JHeimbach LLC

#909

January 23, 2020

Susan J. Carlson, Ph.D., Director
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Carlson:

Pursuant to 21 CFR Part 170, Subpart E, Arla Foods Ingredients Group P/S (Arla), through me as its agent, hereby provides notice of a claim that the addition of fractionated whey protein concentrate containing 41% alpha-lactalbumin to conventional foods is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Arla has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

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

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

Sincerely,

[redacted]

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

Encl.

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

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

Prepared by:
JHeimbach LLC
Port Royal Virginia

September, 2019

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Part 1: Signed Statements and Certification

1.1. GRAS Notice Submission

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

1.2. Name and Address of Notifier

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1.3. Name of Notified Substance

The subject of this Generally Recognized as Safe (GRAS) 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. GRN 000809, 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 GRN 000809 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. For that reason, and to avoid unnecessary redundancy, as is discussed further below, the entirety of GRN 000809 (with the exception of the section on intended use) is incorporated by reference in the current GRAS notice.

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 addition to conventional foods as described in 21 CFR §170.3(o)(20) to increase the dietary intake of the whey protein fraction alpha-lactalbumin

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 CFR §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 the deliberations of an Expert Panel consisting of Ronald E. Kleinman, M.D., Berthold V. Koletzko, M.D., Ph.D., and John A. Thomas, Ph.D., who reviewed a monograph prepared by James T. Heimbach, Ph.D., as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food ingredients. They independently critically reviewed and evaluated the publicly available information and the potential human exposure to fractionated whey protein concentrate containing 41% alpha-lactalbumin anticipated to result from its intended use, and individually and collectively determined that no evidence exists in the available information on fractionated whey protein concentrate containing 41% alpha-lactalbumin that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of fractionated whey protein concentrate containing 41% alpha-lactalbumin.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion regarding the safety of the substance under its intended conditions of use. Therefore, the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin is GRAS by scientific procedures.

1.6. Premarket Exempt Status

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.

Fractionated whey protein concentrate containing 41% alpha-lactalbumin is grandfathered under the definition of whey protein concentrate provided in 21 CFR §184.1979c[1]. Under 21 CFR 184.1979c, whey protein concentrate is produced from whey using physical separation techniques that remove sufficient non-protein constituents from whey so that the finished dry product contains not less than 25 per cent protein. In GRAS Affirmation Petition (GRP) 1G0371, the American Dairy Products Institute (ADPI) cited the final rule that affirmed the GRAS status of whey protein concentrate: "The agency does not intend to limit the processing methods that may be used. Furthermore, the Agency has no objection to the use of newly developed physical separation techniques, if there are no new toxicants introduced as a result of these techniques, and if these techniques do not result in a concentration of natural toxicants in whey products. FDA believes that such results can be avoided by the use of good manufacturing practices and by the establishment of specifications for heavy metals" (September 4, 1981; 46 FR 44435 at 44437). GRN 000809, Sections 2.2.3 and 2.2.4 (pages 12-14), which is incorporated in this GRAS notice by reference, describes the manufacturing process that introduces no new toxicants as well as providing the ALPHA-10 specification including that for trace metals and microbiology.

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

1.7. Data Availability

The data and information that serve as the basis for the conclusion that fractionated whey protein concentrate containing 41% alpha-lactalbumin is GRAS for its intended use will be made available to the FDA upon request. At FDA's option, a complete copy of the information will be sent to FDA in either paper or electronic format, or the information will be available for review at the home office of JHeimbach LLC, located at 923 Water Street, Port Royal VA 22535, during normal business hours.

1.8. Freedom of Information Act Statement

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

1.9. Certification

To the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin.

1.10 FSIS Statement

Not applicable.

1.11. Name, Position and Signature of Notifier

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

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

2.1. Name of the GRAS Substance

The notified substance is 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

Extensive information regarding the source, description, manufacture, and specifications of fractionated whey protein concentrate containing 41% alpha-lactalbumin is contained in GRN 000809, Section 2.2 9 (pages 9-14), which is incorporated by reference. Responses to questions asked by FDA during its review of the GRAS notice are also incorporated by reference and attached as an appendix to this monograph..

2.3. Stability

Extensive information regarding the stability of fractionated whey protein concentrate containing 41% alpha-lactalbumin is contained in GRN 000809, Section 2.3 (pages 15-16), which is incorporated by reference.

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 described in 21 CFR §170.3(o)(20), to increase the dietary intake of the whey protein fraction alpha-lactalbumin.

Part 3: Dietary Exposure

3.1. Intended Conditions of Use

Fractionated whey protein concentrate containing 41% alpha-lactalbumin is intended for addition to the food categories listed in Table 1 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 1. Intended Use of Fractionated Whey Protein Concentrate Containing 41% Alpha-Lactalbumin (In Addition to Infant-Formula Use Described in GRN 000809).

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

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 2+ years were estimated both per capita and per user and expressed per person and on a bodyweight basis.

Overall, 41.3% of NHANES respondents consumed one or more of the intended-use food categories on at least one of the two survey days. The 90th percentile intake of fractionated whey protein concentrate containing 41% alpha-lactalbumin was 21.5 g/day, equivalent to 0.43 g/kg bw/day. Intake of alpha-lactalbumin is 41% of that of fractionated whey protein concentrate, or 8.8 g/day, equivalent to 0.18 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 of ingested alpha-lactalbumin have been studied in human infants, human adults, and several animal models. The published research was discussed in GRN 000809, Section 6.1 (pages 23-24), which is incorporated by reference. A review of the recent literature failed to discover any more recent published studies in this area.

6.2. Animal Studies

Studies in rats and rhesus monkeys were described in GRN 000809, Section 6.2 (pages 24-26), which is incorporated by reference.

6.3. Human Studies

6.3.1. Studies in Infants

The published literature regarding alpha-lactalbumin ingestion by infants was extensively reviewed in GRN 000809, Section 6.3.2 (pages 29-63), which is incorporated by reference. A review of the current literature confirmed that there are no recent published infant studies that were not included in GRN 000809.

6.3.2. Studies in Adults

Since GRN 000809 addressed use of alpha-lactalbumin in infant formula, the relatively small number of available studies in adult humans, generally evaluating the effects of single-dose administration of alpha-lactalbumin on central nervous system function, were dealt with only cursorily. Additionally, a number of adult studies have appeared in the literature since GRN 000809 was prepared; both earlier and recent studies in adults are addressed in this section.

Artym and Zimecki (2013) observed that there is increasing interest in applications of milk-derived proteins and peptides, especially alpha-lactalbumin but also glycomacropeptide, lactoperoxidase, and casein derivatives, and Hill and Newburg (2015) reviewed clinical applications of bioactive milk components including immunoglobulins, lactoferrin, oligo- and polysaccharides, transforming growth factor β, and casein peptides as well as alpha-lactalbumin. They noted that, "a significant advantage of milk-derived bioactive components over other pharmaceutical products is the generally excellent tolerability of milk compounds," citing the "remarkably safe side-effect profile of many milk-derived compounds."

