page 22, please provide the following information:

a. Administered oral doses as mg or g ALA/kg body weight (bw)/d.

b. Express the NOAEL in the same unit (mg or g ALA/kg bw/d).

c. From the NOAEL, derive the ADI in the same unit (mg or g ALA/kg bw/d) as well as mg or g/person/day, and compare the ADI with the proposed 90th percentile EDI.

The reference Zhi *et al.* is in Chinese (other than the abstract) and we have been unable to secure a translated copy given the time provided. That said, using the information given in the abstract and default values for food consumption in rats,[8] we can calculate the administered doses, NOAEL, and ADI.

Zhi *et al.* describes a study utilizing Wistar rats containing 0, 15, 30, or 60% ALA in the diet. The ALA groups consisted of a group which received non-transgenic human ALA and a group administered transgenic human ALA. Using the default assumption of 0.09 for rats in a 90 day (subchronic) study as described by Zhi. For example, a diet containing a test article at 1 mg/kg in feed (1 ppm or 0.0001%) would equate to a dose of 0.09 mg/kg of the test article in the diet. Using this to calculate ALA exposure in the high dose group (60% or 600,000 ppm) would equate to an exposure of 54,000 mg/kg bw/day (600,000 ppm * 0.09= 54,000) or 54 g/kg bw/day. The authors report no adverse effects were observed at any dose, therefore the NOAEL for the study is the high dose, or 54 g/kg bw/day. A human ADI can be derived from the NOAEL by applying an uncertainty factor (UF) to account for differences between rats and humans. A typical UF of 100 can be applied (10x for interspecies extrapolation and 10x for intraspecies extrapolation) would yield an ADI of 0.54 g/kg bw/day. Assuming a typical adult body weight of 70 kg, this would equate to an ADI of 37.8 g ALA/day. This ADI is higher than the calculated mean EDI above of 29.6 g/day but is lower than the 90th percentile EDI of 59.2 g/day.

We again note that the calculated mean and 90th percentile EDI from above is extremely conservative, and overestimates ALA exposure using several "worst case" assumptions as described above. Also, deriving an ADI for macro ingredients (e.g., proteins) from animal studies is notoriously difficult given restrictions on feed consumption in animals and required application of UFs. Even given these limitations, the calculated ADI is higher than the mean EDI. Further, the NOAEL from the Zhi study was the maximum dose tested, indicating a potentially higher NOAEL than can be calculated from the data provided.

12. Tryptophan is an essential amino acid whose requirement decreases with age. The requirement for tryptophan in adults is the lowest of all age groups. The tryptophan content of ALA is the highest among many edible proteins, and it is further enriched through the enrichment of the ALA fraction in the GRAS ingredient. Tryptophan is

[8] EFSA (2012) Scientific Opinion: Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. *EFSA Journal* 10(3):2579

also a precursor of the neurotransmitter serotonin. With this background, please provide the following information:

a. Cite the background daily exposure and the upper safe limit of exposure to tryptophan in adults (from the literature) and confirm that the proposed 90th percentile tryptophan intake through ALA is within the limit of safe exposure.

b. Please affirm with a brief discussion that there is reasonable certainty that an exposure to higher levels of tryptophan in adults from consuming the proposed ALA-enriched products do not lead to effects or outcomes that are aberrant or harmful in healthy individuals. Please include in this discussion the following points and cite relevant references to support your response.

i. Indicate whether an increased exposure to tryptophan through consumption of the ALA-enriched GRAS ingredient can lead to an increased tryptophan accumulation in cells including in neurons, or is the excess tryptophan metabolized to maintain the homeostasis of the intracellular amino acid pool.

ii. If there is an increased tryptophan accumulation in cells including in neurons, then please discuss whether the intracellular tryptophan levels are marginally or significantly elevated, and whether there will be more serotonin synthesis in neurons.

iii. In the event of a significantly increased serotonin accumulation in neurons (from increased tryptophan levels), please discuss why it will not have or lead to any aberrant effects of concern on healthy individuals.

