KELLER AND HECKMAN LLP
Serving Business through Law and Science®

1001 G Street, N.W.
Suite 500 West
Washington, D.C. 20001
tel. 202.434.4100
fax 202.434.4646

Writer's Direct Access
Richard F. Mann
(202) 434-4229
mann@khlaw.com

February 26, 2018

Via FedEx

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Re: GRAS Notice for Alpha-Lactalbumin

Dear Sir or Madam:

We respectfully submit the enclosed GRAS notice (in electronic format, *i.e.*, CD) on behalf of our client, Agropur, Inc. (Agropur), in support of this notice that alpha-lactalbumin – one of the four major whey proteins naturally present in cow's milk – is generally recognized as safe (GRAS) for use in multiple food applications. The enclosed GRAS notice provides detailed information related to the intended uses, manufacturing, and safety of alpha-lactalbumin.

We look forward to FDA's review of this submission and would be happy to answer any questions. If any questions do arise, please contact me at (202) 434-4229 so that we can respond as quickly as possible.

We appreciate your assistance and look forward to receiving additional feedback.

Sincerely,

(b) (6)

Richard F. Mann
Natalie E. Rainer

Enclosures: (1) CD and (2) paper copy

GRN 000763

GRAS Notice for Alpha-Lactalbumin

U.S. Food and Drug Administration
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
4300 River Road
College Park, MD 20740

Prepared by: Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20001

Date: February 26, 2018

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I. Signed Statements and Certification (21 C.F.R. § 170.225)

In accordance with Subpart E ("Generally Recognized as Safe (GRAS) Notice") of 21 C.F.R. Part 170 ("Food additives"), Keller and Heckman LLP submits the enclosed information on behalf of our client, Agropur, Inc., in support of this notice that alpha-lactalbumin – one of the four major whey proteins naturally present in cow's milk – is generally recognized as safe (GRAS) for use in multiple food applications.

Alpha-lactalbumin is intended for use as a food ingredient for functional or nutritional purposes in the following foods: Powdered Nutritional Beverages (at up to 20% w/w), Sports Beverages (at up to 20% w/w), Nutritional Bars (at up to 25% w/w), and Milk Products (including dairy beverages, at up to 10% w/w). This substance is intended to serve as a source of high-quality protein and/or a texturizer. Foods containing alpha-lactalbumin will be consumed by the general population (children over one year and adults).

Agropur, Inc., has determined that alpha-lactalbumin is GRAS for use in a variety of food categories based on scientific procedures in accordance with Section 170.30(a) and (b), and in conformance with the GRAS final rule published by FDA on August 17, 2016, 81 Fed. Reg. 54960. Given the determination that alpha-lactalbumin is GRAS for the intended uses described herein, it is our view that premarket approval required under the Federal Food, Drug, and Cosmetic Act (FD&C Act) is not required.

The analytical data, published studies, and information that are the basis for this GRAS determination are available for FDA review and copying at reasonable times at Keller and Heckman LLP, 1001 G Street, NW, Suite 500W, Washington, DC 20001, or will be sent to FDA upon request.

A. Name and Address of the Notifier

Agropur, Inc.
3500 E. Destination Drive
Appleton, Wisconsin 54915

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

Richard F. Mann
Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20001
Telephone: (202) 434-4229
Facsimile: (202) 434-4646
Email: mann@khlaw.com

B. Certification

I hereby certify that, to the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as

favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance

Signature:

(b) (6)

Richard F. Mahn
Counsel to Agropur, Inc.

Date: February 26, 2018

II. Identity, method of manufacture, specifications, and physical or technical effect (21 C.F.R. § 170.230)

Alpha-lactalbumin (Chemical Abstracts Service Registry Number (CASRN) 9051-29-0) is one of the four major whey proteins present in cow's milk, constituting approximately 20-30% of the overall protein content contributed by the whey proteins present in milk. It is isolated and purified from milk or whey (using physical separation techniques including ion exchange and membrane processing) and then concentrated and spray-dried. Once processed, it is a homogenous, semi-hygroscopic white to light cream colored powder.

A. Manufacturing Process

The manufacturing process for alpha-lactalbumin involves physical processing techniques to isolate alpha-lactalbumin from whey. The process, which is similar to those described in GRN000504 (Milk Protein Concentrate, Milk Protein Isolate) and GRN000633 (Concentrated Milk Protein with $\geq$60:40 whey:casein ratio"), uses a combination of existing dairy production techniques—including Ion Exchange, Membrane Separation, and Centrifugal Separation. Alpha-lactalbumin is manufactured in accordance with Hazard Analysis and Risk-Based Preventive Controls and Good Manufacturing Practices for food, consistent with 21 C.F.R. Part 117.