A similar review of milk-derived proteins and peptides by Lonnerdal (2016) focused on alpha-lactalbumin, lactoferrin, lysozyme, secretory IgA, bile-salt-stimulated lipase, milkfat-globule-membrane proteins, β-casein, κ-casein, and osteopontin, suggesting that one or more of these components may contribute to the advantages of breast milk over formulas. A more recent article, Layman et al. 2018, focused on research on alpha-lactalbumin, reaching extremely laudatory conclusions:

> "α-Lactalbumin is an attractive protein, both because of its physical characteristics, which include a clean flavor profile, high water solubility, and heat stability (which combined allow for diverse food applications), and because of its biological properties derived from its protein quality, which create the potential for diverse nutrition applications. The most frequently studied uses for α-lactalbumin are as a component of infant formulas designed to be more similar to breast milk, and as supplement to enhance sleep or improve mood in adults. However, potential new applications in cancer treatment or to enhance immune response have also garnered interest. To date, most uses of α-lactalbumin derive from its unique amino acid composition, with most attention focused on the essential amino acid

> tryptophan, a precursor to the neurotransmitter serotonin. However, α-lactalbumin is also a rich source of the sulfur amino acids, with a highly unusual 5:1 ratio of cysteine to methionine. Moreover, it contains BCAAs, lysine, and potentially bioactive peptides. Because of its amino acid composition and associated biopeptides, α-lactalbumin has potential as a protein supplement to support infant development, promote gastrointestinal function, and protect muscle mass in aging adults" (Layman et al. 2018).

The published studies described in this section are summarized in Table 1 at the end of the section.

Markus et al. (2000) reported on a prospective, randomized, double-blind crossover study of the ability of alpha-lactalbumin to help high- and low-stress-vulnerable adults cope with stress. Enrollees were 29 highly stress-vulnerable subjects (10M and 19F aged 17-34 years, mean age = 20.5±3.1 years) and 29 relatively stress-invulnerable subjects (9M and 20F aged 17-34 years, mean age = 20.9±3.2 years). Group assignment was based on score on the Inadequacy Scale of the Dutch Personality Inventory (IS-DPI). On each of 2 test days 4 weeks apart (to control for females' menstrual cycles), the participants received chocolate drinks containing a total of 40 g whey protein rich in alpha-lactalbumin[2] or 31 g casein. Each subject was exposed to a computer-assisted battery of mental-arithmetic tests during which skin conductance was measured, salivary cortisol samples were taken, blood was drawn for measurement of amino-acid content, pulse rate was recorded, and a mood-state questionnaire was completed.

The ratio of plasma tryptophan to other large neutral amino acids was significantly higher after the alpha-lactalbumin diet than after the casein diet. In stress-vulnerable subjects, this was accompanied by significantly higher prolactin concentrations, decreased cortisol, and reduced depressive feelings under stress. These were regarded as beneficial changes and no adverse effects were reported. The authors concluded that "consumption of a dietary protein enriched in tryptophan … in stress-vulnerable subjects improved coping ability, probably through alterations in brain serotonin."

Stress-vulnerable subjects may suffer loss of cognitive performance under pressure, and a prospective, randomized, double-blind crossover study assessed whether this could be ameliorated by alpha-lactalbumin (Markus et al. 2002). Twenty-three highly stress-vulnerable subjects (10M and 13F aged 17-33 years, mean age = 20±2 years) and 29 relatively stress-invulnerable subjects (13M and 16F aged 17-33 years, mean age = 21±3 years) were enrolled. Group assignment was based on score on the IS-DPI. On each of 2 test days 4 weeks apart (to control for females' menstrual cycles), the participants received chocolate drinks containing a total of 40 g whey protein rich in alpha-lactalbumin (the same whey protein as was used in Markus et al. [2000]) or 31 g casein. Each subject was exposed to a computer-assisted battery of cognitive-performance tests during which blood was drawn for measurement of amino-acid content.

A significantly greater increase in the ratio of plasma tryptophan to other large neutral amino acids was reported after consumption of the alpha-lactalbumin diet than after the control diet; cognitive performance improved significantly only in the high stress–vulnerable subjects. The authors reported no adverse effects and concluded, "the results suggest that dietary protein rich in α-lactalbumin improves cognitive performance in stress-vulnerable subjects via increased brain tryptophan and serotonin activities" (Markus et al. 2002).

Markus et al. (2005) reported a prospective, randomized, double-blind crossover study on the effect of consumption of a whey protein rich in alpha-lactalbumin during the evening on

[2]The whey protein was provided by Borculo Domo Ingredients, but the actual alpha-lactalbumin content was not reported and does not appear to be available in the company's public literature.

alertness and cognitive function the following morning. Apparently healthy university students with and without sleeping difficulties (14 subjects [7M and 7F] in each group with mean age = 22±2.5 years) consumed chocolate drinks containing a total of 40 g whey protein rich in alpha-lactalbumin (the same whey protein as was used in Markus et al. [2000 and 2002]) or 31 g casein before retiring; blood samples were taken 2 hours after consumption of the drinks for amino-acid analysis. In the morning, the subjects completed a sleepiness questionnaire and took a computer-assisted performance task.

Evening alpha-lactalbumin intake resulted in a significant increase in the ratio of plasma tryptophan to other large neutral amino acids before bedtime, significantly reduced sleepiness, and improved brain-sustained attention processes the following morning. No adverse effects were reported and the authors concluded that, "Evening dietary increases in plasma tryptophan availability for uptake into the brain enhance sustained alertness early in the morning after an overnight sleep, most likely because of improved sleep."

In a prospective, randomized, double-blind crossover study reported by Merens et al. (2005), 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) received diets providing 40 g whey protein rich in alpha-lactalbumin or 31 g casein on 2 separate test days. (The whey protein was the same as was used in previous studies by Markus et al. in 2000, 2002, and 2005.) Subjects were given an "impossible mental arithmetic task performed under noise stimulation," and salivary cortisol samples and blood samples were taken.

The stress test affected mood in both conditions. Although the alpha-lactalbumin diet led to the expected rises in plasma tryptophan and the ratio of tryptophan and other large neutral amino acids, only minimal effects were reported on mood and cortisol response to experimental stress. The results were the same for recovered depressed patients and controls. The authors reported that "α-lactalbumin had no side-effects" but concluded that, "A 1-day diet enriched with α-lactalbumin is not sufficient to prevent a stress-induced mood deterioration or a cortisol response in unmedicated, recovered depressed subjects."

Reporting on the same study (Merens et al. 2005), Booij et al. (2006) concluded that "diet enriched with α-lactalbumin enhanced memory irrespective of history of depression."

Sixteen women aged 18-45 years (mean age = 29±2 years) suffering from premenstrual symptoms were enrolled in a prospective, randomized, double-blind crossover trial of the effect of alpha-lactalbumin on memory (Schmitt et al. 2005). On each of 2 premenstrual test days 4 weeks apart, the woman received chocolate drinks containing a total of 40 g whey protein rich in alpha-lactalbumin or 31 g casein. (The whey protein was the same as was used in previous studies by Markus et al. in 2000, 2002, and 2005 and Merens et al. in 2005.) Approximately 4 hours later, they took tests of short- and long-term memory for words and abstract figures. Women performed significantly better on the long-term memory test for abstract figures after ingesting alpha-lactalbumin than after consuming casein; there were no other significant differences. No adverse effects were reported, and the authors concluded that "the cognitive effect of acute premenstrual administration of alpha-lactalbumin… was modest."

In a prospective, randomized, double-blind, placebo-controlled study (Scrutton et al. 2007), 28 apparently healthy women aged 19-45 years (mean age = 26.8±6.6 years) were randomized to receive 40 g alpha-lactalbumin or casein in flavored water (n = 14/group). Blood was drawn and salivary samples taken just before drink administration and after 2.5 hours, when the women took a variety of psychological tests measuring depression, anxiety, and anger; a facial-expression recognition test; a personality profile; and a memory test. Blood was analyzed for neutral amino acids and saliva for cortisol.