Elango 2023 reports on both background (i.e., current consumption) of individual amino acids derived from European consumers (EPIC 2022) and US consumers (NHANES 2005).[9] Mean and 75th percentile exposures to tryptophan based on EPIC 2022 data are 0.8 and 1.0 g/day respectively, while mean and 99th percentile exposures based on NHANES 2005 data are 1.0 and 2.1 g/day respectively. From data provided above, the average tryptophan content of Arla's ALA ingredient is 2.4 g/100g. This combined with the above calculated 90th percentile exposure to Arla's ALA ingredient of 59.2 g/day would equate to a tryptophan exposure of approximately 1.4 g Trp/day (2.4 g Trp/100g protein * 0.592) which is well within the range of background exposure to tryptophan as reported by Elango of 1.0 g/day at the mean and 2.1 g/day for the 99th percentile for the US population. As discussed, the calculated ALA exposure is extremely conservative and includes 66% (as animal protein) of all

[9] Elango (2023) Tolerable upper intake level for individual amino acids in humans: a narrative review of recent clinical studies. *Advances in Nutrition*. 14(4): 885-894

protein consumed assumed to be ALA for this purpose. Even though this calculation does not include potential exposure to Trp from plant proteins, Trp is typically present at lower levels in proteins derived from plants than proteins derived from animals.

Further information available in the public literature gives a tolerable upper intake limit for tryptophan of 4.5 g/d.[10] Given that the tolerable upper limit for tryptophan is more than 3x that expected from Arla's ALA ingredient, even given the extremely conservative approach to exposure calculations employed here, there is no reason to suspect that Arla's ALA ingredient would cause adverse effects due to tryptophan content. We can therefore state with (more than) reasonable certainty that exposure to tryptophan from Arla's ALA ingredient would not lead to increased tryptophan in cells and any increased exposure that did occur would be marginal and expected to be metabolized to maintain homeostatsis.

13. **Please provide updated information on the literature search(es) performed to prepare the notice. This includes the date(s) (e.g., month and year) of the search(es), the resource database(s) used (e.g., PubMed), the principal search terms used, and the time period that the search spanned (e.g., 1/2000 to 3/2023).**

The literature search for GRN 1100 was performed in May 2022. We have conducted an updated literature search in July 2023 using the following search databases: PubMed, ToxPlanet, Google Scolar, and SciFinder. Search terms were (and/or): alpha-lactalbuimin, ALA, whey, whey protein, toxicology, adverse effects, whey consumption, milk consumption. No additional relevant studies were identified in the July 2023 search.

* * *

We appreciate the Agency's review of GRAS Notice 1100. Please let us know if you have any other questions or if you need any additional information.

Cordially yours,

Frederick A. Stearns

[10] Hiratsuka *et al.* (2013) Supplementing healthy women with up to 5.0 g/d of L-tryptophan has no adverse effects. *J Nutr*. 143(6):859-866; Cynober *et al.* (2016) Proposals for upper limits of safe intake for arginine and tryptophan in young adults and an upper limit of safe intake for leucine in the elderly. *J Nutr*. 146(12):2652S-2654S.

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August 15, 2023

Via Electronic Mail

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Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
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Re: Response to FDA's Second Set of Questions for GRAS Notice No. 001100

Dear Dr. Deng:

On behalf of Arla Food Ingredients P/S (Arla), we are writing in response to FDA's second set of questions (received on August 4, 2023) regarding GRAS Notice No. 001100 for fractionated whey-derived protein concentrate containing ≥41% alpha-lactalbumin (fractionated WPC (41% ALA)). For ease of reference, we reproduce each question below, followed by Arla's response.

1. **The units for heavy metals in Table 2 (p. 5) of the amendment dated July 19, 2023 are given as ug/kg (presumed to be µg/kg). While these units appear correct for the results of the batch analyses, they are incorrect for the specifications which, as previously stated, are in units of mg/kg. We request that the notifier provide a corrected Table 2 for the record where the units reflect both the results and specifications.**

As requested, we converted the data for arsenic, cadmium, and lead from µg/kg to mg/kg. Mercury did not need to be converted because the data were already declared in terms of mg/kg, but the unit was incorrectly listed as µg/kg. A corrected Table 2 (updated from the July 19, 2023 amendment) is provided below.

CORRECTED Table 2: Heavy metal batch analysis data

Parameter	Spec	Method of analysis	Lacprodan® Alpha-10 batches				
			A	B	C	D	E
Arsenic (mg/kg)	<0.1	ICP-HRMS ISO 17294m:2016	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (mg/kg)	<0.05	ICP-HRMS ISO 17294m:2016	<0.0006	<0.0006	<0.0006	0.0015	0.0023
Lead (mg/kg)	<0.05	ICP-HRMS ISO 17294m:2016	<0.003	<0.003	<0.003	0.0075	0.0083
Mercury (mg/kg)	<0.05	ICP-HRMS ISO 17294m:2016	<0.005	<0.005	<0.005	<0.005	<0.005

2. **The sampling size for ISO 6579-1:2017 method for Salmonella testing is 25 g, as listed in the notice of GRN 001100. We note that a typo of "250 g" is in Table 1 (p. 5) of the amendment date July 19, 2023.**

We confirm that the sampling size for ISO 6579-1:2017 for *Salmonella* is 25g, and not 250g. We have included a corrected Table 1 (updated from the July 19, 2023 amendment) below.