To produce alpha-lactalbumin, fresh sweet dairy whey from the cheese manufacturing process is clarified to remove cheese fines and skimmed to remove whey fat. As an alternative to dairy whey from cheese manufacturing, a purified whey protein solution may be used.[1]

To ensure pathogen control and compliance with regulatory requirements, the clarified and separated liquid sweet dairy whey is pasteurized in accordance with Pasteurized Milk Ordinance (PMO) requirements. Specifically, the product is pasteurized using High Temperature Short Time (HTST) at a minimum temperature of 161°F for a minimum of 15 seconds.

Techniques used to concentrate protein and ensure highly purified protein include a combination of ion exchange, membrane filtration, and centrifugal separation. All food-contact membrane materials employed in the production of alpha-lactalbumin comply with applicable food-contact regulations (*e.g.*, 21 C.F.R. § 177.2910) ("Ultra-filtration membranes"). Major whey proteins are fractionated by ion exchange to remove extraneous components, resulting in a purified liquid protein.

The pH and ionic strength of the purified whey protein liquid is adjusted using food-grade acid and food-grade salts (e.g., sodium chloride and/or potassium chloride and/or calcium chloride). Any food-grade acids and salts used in the processing of alpha-lactalbumin are either GRAS for their intended use or the subject of applicable food additive regulations.

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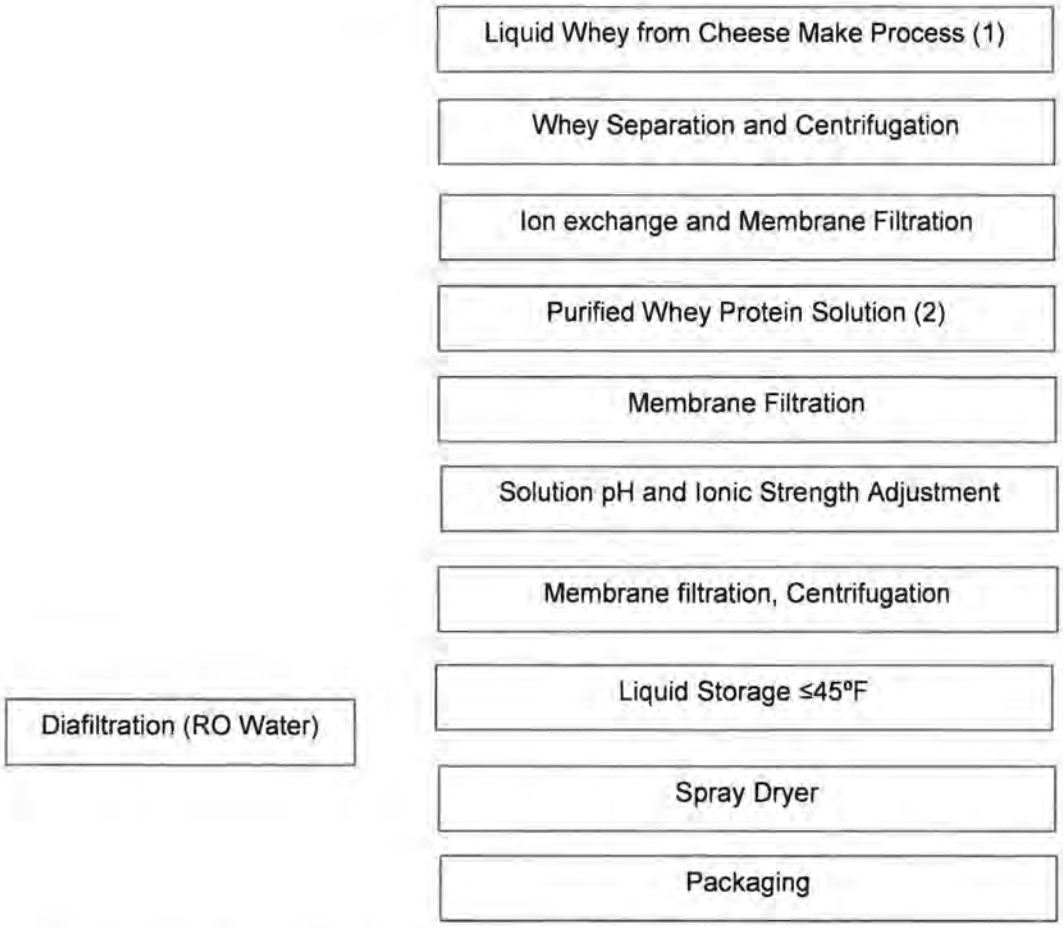
[1] This process involves acidifying and heat-treating fresh milk. An organic solvent is added, and insoluble protein precipitates out of solution. Alpha-lactalbumin remains soluble in the whey protein solution.