Emotional processing and cortisol levels were not different between groups, but alpha-lactalbumin significantly raised blood tryptophan and the ratio of tryptophan to other neutral amino acids. Women receiving alpha-lactalbumin experienced significantly more nausea than those receiving casein, an effect the authors suggested might reflect increased brain serotonin activity. The authors concluded that, "this study suggests limited effects of α-lactalbumin on both tryptophan availability and emotional processing," but also suggested that the modest increase in tryptophan availability resulting from the single dose of alpha-lactalbumin may have been insufficient to produce significant effects.

The effect of alpha-lactalbumin on satiety was determined in a prospective, randomized, single-blind crossover study in which 24 apparently healthy adults, 11M and 13F, aged 19-37 years (mean age = 21±0.8 years) received 10%-protein breakfasts containing alpha-lactalbumin, gelatin, or gelatin+tryptophan as the sole protein source (Nieuwenhuizen et al. 2009). At lunchtime, hunger, glucagon-like peptide-1, ghrelin, and amino acid concentrations were measured, as was the amount of lunch consumed. Plasma tryptophan and suppression of hunger at lunchtime were significantly higher and total amino-acid concentration was lower after the alpha-lactalbumin breakfast than the other meals. Hormones related to satiety, glucagon-like peptide-1 and ghrelin, were not related to the type of breakfast consumed. No adverse effects were reported. The authors concluded that, "the present study shows that a breakfast containing α-lactalbumin as the only protein source results in a more prolonged suppression of hunger than a breakfast containing gelatin."

In a prospective, randomized, single-blind crossover satiety study (Veldhorst et al. 2009) with 24 apparently healthy subjects aged 18-45 years (mean age = 25±2 years), 10M and 14F, energy intake at lunch was evaluated following a breakfast 3 hours earlier containing one of a variety of proteins: alpha-lactalbumin, gelatin, casein, soy, whey, glycomacropeptide-depleted whey, or gelatin+tryptophan providing either 10% or 25% of the energy. The alpha-lactalbumin, gelatin and gelatin+tryptophan breakfasts resulted in significantly reduced energy intake at lunch compared to experimental conditions in which the subjects consumed breakfasts containing the other proteins. No adverse effects were reported, and the authors concluded that, "different proteins (alpha-lactalbumin, gelatin, and gelatin+tryptophan) that are 30–50% more satiating than other proteins (casein, soy, whey, and glycomacropeptide-depleted whey) induce a related 17–24% reduction of subsequent energy intake at the following meal."

In a prospective, randomized, double-blind crossover trial (Verschoor et al. 2010), 13 high- and 12 low-trait anxiety individuals (university students) participated in a 2-way design. The high-anxiety group included 2M and 11F aged 21.5±5.7 years while the low-anxiety group had 1M and 11F aged 21.0±1.7 years.) Participants consumed lunch containing either 40 g alpha-lactalbumin or 40 g casein on 2 consecutive test days; lunch was followed by a stress-inducing mental-arithmetic task. After a one-week washout, the interventions were reversed. Mood, appetite, and food hedonics were assessed each day.

The intervention did not exert any immediate effects on stress, mood, or appetite ratings, but high-trait anxiety individuals receiving alpha-lactalbumin exhibited significantly decreased liking of foods in general and reduced preference for sweet foods. There was no effect on low-trait anxiety participants. The authors attributed the effect to the tryptophan content of alpha-lactalbumin. These were regarded as beneficial effects, and no adverse effects were reported.

In an attempt to differentiate the effects on energy expenditure and satiety of total whey protein and alpha-lactalbumin, a prospective, randomized, double-blind crossover trial was conducted (Hursel et al. 2010). Thirty-five apparently healthy university students, 17M and 18F aged 20.9±1.9 years, received a breakfast yogurt that was unenriched, enriched with additional whey, or enriched with whey protein enriched in alpha-lactalbumin and depleted in caseinomacropeptide. The enriched yogurt had 14% energy from casein and 27% energy from

whey or alpha-lactalbumin-enriched whey and each subject consumed all 3 yogurts a week apart. Resting energy expenditure and diet-induced thermogenesis were measured before and after yogurt consumption and the subjects completed a satiety questionnaire.

No difference due to yogurt type was reported in resting-energy expenditure, but addition of either whey protein significantly increased thermogenesis and protein balance. Addition of alpha-lactalbumin-enriched whey protein, but not total whey, significantly suppressed self-reported hunger and desire to eat. The authors reported that "No adverse events occurred, and no subjects reported any feelings of discomfort after consuming the yoghurt drinks," and concluded that "the addition of alpha-lactalbumin, i.e. whey, to a normal-protein milk protein yoghurt drink increases diet-induced thermogenesis; additional alpha-lactalbumin also suppresses hunger and the desire to eat."

Sashihara et al. (2013) reported a prospective, randomized, double-blind, placebo-controlled trial of the effect of *Lactobacillus gasseri* strain OLL2809, with or without added alpha-lactalbumin, on athletes undergoing strenuous exercise. Forty-four male university students were randomized to 3 groups performing strenuous exercise on a braked cycle ergometer daily for 4 weeks while receiving meals providing daily doses of placebo (n = 14, age 20.2±1.1 years), 10^{10} cfu *L. gasseri* alone (n = 15, age 19.8±0.9 years), or *L. gasseri* with 900 mg alpha-lactalbumin (n = 15, age 19.1±0.9 years). Participants completed questionnaires of mood state at the beginning and end of the study and provided blood samples before and after exercise for analysis of NK cell activity, reactive oxygen species, transforming growth factor (TGF) β1, hematology parameters, and clinical chemistries.

No subject withdrew from the study and all completed the trial successfully. Ingestion of *L. gasseri*, both with and without alpha-lactalbumin, resulted in significantly reduced scores on tension-anxiety; the "fatigue" score was significantly reduced in the group receiving both *L. gasseri* and alpha-lactalbumin, but not that ingesting the probiotic alone. There were no differences in exercise performance. NK activity was reduced after exercise in the placebo group, but not in either test group. The combination of *L. gasseri* and alpha-lactalbumin significantly reduced post-exercise serum reactive oxygen metabolites and TGF-β1 levels, both regarded as helpful effects. 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."

The ability of yogurt alone or yogurt enriched with alpha-lactalbumin, casein tripeptides, and B vitamins to reduce anxiety in adults scoring highly on an anxiety-trait pretest was evaluated in a prospective, randomized, double-blind, placebo-controlled trial (Jaatinen et al. 2014). A total of 67 adults with high anxiety scores, 7 men and 60 women aged 18-63 years (mean age = 39.3±3.2 years), was enrolled and randomized to receive 125 g yogurt twice a day for 4 weeks, either regular strawberry yogurt (n = 34) or yogurt providing daily doses of 17 g alpha-lactalbumin, 4.2 g casein tripeptides (isoleucine-proline-proline and valine-proline-proline), 1.2 mg vitamin B_6, 121 µg folacin, and 2.6 µg vitamin B_{12} (n = 33). Anxiety level was measured by scores on a stress questionnaire, heart-rate variability, shifts from autonomic to sympathetic cardiac dominance, sleep-monitor actigraphy, blood pressure measures, salivary cortisol level, and blood measures of IgA, C-reactive protein, and adrenocorticotropic hormone (ACTH).

The test yogurt with bioactive components significantly increased self-ratings of vigor, heart-rate variability, and recovery index. The authors concluded that "daily intake of yogurt enriched with bioactive components may aid in stress coping." No adverse effects were reported.