CORRECTED Table 1: Physiochemical and microbiological batch analysis data

Parameter	Spec	Method of analysis	Lacprodan® Alpha-10 batches				
			1	2	3	4	5
Protein (%)	81-87	ISO 8968-3:2007/IDF 20-3:2007	83	84	83.5	83.5	84
ALA % of protein	≥41.0	HPLC (in-house; validated)	54.8	51.2	49.1	52.1	58.3
Ash (%)	≤5.0	NMKL 173:2005	3.3	3.4	3.4	3.5	3.3
Moisture (%)	≤5.5	ISO 6731:2010(E)/IDF 21:2010(E)	4.7	4.4	4.5	4.6	4.5
Lactose (%)	≤10.0	DS/ISO 5765-2:2002, IDF 79-2:2002	8.5	8	8.5	8.5	8.5
Fat (%)	≤2.0	ISO 1736:2008, IDF 9:2008	0.1	0.1	0.5	0.1	0.1

Sodium (%)	≤0.45	AOAC 984.27, modified	0.37	0.39	0.35	0.32	0.37
Chloride (%)	≤0.20	ISO 5942:2006 / IDF 88:2006	0.07	0.05	0.05	0.05	0.07
Phosphorus (%)	≤0.40	AOAC 984.27, modified	0.22	0.22	0.22	0.21	0.20
Calcium (%)	≤0.60	AOAC 984.27, modified	0.47	0.45	0.45	0.47	0.48
Potassium (%)	≤0.90	AOAC 984.27, modified	0.55	0.59	0.58	0.55	0.53
TPC 30ºC (CFU/g)	≤10,000	ISO 4833-1:2013/A1:2022, modified	<100	20	300	20	200
Mold/yeast (CFU/g)	<10	ISO 6611:2004(E), IDF 94:2004(E), modified	<10	<10	<10	<10	<10
B. cereus (CFU/g)	<50	EN ISO 7932:2005E	<10	<10	<10	<10	<10
Enterobacteriaceae (in 10 x 10g)	<10	ISO 21528-1:2017	Absent	Absent	Absent	Absent	Absent
S. aureus (in 1g)	Absent	ISO 6888-3:2003	Absent	Absent	Absent	Absent	Absent
Salmonella (in 25g)	Absent	ISO 6579-1:2017	Absent	Absent	Absent	Absent	Absent

* * *

We appreciate the Agency's continued review of GRAS Notice 1100. Please let us know if you have any other questions or if you need any additional information.

Cordially yours,

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September 28, 2023

Via Electronic Mail

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Re: Response to FDA's Third Set of Questions for GRAS Notice No. 001100

Dear Dr. Deng:

On behalf of Arla Food Ingredients P/S (Arla), we are writing in response to FDA's third set of questions regarding GRAS Notice No. 001100 for fractionated whey-derived protein concentrate containing ≥41% alpha-lactalbumin (fractionated WPC (41% ALA)). For ease of reference, we reproduce each question below, followed by Arla's response.

1. **From the information provided in GRN 001100 and the July 19, 2023, amendment, we have compiled a table of maximum use levels for fractionated WPC (41% ALA).**

Table 1: Maximum use level of fractionated WPC (41% ALA) in specified food categories.

Food category	Maximum use level (g/100 g)
Nutrition bars including meal replacement and "protein" bars	30
Crackers primarily used as snacks	30
Cookies	30
Milk-based nutritional beverages including meal replacements and meal supplements	5
Flavored milk drinks, ready to drink (RTD) and prepared from powder	4
Sports drinks	4
Enhanced or fortified waters	4
Fruit juice drinks and smoothies	4
Yogurt (dairy and non-dairy)	5
Fermented milk drinks (e.g., yogurt drinks, kefir)	5
Buttermilk	5
Beverage powders	5

We have highlighted points of ambiguity in this table referred to in the questions below. For clarity of the record, we request that you address the following:

Given the overlap in foods within the categories of protein-enriched milks (herein termed "flavored milks") and "milk-based nutritional beverages", please clarify the distinction between these categories or indicate a single maximum value (e.g., 5%) that applies to both categories.