The solution is processed with centrifugal separation to selectively purify alpha-lactalbumin from the whey protein solution. The concentrated alpha lactalbumin liquid is further purified by membrane filtration (incorporating diafiltration) to remove water, food-grade salts, other minerals, and low molecular weight constituents from the final product, resulting in a highly-purified alpha-lactalbumin liquid. The final product is pH adjusted with sodium hydroxide meeting the specifications under 21 C.F.R. § 184.1763. The solution is further concentrated with membrane equipment to remove water. The typical composition of protein in the finished product is 90-96% alpha-lactalbumin, 3-5% beta-lactoglobulin, 0-5% bovine serum albumin, and 0-2%, and immunoglobulin G 0-2%.

The concentrated liquid is stored at 45°F or less, followed by spray drying (using normal dairy drying techniques) prior to packaging.

A manufacturing process flow chart is provided below in **Figure 1**.

Figure 1. Manufacturing Process Flow Chart



(1) Start of process if using Whey from Cheese Making.

(2) Start of process if using alpha-lactalbumins isolated directly from Milk.

B. Product Specifications

Agropur provides compositional and microbiological specifications for its alpha-lactalbumin, as summarized in **Tables 1** and **2** below.

Table 1. Compositional Specifications

Parameter	Specification	Method
Moisture (%)	≤6.0	Vacuum Oven AOAC 927.05
Protein, Dry Basis (N*6.25, %)	≥95.0	Leco Combustion AOAC 990.03
Alpha-lactalbumin (% of protein)	≥90.0	HPLC-USP NF
Fat (%)	≤0.5	Mojonnier AOAC 989.05
Ash (%)	≤3.5	Residue on Ignition AOAC 930.30
Lactose (%)	≤0.2	Enzymatic AOAC 984.15
pH	6.0-7.5	10% Sol. @ 20C, AOAC 945.27
Scorched Particles, mg/25g	≤15.0	ADPI, AOAC 952.21

Table 2. Microbiological Specifications

Parameter	Specification	Method
Aerobic Plate Count/g	<10,000	FDA/BAM, AOAC 966.23
Coliform (MPN)/g	<10	FDA/BAM, AOAC 966.23
E.coli (MPN)/g	Negative	FDA/BAM, AOAC 966.23
Yeast & Mold/g	≤10	FDA/BAM
Coagulase-Positive *Staphylococcus* (MPN)/g	<10	FDA/BAM, AOAC 966.23
Salmonella sp./375g	Negative	FDA/BAM, ELISA AOAC 2004.03
Listeria sp./25g	Negative	FDA/BAM, ELISA AOAC 999.06

Five batch analyses demonstrating compliance with specifications are provided below.

Table 3. Product Analysis

Parameter	Specification	Batch 1 JE 015-6-414	Batch 2 JE 024-6-414	Batch 3 JE 001-7-414	Batch 4 JE 008-7-414	Batch 5 JE 010-7-414
Moisture	<6.0	5.4	5.4	4.9	5.4	4.9
Protein, Dry basis	≥95.0	97.6	98.1	97.3	98.1	97.2
Alpha-lactalbumin (% of protein)	>90.0	91.7	92.6	93.9	92.6	90.1
Fat (%)	<0.5	0.1	0.1	0.1	0.1	0.1
Ash (%)	<3.5	2.2	1.7	2.4	1.7	2.6
Lactose (%)	<0.2	0.0	0.0	0.0	0.0	0.0
pH	6.0-7.5	6.3	6.0	6.5	6.0	6.8
Scorched Particles (mg/25 g)	≤15.0	7.5	7.5	7.5	7.5	7.5
Aerobic Plate Count (per gram)	<10,000	<250	<250	<250	<250	<250