The effects of dietary protein on postprandial lipemia were assessed in a prospective, randomized, double-blind crossover trial reported by Mariotti et al. (2015). Ten apparently healthy but overweight men aged 34±9 years consumed meals comprising 233 g high-fat milk cream, 45 g

sucrose, and protein isolates of 54 g casein, 55 g whey, or 49 g alpha-lactalbumin-enriched whey; they consumed each meal at least 2 weeks apart. The men were examined to assess weight; body composition; blood pressure; and plasma concentrations of glucose, triacylglycerol, fatty acids, free α-amino nitrogen, insulin, IL-6, TNF-α, ApoB-48, monocyte chemo-attractant protein-1, vascular cell adhesion molecule-1, intercellular adhesion molecule-2, plasminogen activator inhibitor-1, hydrogen peroxide, malondialdehyde, and F_2-isoprostane 15(S)-8-iso-prostaglandin $F_{2\alpha}$.

The type of protein did not affect postprandial plasma glucose, amino acids, insulin, or nonesterified fatty acids, but, compared with whey and alpha-lactalbumin-enriched whey, which did not differ, casein significantly reduced postprandial triacylglycerol. There were no significant differences among the meals in postprandial oxidative stress (plasma hydroperoxides and malondialdehyde), endothelial dysfunction, or low-grade inflammation. 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."

The relative effects of isolates of total whey protein, alpha-lactalbumin, and beta-lactoglobulin were compared in a prospective, randomized, single-blind crossover study (Chungchunlam et al. 2017). Twenty apparently healthy women aged 24.2±0.8 years, with normal bodyweight, consumed meals preloaded with ~50 g protein in the form of each of the 3 isolates; 2 hours later they provided subjective ratings of appetite.

Energy intake at the test meal and total energy intakes (preload+test meal) did not differ among the three preload meals. There were no significant differences observed for the appetite self-ratings. No adverse effects were reported of any of the protein isolates. The authors concluded that "the satiating effect of whey protein was similar to that of beta-lactoglobulin or alpha-lactalbumin individually and suggest that the major whey protein components beta-lactoglobulin and alpha-lactalbumin do not mediate the satiating effect of whey protein."

Twelve apparently healthy male endurance runners aged 30.4±2.8 years were enrolled in a prospective, randomized, double-blind crossover study to compare the effects of alpha-lactalbumin and total whey protein on muscle damage, muscle pain, and mood states during short-term recovery after prolonged strenuous exercise (Qin et al 2017). Creatine kinase, IL-6, blood glucose, blood lactic acid, hemoglobin, salivary cortisol, self-rating of muscle pain, pressure-pain threshold and mood states were evaluated before and immediately after exercise and at 2 and 4 hours post-exercise; creatine kinase, IL-6, glucose, lactic acid, muscle pain, and pressure-pain threshold were evaluated at 24 hours. During the first 2 hours after exercise, subjects consumed whey isolate or alpha-lactalbumin at a rate of 0.34 g/kg bw/hour.

IL-6 was significantly higher immediately and 2 hours after exercise than before exercise, and creatine kinase was significantly higher 24 hours after exercise than before exercise with both interventions, but whey isolate and alpha-lactalbumin did not differ in their effects. Salivary cortisol was elevated immediately after exercise in both trials but significantly lower at 24 hours than pre-exercise when alpha-lactalbumin was consumed. The pressure-pain threshold was significantly higher and the feeling of fatigue was significantly lower after consumption of alpha-lactalbumin than after whey isolate. No adverse effects were reported, and the authors concluded that, compared with ingestion of whey isolate, ingestion of alpha-lactalbumin "reduced sensitivity to the muscle pain and was more beneficial in reducing the feeling of fatigue and cortisol responses during 4-hour recovery following 90 minutes running."

Ong et al. (2017) reported a pilot study of the effect of alpha-lactalbumin intake on sleep quality and duration. Ten apparently healthy males aged 26.9±5.3 years were enrolled in a

prospective, randomized, double-blind crossover trial in which they received 20 g of either alpha-lactalbumin or sodium caseinate one hour before bedtime for 2 days, followed by a 1-day washout, and the other treatment for 2 days. Subjects wore a wrist actigraphy sensor to measure total sleep time, sleep onset latency, and waking episodes, and kept a sleep log recording bedtime, time to fall asleep, wakenings during the night, and rising time.

Consumption of alpha-lactalbumin vs. placebo significantly increased total sleep time and decreased sleep onset latency and the number of waking episodes. No adverse effects attributable to alpha-lactalbumin were reported. The authors concluded that, "increased objective and subjective sleep duration and objective sleep efficiency were observed after an evening intake of alpha-lactalbumin compared to a placebo in healthy male adults."

Pujos-Guillot et al. (2018) reported on a prospective, randomized, double-blind crossover study in which 10 apparently healthy but overweight men aged 21-50 years (mean age = 34±9 years) were provided meals that included 233 g high-fat milk cream, 45 g sucrose, and protein isolates of 54 g casein, 55 g whey, or 49 g alpha-lactalbumin-enriched whey; they consumed each of the 3 meals with 2-week washout intervals. Urine samples were collected for metabolomics analysis before the meal and at 0, 2, 4, and 6 hours afterwards; blood was drawn prior to the meal and 0.5, 1, 1.5, 2, 3, 4, and 6 hours after eating and analyzed for triacylglycerol and apoB-48.

Seventeen metabolites were identified that significantly explained the effect of protein type on postprandial metabolomics changes. Acylcarnitines and other acylated metabolites related to fatty-acid or amino-acid oxidation were the main discriminants. The difference in metabolic profiles was mainly explained by urinary acylcarnitines and some other acylated products, with a significantly greater increase after consumption of whey isolate and alpha-lactalbumin-enriched whey as compared with casein. 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."

In a prospective, randomized, double-blind, placebo-controlled trial reported by Lagana et al. (2018), 50 pregnant women aged 26.6±6.1 years, between 19 and 21 gestational weeks, suffering from iron-deficiency anemia (hemoglobin <10.5 g/dL and ferritin < 10-15 µg/L) but otherwise apparently healthy were treated daily with 30 mg micronized dispersible ferric pyrophosphate plus 300 mg alpha-lactalbumin (n = 25) or 80 mg ferrous gluconate for 30 days. At baseline and after 15 and 30 days, blood was analyzed for hemoglobin, ferritin, red blood cells, serum iron, hematocrit. Any adverse effects were recorded at 15 and 30 days.

Both groups showed significant improvements in iron-status parameters, but those receiving ferric pyrophosphate and alpha-lactalbumin showed significantly greater progress than those receiving ferrous gluconate. 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/alpha-lactalbumin group did not show any significant side effect." They concluded that, "overall, ferric-pyrophosphate/alpha-lactalbumin is more effective and safe than ferrous gluconate for the treatment of iron-deficiency anemia in pregnant women."

Qin et al. (2019) reported that 11 apparently healthy male endurance runners aged 31±2 years were enrolled in a prospective, randomized, double-blind crossover trial in which they consumed either alpha-lactalbumin or whey protein isolate 2 hours before a 21-kilometer run. Creatine kinase, IL-6, muscle pain, pressure-pain threshold, and mood states were evaluated 2 hours and immediately before running and immediately after completion.

No difference was reported in running performance, but ingestion of alpha-lactalbumin resulted in significantly higher pressure-pain threshold and significantly lower fatigue and salivary cortisol. No adverse effects attributable to alpha-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.”