Arla acknowledges the overlapping characteristics between the categories "Milk-based nutritional beverages including meal replacements and meal supplements" and "Flavored milk drinks, ready to drink (RTD) and prepared from powder." Therefore, Arla has combined the two into a single food category, entitled "Flavored milk-based beverages including meal replacements and meal supplements," with a maximum use level of 5g/100g. Reproduced below is a revised version of GRN 1100's Table 4. "Intended Use of Fractionated Whey Protein Concentrate Containing 41% Alpha-Lacatalbumin (in addition to Infant-Formula Use Described in GRAS Notice 809)." Revisions are provided in red text.

REVISED Table 4. Intended Use of Fractionated Whey Protein Concentrate Containing 41% Alpha-Lacatalbumin (in addition to Infant-Formula Use Described in GRAS Notice 809)

Food/Beverage Category	NHANES Description	Serving Size	Max Whey Protein Concentrate Addition (g)	Max Whey Protein Concentrate Addition (%)	Max Alpha-Lactalbumin Addition (g)
Protein-enriched baked products; nutrition bars	All nutrition or meal replacement bars	40 g	12 g	30%	4.9 g
Flavored milk-based beverages including meal replacements and meal supplements	All nutritional beverages, RTD flavored milk, and all sport drinks	240-360 ml	12 g	5%	4.9 g
Yogurt	All yogurts, including regular, Greek, and non-dairy yogurts	170 g	8.5 g	5%	3.5 g

Fermented milk drinks, drinkable yogurts	Buttermilk, kefir, liquid yogurt	240 g	12 g	5%	4.9 g
Other nutritional beverages & powders	Non-reconstituted protein and nutritional powders	Amount to make 240 ml	12 g	5% (as consumed)	4.9 g

2. **For "non-reconstituted protein and nutritional powders" (herein referred to as beverage powders), the reference amount customarily consumed (RACC) is given as the amount (of powder) to make 240 mL. We note that, as written, the use level appears to be expressed for the powder form. If the intended use is in the powder and is concentrated (in proportion to the ratio of liquid to powder) to provide up to 5% of the ingredient in the beverage "as consumed" or reconstituted, please clearly state this for the record.**

We confirm that the 5% intended use level for the "non-reconstituted protein and nutritional powders" is "as consumed." We have added the "as consumed" qualifier to the REVISED Table 4 above.

* * *

We appreciate the Agency's continued review of GRAS Notice 1100. Please let us know if you have any other questions or if you need any additional information.

Cordially yours,

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October 12, 2023

Via Electronic Mail

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Re: Response to FDA's Fourth Set of Questions for GRAS Notice No. 001100

Dear Dr. Deng:

On behalf of Arla Food Ingredients P/S (Arla), we are writing in response to FDA's fourth set of questions regarding GRAS Notice No. 001100 for fractionated whey-derived protein concentrate containing ≥41% alpha-lacatalbumin (fractionated WPC (41% ALA)). For ease of reference, we reproduced your question below, followed by Arla's response.

QUESTION:

In your response to FDA's third set of questions dated 9/28/2023, you have grouped several beverages in a single category "All nutritional beverages, RTD flavored milk, and all sports drinks" with a maximum use level of 5% (w/w) as consumed. In the "Revised Table 4" (provided in the amendment), you no longer include "enhanced or fortified waters", "fruit juice drinks", or "fruit smoothies". Further, the categories of "crackers primarily used as snacks" and "cookies" are no longer included. Please clarify if these omissions were intentional. We have captured our understanding of the intended uses in Table 4 (rev. 2) below, based on the totality of the amendments you have provided. Please confirm if Table 4 (rev. 2) as shown herein correctly reflects the proposed uses in GRN 001100. If particular food categories, listed below, are now omitted from the intended uses, please clearly state this for the record.

Table 4 (rev. 2): Maximum use level of fractionated WPC (41% ALA) in specified food categories.

Food category	Maximum use level (g/100g)
Nutrition bars including meal replacement and "protein" bars	30
Crackers primarily used as snacks	30
Cookies	30
Dairy-based beverages, ready-to-drink (RTD) or prepared from powder, including the following: flavored and/or fermented milks, buttermilk, nutritional beverages (e.g., meal replacements, meal supplements), milk shakes and other dairy drinks, and smoothies	5
Sports drinks, RTD or prepared from powder	5
Enhanced or fortified waters, RTD or prepared from powder	5
Fruit juice drinks and smoothies, RTD or prepared from powder	5
Yogurt (dairy and non-dairy)	5

Arla confirms that Table 4 (rev. 2), reproduced above, is correct and reflects the proposed uses in GRAS Notice 1100.

* * *

We appreciate the Agency's continued review of GRAS Notice 1100. Please let us know if you have any other questions or if you need any additional information.

Cordially yours,

Frederick A. Stearns