Parameter	Specification	Batch 1 JE 015-6-414	Batch 2 JE 024-6-414	Batch 3 JE 001-7-414	Batch 4 JE 008-7-414	Batch 5 JE 010-7-414
Coliform (MPN/g)	<10	<10	<10	<10	<10	<10
E. coli (MPN/g)	Negative	Negative	Negative	Negative	Negative	Negative
Yeast & Mold (per gram)	≤10	<10	<10	<10	<10	<10
Coagulase-Positive *Staphylococcus* (MPN/g)	<10	<10	<10	<10	<10	<10
Salmonella sp. (per 375 g)	Negative	Negative	Negative	Negative	Negative	Negative
Listeria sp. (per 25 g)	Negative	Negative	Negative	Negative	Negative	Negative

C. Physical and Technical Effect

The value of the protein digestibility-corrected amino acid score (PDCAAS) for whey protein derived from cheese making has been shown to be 1.14, higher than for beef (0.9), soy (0.93) or wheat (0.42).[2] Because alpha-lactalbumin will be obtained from either whey from cheese making or directly from milk, the PDCAAS for alpha-lactalbumin is expected to be equivalent to whey protein derived from cheese making.

Agropur has conducted testing using the Digestible Indispensable Amino Acid Score (DIAAS), an alternative to PDCAAS, for testing protein quality that has been recommended by the Food and Agriculture Organization (FAO) of the United Nations.[3] A DIAAS measurement was performed at the Riddet Institute at Massey University, New Zealand using standard protocols previously reviewed and published. The DIAAS score for alpha-lactalbumin was determined to be well over 100, indicating complete amino acid availability.

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[2] Schaafsma G. The Protein Digestibility-Corrected Amino Acid Score. J Nutr 2000; 130:1865S-1867S (internal citations omitted).

[3] *See* Dietary protein quality evaluation in human nutrition: Report of an FAO Expert Consultation, FAO Food and Nutrition Paper 92 (FAO, 2013). *See also* Rutherford *et al.*, Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J Nutr. 2015 Feb;145(2):372-9. 2014 Nov 26, summary available at https://www.ncbi.nlm.nih.gov/pubmed/25644361 (last accessed November 28, 2017). We note that FAO has decided to continue recommending PDCAAS testing in follow-up formula. *See* Joint FFAO/WHO Food Standards Programme Codex Committee on Nutrition and Foods for Special Dietary Uses, Thirty-Eighth Session (Hamburg, Germany, 5 – 9 December 2016), Review Of The Standard For Follow-Up Formula (Codex Stan 156-1987), Physical Working Group Report, at http://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-720-38%252FCRDs%252FCRD_13.pdf (last accessed November 28, 2017).

The nutritional utilization of milk proteins has been studied in both animals and humans.[4] It has been shown that milk proteins are of particularly excellent nutritional value in humans with a true digestibility and a net postprandial protein utilization of 95-96% and 74%, respectively.[5]

D. Consideration of Potential Contaminating Materials

1. Pesticide Residues

Agropur screens its alpha-lactalbumin biannually for agricultural residues using Multi Residue Methods (MRM) for common pesticides, insecticides, herbicides, and fungicides (including organochlorides, organophosphates, organonitrogen, carbamates and pyrethroids). Historical MRM screening have not indicated unlawful residues in Agropur products, including alpha-lactalbumin.

2. Melamine

Alpha-lactalbumin is screened biannually to verify the absence of melamine. The results show that melamine is not present in this product. All equipment used in the manufacturing of this product and its packaging materials are melamine free. Agropur uses FDA's method described in Laboratory Information Bulletin (LIB) 4422 ("Melamine and Cyanuric Acid Residues in Foods") to test for melamine.

3. Drug Residues

The milk used in the production of alpha-lactalbumin is tested for drug residues as described under Appendix N of the PMO, per procedures for sampling, analysis, and reporting described therein.[6] The document states "industry shall screen all bulk milk pickup tankers, regardless of final use, for Beta lactam drug residues. Additionally, other drug residues shall be screened for by employing a random sampling program on bulk milk pickup tankers when the Commissioner of the FDA determines that a potential problem exists as cited in [Section 6 of the PMO]. The random bulk milk pickup tanker sampling program shall represent and include, during any consecutive six (6) months, at least four (4) samples collected in at least four (4)