Table 2. Studies of Alpha-Lactalbumin in Adults.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	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	Energy intake at the test meal and total energy intakes (preload+test meal) did not differ among the three preload meals. There were no significant differences observed for the appetite self-ratings. No adverse effects were reported of any of the protein isolates. The authors concluded that "the satiating effect of whey protein was similar to that of β-lactoglobulin or α-lactalbumin individually and suggest that the major whey protein components beta-lactoglobulin and α-lactalbumin do not mediate the satiating effect of whey protein."
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	No difference due to yogurt type was reported in resting-energy expenditure, but addition of either whey protein significantly increased thermogenesis and protein balance. Addition of α-lactalbumin-enriched whey protein, but not total whey, significantly suppressed self-reported hunger and desire to eat. The authors reported that "No adverse events occurred, and no subjects reported any feelings of discomfort after consuming the yoghurt drinks," and concluded that "the addition of α-lactalbumin, i.e. whey, to a normal-protein milk protein yoghurt drink increases diet-induced thermogenesis; additional α-lactalbumin also suppresses hunger and the desire to eat."

Table 2. Studies of Alpha-Lactalbumin in Adults.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
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}	The test yogurt with bioactive components significantly increased self-ratings of vigor, heart-rate variability, and recovery index. The authors concluded that "daily intake of yogurt enriched with bioactive components may aid in stress coping." No adverse effects were reported.
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	Both groups showed significant improvements in iron-status parameters, but those receiving ferric pyrophosphate and α-lactalbumin showed significantly greater progress than those receiving ferrous gluconate. 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."

Table 2. Studies of Alpha-Lactalbumin in Adults.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
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	The type of protein did not affect postprandial plasma glucose, amino acids, insulin, or nonesterified fatty acids, but, compared with whey and α-lactalbumin-enriched whey, which did not differ, casein significantly reduced postprandial triacylglycerol. There were no significant differences among the meals in postprandial oxidative stress (plasma hydroperoxides and malondialdehyde), endothelial dysfunction, or low-grade inflammation. 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."
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	The ratio of plasma tryptophan to other large neutral amino acids was significantly higher after the α-lactalbumin diet than after the casein diet. In stress-vulnerable subjects, this was accompanied by significantly higher prolactin concentrations, decreased cortisol, and reduced depressive feelings under stress. These were regarded as beneficial changes and no adverse effects were reported. The authors concluded that "consumption of a dietary protein enriched in tryptophan … in stress-vulnerable subjects improved coping ability, probably through alterations in brain serotonin."

Table 2. Studies of Alpha-Lactalbumin in Adults.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
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	A significantly greater increase in the ratio of plasma tryptophan to other large neutral amino acids was reported after consumption of the α-lactalbumin diet than after the control diet; cognitive performance improved significantly only in the high stress–vulnerable subjects. The authors reported no adverse effects and concluded, "the results suggest that dietary protein rich in α-lactalbumin improves cognitive performance in stress-vulnerable subjects via increased brain tryptophan and serotonin activities."
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	Evening α-lactalbumin intake resulted in a significant increase in the ratio of plasma tryptophan to other large neutral amino acids before bedtime, significantly reduced sleepiness, and improved brain-sustained attention processes the following morning. No adverse effects were reported and the authors concluded that, "Evening dietary increases in plasma tryptophan availability for uptake into the brain enhance sustained alertness early in the morning after an overnight sleep, most likely because of improved sleep."

Table 2. Studies of Alpha-Lactalbumin in Adults.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
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	The stress test affected mood in both conditions. Although the α-lactalbumin diet led to the expected rises in plasma tryptophan and the ratio of tryptophan and other large neutral amino acids, only minimal effects were reported on mood and cortisol response to experimental stress. The results were the same for recovered depressed patients and controls. The authors reported that "α-lactalbumin had no side-effects" but concluded that, "A 1-day diet enriched with α-lactalbumin is not sufficient to prevent a stress-induced mood deterioration or a cortisol response in unmedicated, recovered depressed subjects."
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	Plasma tryptophan and suppression of hunger at lunchtime were significantly higher and total amino-acid concentration was lower after the α-lactalbumin breakfast than the other meals. Hormones related to satiety, glucagon-like peptide-1 and ghrelin, were not related to the type of breakfast consumed. No adverse effects were reported. The authors concluded that, "the present study shows that a breakfast containing α-lactalbumin as the only protein source results in a more prolonged suppression of hunger than a breakfast containing gelatin."
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 for 2 days	Consumption of α-lactalbumin vs. placebo significantly increased total sleep time and decreased sleep onset latency and the number of waking episodes. No adverse effects attributable to α-lactalbumin were reported. The authors concluded that, "increased objective and subjective sleep duration and objective sleep efficiency were observed after an evening intake of α-lactalbumin compared to a placebo in healthy male adults."

Table 2. Studies of Alpha-Lactalbumin in Adults.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
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	Seventeen metabolites were identified that significantly explained the effect of protein type on postprandial metabolomics changes. Acylcarnitines and other acylated metabolites related to fatty-acid or amino-acid oxidation were the main discriminants. The difference in metabolic profiles was mainly explained by urinary acylcarnitines and some other acylated products, with a significantly greater increase after consumption of whey isolate and α-lactalbumin-enriched whey as compared with casein. 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	IL-6 was significantly higher immediately and 2 hours after exercise than before exercise, and creatine kinase was significantly higher 24 hours after exercise than before exercise with both interventions, but whey isolate and α-lactalbumin did not differ in their effects. Salivary cortisol was elevated immediately after exercise in both trials but significantly lower at 24 hours than pre-exercise when α-lactalbumin was consumed. The pressure-pain threshold was significantly higher and the feeling of fatigue was significantly lower after consumption of α-lactalbumin than after whey isolate. No adverse effects were reported, and the authors concluded that, compared with ingestion of whey isolate, ingestion of α-lactalbumin "reduced sensitivity to the muscle pain and was more beneficial in reducing the feeling of fatigue and cortisol responses during 4-hour recovery following 90 minutes running."
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	No difference was reported in running performance, but ingestion of α-lactalbumin resulted in significantly higher pressure-pain threshold and significantly lower fatigue and salivary cortisol. 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."

Table 2. Studies of Alpha-Lactalbumin in Adults.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
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	No subject withdrew from the study and all completed the trial successfully. Ingestion of *L. gasseri*, both with and without α-lactalbumin, resulted in significantly reduced scores on tension-anxiety; the "fatigue" score was significantly reduced in the group receiving both *L. gasseri* and α-lactalbumin, but not that ingesting the probiotic alone. There were no differences in exercise performance. NK activity was reduced after exercise in the placebo group, but not in either test group. The combination of *L. gasseri* and α-lactalbumin significantly reduced post-exercise serum reactive oxygen metabolites and TGF-β1 levels, both regarded as helpful effects. 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	Women performed significantly better on the long-term memory test for abstract figures after ingesting α-lactalbumin than after consuming casein; there were no other significant differences. No adverse effects were reported, and the authors concluded that "the cognitive effect of acute premenstrual administration of α-lactal-bumin… was modest."
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	Emotional processing and cortisol levels were not different between groups, but α-lactalbumin significantly raised blood tryptophan and the ratio of tryptophan to other neutral amino acids. Women receiving α-lactalbumin experienced significantly more nausea than those receiving casein, an effect the authors suggested might reflect increased brain serotonin activity. The authors concluded that, "this study suggests limited effects of α-lactalbumin on both tryptophan availability and emotional processing," but also suggested that the modest increase in tryptophan availability resulting from the single dose of α-lactalbumin may have been insufficient to produce significant effects.

Table 2. Studies of Alpha-Lactalbumin in Adults.

Reference	Study Design, Duration & Objective	Subjects	Description of Test Articles	Results
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	The α-lactalbumin, gelatin and gelatin+tryptophan breakfasts resulted in significantly reduced energy intake at lunch compared to experimental conditions in which the subjects consumed breakfasts containing the other proteins. No adverse effects were reported, and the authors concluded that, "different proteins (α-lactalbumin, gelatin, and gelatin+tryptophan) that are 30–50% more satiating than other proteins (casein, soy, whey, and glycomacropeptide-depleted whey) induce a related 17–24% reduction of subsequent energy intake at the following meal."
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	The intervention did not exert any immediate effects on stress, mood, or appetite ratings, but high-trait anxiety individuals receiving α-lactalbumin exhibited significantly decreased liking of foods in general and reduced preference for sweet foods. There was no effect on low-trait anxiety participants. The authors attributed the effect to the tryptophan content of α-lactalbumin. These were regarded as beneficial effects, and no adverse effects were reported.

6.4. Safety Assessment and GRAS Determination

This section presents an assessment that demonstrates that the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin is safe and is GRAS based on scientific procedures.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of the intended use of 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 GRN 000809. 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.

6.4.2. Conclusion of the Expert Panel

The intended addition of fractionated whey protein concentrate containing 41% alpha-lactalbumin to conventional foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was shown by reviewing the extensive published reports of studies of infants consuming alpha-lactalbumin enriched formulas (in GRN 000809, Section 6.3.2, pages 28-63, incorporated by reference) and adults consuming alpha-lactalbumin in two-arm or crossover trials, and concluding that the expected exposure to fractionated whey protein concentrate containing 41% alpha-lactalbumin is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin has been made through the deliberations of an Expert Panel consisting of Ronald E. Kleinman, M.D., Berthold V. Koletzko, M.D., Ph.D., and John A. Thomas, Ph.D., who reviewed a monograph prepared by James T. Heimbach, Ph.D., as well as other information available to them. These individuals, qualified by scientific training and experience to evaluate the safety of food ingredients, independently critically reviewed and evaluated the publicly available information and the potential human exposure to alpha-lactalbumin anticipated to result from its intended use. They individually and collectively determined that no evidence exists in the available information on fractionated whey protein concentrate containing 41% alpha-lactalbumin that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of fractionated whey protein concentrate containing 41% alpha-lactalbumin.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion regarding the safety of the substance under its intended conditions of use. Therefore, the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin in conventional foods is GRAS by scientific procedures.

6.5. Statement Regarding Information Inconsistent with GRAS

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with our conclusion of GRAS status of the intended use of fractionated whey protein concentrate containing 41% alpha-lactalbumin.

6.6. Statement of the Expert Panel

We, the undersigned members of the Expert Panel, are qualified by scientific education and experience to evaluate the safety of substances intended for addition to foods. We have individually and collectively critically evaluated the publicly available information on fractionated whey protein concentrate containing 41% alpha-lactalbumin summarized in a monograph, *Generally Recognized As Safe (GRAS) Determination for the Intended Use of Fractionated Whey Protein Concentrate Containing 41% Alpha-Lactalbumin in Conventional Foods* (July 2019), prepared by James T. Heimbach, Ph.D., and other material deemed appropriate or necessary.

We have individually and collectively determined that no evidence exists in the available information on fractionated whey protein concentrate containing 41% alpha-lactalbumin that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of fractionated whey protein concentrate containing 41% alpha-lactalbumin.

We unanimously conclude that the intended addition of fractionated whey protein concentrate containing 41% alpha-lactalbumin, produced consistent with current good manufacturing practice (cGMP) and meeting the food-grade specifications presented in the monograph, to conventional foods at the levels specified in this monograph is safe and is GRAS by scientific procedures.

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

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

Signature: _____ Date: _____

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

Signature: _____ Date: _____

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

Signature: _____ Date: _____

6.6. Statement of the Expert Panel

We, the undersigned members of the Expert Panel, are qualified by scientific education and experience to evaluate the safety of substances intended for addition to foods. We have individually and collectively critically evaluated the publicly available information on fractionated whey protein concentrate containing 41% alpha-lactalbumin summarized in a monograph, *Generally Recognized As Safe (GRAS) Determination for the Intended Use of Fractionated Whey Protein Concentrate Containing 41% Alpha-Lactalbumin in Conventional Foods* (July 2019), prepared by James T. Heimbach, Ph.D., and other material deemed appropriate or necessary.

We have individually and collectively determined that no evidence exists in the available information on fractionated whey protein concentrate containing 41% alpha-lactalbumin that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of fractionated whey protein concentrate containing 41% alpha-lactalbumin.

We unanimously conclude that the intended addition of fractionated whey protein concentrate containing 41% alpha-lactalbumin, produced consistent with current good manufacturing practice (cGMP) and meeting the food-grade specifications presented in the monograph, to conventional foods at the levels specified in this monograph is safe and is GRAS by scientific procedures.

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

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

Signature: [redacted] Date: Sept. 3, 2019

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

Signature: _____ Date: _____

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

Signature: _____ Date: _____

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

Signature: _____ Date: _____

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

Signature: _____ Date: 10 Sept 2019

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

Signature: _____ Date: _____

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We have individually and collectively determined that no evidence exists in the available information on fractionated whey protein concentrate containing 41% alpha-lactalbumin that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of fractionated whey protein concentrate containing 41% alpha-lactalbumin.

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

Ronald E. Kleinman, M.D.
Professor of Pediatrics
Harvard Medical School
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Signature: _____ Date: _____

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

Signature: _____ Date: _____

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

Signature:  Date: Sept. 4, 2019

Part 7: List of Supporting Data and Information

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APPENDIX

FDA QUESTIONS ON GRN0000809 AND RESPONSES

General Comments

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

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

Chemistry

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

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

We used the following values in our calculations:

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

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

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

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

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

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

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

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

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

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

To reach average human milk levels of ALA using various combinations of whey and casein, the replacement level of total whey or casein protein by fractionated WPC (41% ALA) needs to be in the range of 27.5% to 42.8%. Such addition would provide ALA levels in formulas that would reach 2.5 g/RL for declared content or approximately 7.5 g/L of fractionated WPC (41% ALA) covering our intended use levels. To account for analytical and manufacturing variabilities, we requested a 10% overage level of 8.3 g/L and the overage amount is not reflected in the above calculations. The 100% replacement of regular whey with fractionated whey (41% ALA) is still limited in reaching human milk average levels of 2.5 g/L (Appendix 1, rows 24 and 25) for whey:casein ratios 40:60 and 50:50, either at 14g total protein or at 15g total protein formulas. Using fractionated WPC (41% ALA) to bring alpha lactalbumin levels closer to human milk average of 2.5g/L is best suited for whey dominant formulas only.

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

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

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

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

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

Analyses were conducted in-house and by a third-party laboratory for ALA, β-lactoglobulin, casein glycomacropeptide (CGMP), proteose peptones (PP8 and PP5), immunoglobulin G-1 (IgG-1), bovine serum albumin (BSA), and lactoferrin (LF). Our findings compare well with the published literature regarding the protein profile of a variety of whey products (Elgar DF, Norris CS, Ayers JS, Pritchard M, Otter DE, and Palmano KP (2000). Simultaneous separation and quantitation of the major bovine whey proteins including proteose peptone and caseinomacropeptide by reversed-phase high-performance liquid chromatography on polystyrene-divinylbenzene. *J Chromatogr A* 878:183-186.).

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.