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[4] Cook BB, Morgan AF, Singer B, Parker J. The effect of heat treatment on the nutritive value of milk proteins. II. Rat growth studies with casein and lactalbumin and their lactose derivatives. J Nutr 1951;44:63–81; Gilani GS, Sepehr E. Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. J Nutr 2003;133:220–5; Rutherfurd SM, Moughan PJ. The digestible amino acid composition of several milk proteins: application of a new bioassay. J Dairy Sci 1998; 81:909–17; Bos C, Gaudichon C, Tome D. Nutritional and physiological criteria in the assessment of milk protein quality for humans. J Am Coll Nutr 2000;19(suppl):191S–205S; Gaudichon C, Mahe S, Benamouzig R, *et al.* Net postprandial utilization of [15N]-labeled milk protein nitrogen is influenced by diet composition in humans. J Nutr 1999;129:890–5.

[5] Bos C, Mahe S, Gaudichon C, *et al.* Assessment of net postprandial protein utilization of 15N-labelled milk nitrogen in human subjects. Br J Nutr 1999;81:221–6; Bos C, Metges CC, Gaudichon C, *et al.* Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. J Nutr 2003;133:1308–15; Gausseres N, Mahe S, Benamouzig R, *et al.* [15N]-labeled pea flour protein nitrogen exhibits good ileal digestibility and postprandial retention in humans. J Nutr 1997;127:1160–5; Morens C, Bos C, Pueyo ME, *et al.* Increasing habitual protein intake accentuates differences in postprandial dietary nitrogen utilization between protein sources in humans. J Nutr 2003;133:2733–40.

[6] FDA, Grade "A" Pasteurized Milk Ordinance (2013 Revision), at Appendix N ("Drug Residue Testing and Farm Surveillance – Industry Responsibilities – Monitoring and Surveillance").

separate months, except when three (3) months show a month containing two (2) sampling dates separated by at least twenty (20) days. Samples collected under this random sampling program shall be analyzed as specified by FDA." The Notifier follows PMO guidelines with respect to monitoring for contaminants during the production of alpha-lactalbumin.

III. Estimated Consumption of Alpha-Lactalbumin (21 C.F.R. § 170.235)

Alpha-lactalbumin is intended for use as a food ingredient for functional (texturizer) or nutritional (high-quality protein) purposes in Nutritional Products and Dairy and Dairy-Based Products. Specifically, alpha-lactalbumin is intended for use at up to 20% w/w in Powdered Nutritional Beverages and Sports Beverages, 25% w/w in Nutritional Bars, and 10% w/w in Milk Products (including dairy beverages).

Due to the relative novelty of high purity alpha-lactalbumin products, specific consumption data are not available at this time. However, because alpha-lactalbumin products are equivalent to traditional whey protein products from the standpoint of nutritional properties and safety, and because alpha-lactalbumin products effectively will substitute for traditional whey protein products in the marketplace, we anticipate no issues related to dietary exposure.

As a conservative numeric estimate, we have calculated dietary exposure using data available for an analogous dairy protein product, micellar casein. In 2010 the total domestic market for micellar casein (all protein levels) was estimated to be 5,000 metric tons (5.0×10^6 kg).[7] The total population of the United States in 2010 was about 310 million people.[8] The mean daily consumption of micellar casein per capita is as follows:

$$5.0 \times 10^6 \text{ (kg/year)} \times 10^3 \text{ (g/kg)} \div 310 \times 10^6 \text{ (persons)} \div 365 \text{ (days/year)} = 0.044 \text{ g/person/day}$$

We conservatively assume that the protein content of the micellar casein comprises 90% of the product. The mean daily protein intake from micellar casein per capita would thus be:

$$0.044 \text{ g/person/day} \times 90\% = 0.040 \text{ g/person/day}$$

If we assume that the entire amount of micellar casein produced in the United States is consumed by only 10% of the population ("eaters-only"), the daily consumption of micellar casein per capita for the eaters-only population would be 0.44 g/person/day, and the daily protein intake from micellar casein per capita would thus be 0.40 g/person/day. **Table 4** provides recommendations for milk intake taken from GRN000504 for concentrated milk proteins:

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[7] Leprino Foods Company, GRN000633 regarding Whey Protein Concentrate, available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm505250.pdf (last accessed November 28, 2017), citing DH Business Consulting and Associates, Micellar casein ingredients business & market analysis (2013).