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

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

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

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

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

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

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

*Essential amino acids in bold font

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

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

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

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

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

Thus, on an average, CGMP exposure is about 25% greater at the maximal WPC (41% ALA) concentration of 8.3 g/RL specified in the petition. As can be seen in response to Q1a, for whey dominant formulas only a portion of standard WPC 80 is replaced by fractionated WPC (41% ALA). Therefore, in most formulas, the increase in CGMP intake would be less than the maximum projected level. Sandstrom O., Lonnerdal B, Graverholt G, and Hernell O. (2008. Effects of alpha-lactalbumin-enriched formula containing different concentrations of glycomacropeptide on infant nutrition. *Am J Clin Nutr* 87:921–928.) studied the fraction with high (15% protein as CGMP, 294 mg/100 Kcal) and low CGMP (10% protein as CGMP, 196 mg/100 Kcal) content and found them both to be safe.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Toxicology

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

We appreciate FDA's suggestion.

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

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

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

Additional FDA Questions and Responses

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

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

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

Reference:

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

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

Yes. Sorry for the confusion.

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

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

4. *In the absence of data regarding levels of growth factors such as IGF-1 or TGF-β in fractionated WPC (41% ALA), please comment on whether these components would be concentrated in the final ingredient based on the molecular weight cutoff of the ultrafiltration membrane used in your method of manufacture or the use of pasteurized milk for whey production from cheesemaking. (We note that effects of pasteurization and membrane processing on levels of TGF-β1 and IGF-1 in ultrafiltered whey have been discussed in the published literature (Ollikainen et al., 2012; Akbache et al., 2009)).*

Thank you for the forwarded literature.

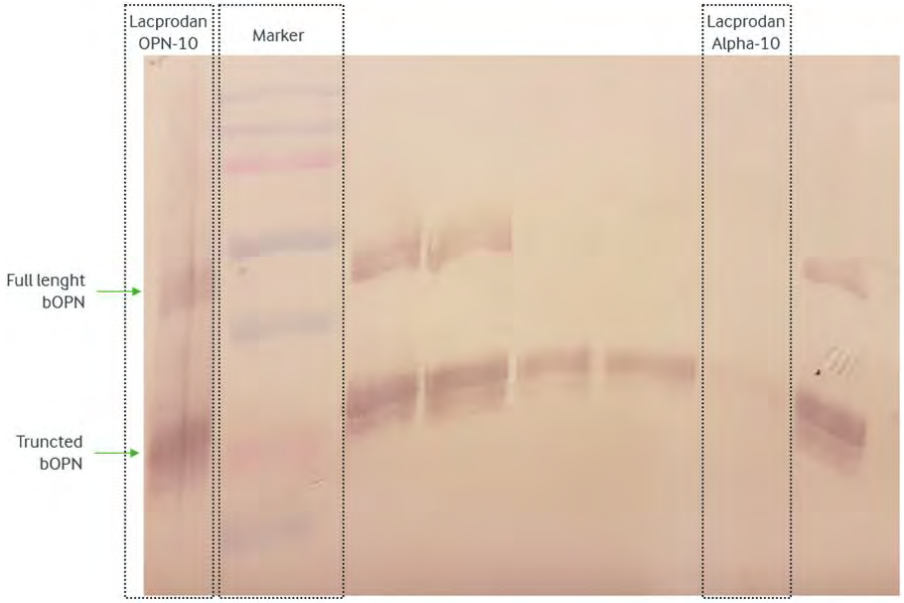
The manufacture of Lacprodan Alpha-10 utilizes an even tighter separation material than that employed by Ollikainen et al. (2012), which means that most TGF-β from the starting material (WPC) should be in the reduced retentate fraction rather than in the notified GRAS fraction, fractionated WPC (41%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

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

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

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

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



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

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

RESPONSES TO FDA QUESTIONS ON GRN 909

1. *In Section 1.5 of the notice, you note that "Fractionated whey protein concentrate containing 41% alpha-lactalbumin is grandfathered under the definition of whey protein concentrate provided in 21 CFR § 184.1979c." We note that, although both ingredients use physical separation techniques, because the method of production of the GRN 000909 ingredient entails removal of a fraction of the proteins in whey, it does not meet the regulatory definition of whey protein concentrate which is produced by removal of only non-protein constituents from whey.*

We have removed the claim that the product is grandfathered. (See Section 1.6 on page 5.)

2. *You note on p. 4 that the intended use is "to increase the dietary intake of the whey protein fraction alpha-lactalbumin"; further, the statement appears to be incomplete. Please clarify the intended technical effect to reflect that it is either use as a source of protein or as an ingredient.*

Throughout the document, we have specified that the intended use is as a source of protein. (See Section 1.4 on page 4, Section 2.4 on page 13, Section 3.1 on page 14, and Section 6.4.2 on page 39.)

3. *Please clarify if the proposed uses include use in toddler drinks and foods. We note that the serving sizes you have listed in Table 1 (p. 8) are for consumers ages 4 years and above. If your uses include toddler foods, please add the RACCs for children ages 1-3 years for applicable food categories in Table*
   1. *https://www.federalregister.gov/documents/2018/05/04/2018-09476/food-labeling-revision-of-the-nutrition-and-supplement-facts-labels-and-serving-sizes-of-foods-that*

Yes, the intended use includes toddler foods and drinks. We are aware that RACCs for children aged 1-3 years are smaller than those for adults, but that does not affect the intake analyses. The addition level of fractionated WPC for purposes of intake estimation is expressed in g WPC/g food or drink (i.e., as a %). (E.g., addition of 12 g WPC to a 40-g serving of protein-enriched baked products is expressed as addition of 0.3 g WPC/g food [12/40].) The concentration remains the same in products intended for toddlers, but the intake of WPC is lower because consumption of the food or drink is lower. And, of course, the intake estimates are based on actual consumption as reported in the surveys, not on RACCs.

We have added a column to Table 5 (page 14) expressing the WPC addition level as a % to clarify this.

4. *You note on p. 5 that the Expert Panel reviewed a monograph as well as other information available to them, but you do not include the monograph in the notice. For the administrative record, please clarify if the Expert Panel reviewed materials not included in GRN 000909.*

We have clarified that the panel reviewed the present monograph (now a GRAS notice), prepared by James T. Heimbach. (See Section 1.5 on page 5 and Section 6.4.2 on page 39.) Additionally, GRAS Panel members are always free to look at the literature on their own or to employ any other knowledge in their possession. If they have done so, this was neither reported nor discussed.

5. *Please express the maximum use levels for Table 1 column 3, (p. 8) in terms of grams per 100 g of food. As written, since the serving size may vary for foods within a given category, If the concentrations are different in foods for children ages 1-3 years, please clarify the maximum concentrations in toddler foods.*

We have done this, as explained in our response to Q3. And, as noted in that response, concentrations of WPC in foods are the same for products intended for consumption by toddlers as they are for adults. As stated in response to Q3, we have added a column to Table 5 (page 14) expressing the WPC addition level as a % to clarify this.

6. *Please clarify what is meant by "spoonable milk products." Are these products refrigerated or frozen? Does this include puddings, dairy-based dips and spreads, and/or frozen dairy based desserts?*

"Spoonable milk products" is a term introduced by E^{x}ponent in reporting their dietary assessment. According to E^{x}ponent's report, all products included in the category are types of yogurt, including Greek yogurt, yogurt "not specified as to type of milk," soy yogurt, coconut milk yogurt, and yogurt with oats as well as full-fat, low-fat, and non-fat yogurts of various flavors.

7. *Please provide a <u>mean</u> dietary exposure estimate for the use of fractionated WPC containing 41% alpha-lactalbumin for all relevant ages (addressing the additional points below).*

Done. See Section 3.2 on page 14.

8. *The dietary exposure estimate you provide is for ages 2+ years, while the RACCs you use in describing use levels are for ages 4+ years. Please describe the estimated dietary exposure for ages 1-3 y if infant and toddler foods are included in the intended use. We note that, the use of this ingredient in infant formula as described in GRN 000809 did not include toddler formulas or other toddler foods.*

Rather than provide estimated intakes for all individuals aged 2+, we have provided intake estimates for users of target foods aged 1-3 years and 4+ years. See Section 3.2 on page 14.

9. *Please confirm that the estimates of intake were based on the maximum use levels given in Table 1 (p. 8) and verify the levels (previously noted in question 5).*

We confirm that all intake estimates are based on maximum intended use levels.

10. *Alpha-lactalbumin is also present in milk/milk products and in other alpha-lactalbumin ingredients (e.g., the subject of GRN 000763). Your dietary exposure estimate does not address current intake of alpha-lactalbumin. We request that you address the following:*
    a. *Indicate whether uses of your ingredient are substitutional, partially substitutional, or additive in relation to background intakes of milk products containing alpha-lactalbumin.*
    b. *Indicate whether uses of WPC containing 41% alpha-lactalbumin are substitutional or additive to other alpha-lactalbumin ingredients currently on the market and also used in food categories listed in GRN 909.*
    c. *Please address estimated cumulative dietary exposure to alpha-lactalbumin from uses of the fractionated WPC containing 41% alpha-lactalbumin, and background dietary intakes of milk products and other alpha-lactalbumin ingredients on the market.*

In response to Q10a and Q10b, alpha-lactalbumin enriched whey protein concentrate in proposed product offerings will be used as a protein source and will be substitutional to replace other dairy based protein sources containing alpha-lactalbumin.
In response to Q10c, we first note that, since the use of WPC containing 41% alpha-lactalbumin 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 alpha-lactalbumin (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 alpha-lactalbumin and about 7.9% to the alpha-lactalbumin intake of those aged 4 years and older.

Haug A, AT Hostmark, OM Harstad. 2007. Bovine milk in human nutrition—a review. *Lipids Health Dis* doi: 10.1186/1476-511X-6-25.

Layman DK, B Lonnerdal, JD Fernstrom. 2018. Applications for α-lactalbumin in human nutrition. *Nutr Rev* 76:444-460.

Sebastian RS, JD Goldman, CW Enns, RP LaComb. 2010. Fluid milk consumption in the United States. *Food Surveys Research Group Dietary Data Brief No. 3*.

11. *In Section 6.1, although you indicate that the pharmacokinetic information of alpha-lactalbumin is incorporated by reference from GRN 000809, it is not clear which studies are relevant to the current intended use. Please provide a brief summary of relevant study (studies), key pharmacokinetic information and conclusions in the context of safety.*

We have brought over the complete discussion of PK data that was presented in GRN 809. (See Section 6.1, pages 17-18.)

12. *The current toxicology narrative in Section 6.2 doesn't provide adequate toxicological information, please revise these sections to support alpha-lactalbumin's safe use in food at the use levels described in the notice. Please identify the toxicological study (studies) that the current GRAS conclusion is based on and provide a narrative that summarize the key toxicological information. Please also address how the toxicological studies cited in GRN 000809 and their endpoints (e.g. weight gain in infant animals) can be extrapolated to support the safety of the current intended use in the general (non-infant) population.*

Toxicological information regarding ALA is scant, presumably because it is a natural component of milk that has been consumed for millennia with no apparent harm. We note that previous GRAS notices for ALA have included no reports of toxicological studies. For example, GRN 763 includes the statement, " Given the long history of human consumption, milk and milk proteins are of little toxicological concern to humans or animals. With the exception of particularly

sensitive populations -namely milk-allergic and lactose-intolerant individuals -we are not aware of adverse effects associated with consumption of alpha lactalbumin. In addition, a literature search does not yield any reported adverse effects."

The animal studies reported in GRN 809 and now included in this notice (Section 6.2 on pages 18-20), including rats and rhesus monkeys, were focused on growth and nutritional effects, but reported a number of endpoints relevant to safety assessment. (While FDA questions the value of measurement of weight gain as an indicator of safety, it might be noted that many toxicologists regard weight gain as the single most important endpoint in toxicological studies that include organ weights, histopathology, urinalysis, hematology, and clinical chemistries.) Some of these additional endpoints are addressed in the rhesus monkey studies reported by Kelleher et al. (2003) and Bruck et al. (2003).

An intensive search of the literature located a Chinese study reported by Zhi et al. (2011). In this study, Wistar rats received chow containing admixtures of 15, 30, or 60% either transgenic or non-transgenic human ALA milk powders (or control diet) for 90 days. No adverse effects were reported on feed consumption, weight gain, absolute or relative organ weights, hematology, clinical chemistry, or histopathology. A discussion of this study has been added to the GRAS notice. (Section 6.2.3 on page 20.)

13. *Please indicate whether a literature search on animal studies has been performed, and if so, please specify the source and publication period (month, year)that your literature search covered.*

PubMed and Google Scholar were searched through 2019 for animal studies using Arla's brands of ALA products. Three rat studies and two studies in rhesus monkeys were identified and are reported in Sections 6.2.1 and 6.2.2. (Note that these studies were discussed in GRN 809 and were originally incorporated in this GRAS notice by reference. They have now been included (on pages 18-20) rather than merely incorporated by reference.

14. *In Section 6.3 (p. 28), you state that "corroborating evidence is provided by the 19 additional published studies". We note that the 19 human (adult) studies were designed to investigate the pharmacological or health benefits of the product, rather than to investigate the safety. Please note that FDA does not evaluate the health benefit effects and efficacy data cannot be used in place of safety evidence, while reports of adverse effects would be important to include in the notice. Please revise the narrative to specify the toxicological endpoints evaluated in these studies and how they support safety in the notified use.*

As FDA noted, human trials of ALA-enriched diets were all focused on putative benefits as the primary endpoints. The published reports were reviewed to obtain all information provided regarding side effects, adverse events, and study dropouts. Discussion of reported benefits has been removed from the tabular presentations of the trials, leaving only the (often brief) reports addressing safety. These have been reported in full. It is true that studies in humans cannot address many toxicological endpoints that require invasive examination, but at the same time it is true that toxicological studies in animals cannot address questions of interspecies translations to humans. This is why we present the data available from both types of studies.

15. *Many of the clinical studies discussed in this notice were conducted in individuals with a particular health condition. Given the biological effects discussed in the notice, please revise the narrative to explain why the intended use would not pose a health risk for the general healthy population.*

As noted in response to Q14, we have removed all discussion of biological effects other than those related to safety. As FDA observes, some of the published literature involves participants with health conditions such as anxiety, iron deficiency, or overweight. Of the 19 reported studies in adults, 10 were of completely healthy adults, 6 were of adults physically healthy but with psychological or emotional issues, and 3 were of physically compromised adults. Findings of no adverse effects due to the intervention in the latter two groups can legitimately be inferred to indicate a likely absence of adverse effects in completely healthy individuals.

16. *In Table 2, the information of study duration is missing for all studies in column 2 (entitled "Study Design, Duration and Objective"). Please provide the missing study details. Please also specify the source and publication period that your literature search covered.*

Information regarding study duration has been added to all study reports for which the information was not previously given. The literature review was based on PubMed and Google Scholar through approximately May, 2019.