[8] The 2017 population of the United States is slightly higher than this figure, but we have used a 2010 population estimate to remain conservative and consistent with the available market data for micellar casein.

Table 4. Recommendations for Milk Intake

Male and Female Age Groups	Number of 8 ounce cups per Day	Milk Protein Equivalent grams/daily
<1-3 years	1-2	8-16
4-8 years	2-3	16-24
9-18 years	4	32
19-50 years	3	24
51+ years	3	24

The recommended daily protein intake from milk for the general population, excluding infants, ranges between 8 and 32 grams per day, depending on age cohort. In addition, FDA has established a Daily Reference Value (DRV) of 50 g/day for protein for adults and children aged 4 or older.[9] The Institute of Medicine (IOM) has established a Recommended Dietary Allowance (RDA) for protein of 56 g/day for adult males and 46 g/day for adult females.[10] The estimated daily protein intake from micellar casein is approximately 0.040 g/person/day, which is a fraction of the recommended protein intake. Even considering the eaters-only population, which provides the most conservative estimate of consumption, the daily protein intake from micellar casein of 0.40 g/person/day is far less than the recommended protein intake described above. This data serves as a proxy for an alpha-lactalbumin consumption estimate, and they indicate there is no safety concern at the intended use levels.

Most of the population's protein intake is derived from, and will continue to be derived from unprocessed foods, including meat, poultry, fish, and legumes. Moreover, for those processed foods to which the alpha-lactalbumin will be added, there are competitive products on the market. Thus, the addition of alpha-lactalbumin simply will serve as a replacement for these other competitive protein sources and will not increase consumer exposure to protein. Therefore, we do not realistically expect that the actual consumption of foods containing alpha-lactalbumin will contribute to a significant portion of total protein intake.

IV. Self-Limiting Levels of Use (21 C.F.R. § 170.240)

The use of alpha-lactalbumin is not self-limiting. The maximum use levels in food are described above.

V. Experience based on common use in food before 1958 (21 C.F.R. § 170.245)

While the basis for this GRAS Notice is scientific procedures, rather than common use in food, we note that alpha-lactalbumin is a component of milk. Milk and products derived from milk, such as whey, have a long history of safe consumption by humans at all ages in the form of fluid milk, in dried form (*i.e.*, milk powder), or as milk-derived ingredients. Therefore, the

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[9] 21 C.F.R. § 101.9(c)(9)

[10] Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Total Water and Macronutrients. Food and Nutrition Board, Institute of Medicine, National Academies, available at https://www.ncbi.nlm.nih.gov/books/NBK56068/table/summarytables.t4/?report=objectonly (last accessed on November 28, 2017).

history of milk consumption provides supplemental support for the safety of alpha-lactalbumin's intended use.

VI. GRAS Notice Narrative (21 C.F.R. § 170.250)

As discussed further below, several substances that are similar to alpha-lactalbumin have already been affirmed as GRAS or have been the subject of GRAS Notices. Milk proteins are classified under two major groups: whey proteins (20%) and caseins (80%).[11] Whey proteins, in particular, are the soluble proteins that remain when milk coagulates. Like the GRAS substances described below, the alpha-lactalbumin that is the subject of the current GRAS Notice is manufactured through physical separation techniques. Therefore, the constituents of the final product are no different in substance than the other milk protein products described below. Due to the similarities between alpha-lactalbumin and the substances described below, FDA's acceptance of the GRAS status of the following substances has direct implications for the GRAS status of alpha-lactalbumin.

Whey protein concentrate is GRAS affirmed at 21 C.F.R. § 184.1979(c). The regulation states that whey protein concentrate is the substance obtained by the removal of sufficient non-protein constituents from whey so that the finished dry product contains no less than 25% total protein. Whey protein concentrate is produced by physical separation techniques, such as precipitation, filtration, or dialysis. As with whey, whey protein concentrate can be used as a fluid, concentrate, or dry product form.

In GRN000011, FDA had no questions regarding the GRAS determination of a "mixture of calcium casein peptone and calcium phosphate (CCP-CP)" for use as a texturizer in chewing gum at a level not to exceed 5%.[12] CCP-CP is produced by the enzymatic hydrolysis of casein to form casein peptone, which is then complexed with amorphous calcium phosphate to form a calcium casein peptone-calcium phosphate complex. Casein peptones have been GRAS affirmed for the direct addition to human foods at 21 C.F.R. § 184.1553.

In GRN000037, FDA had no questions regarding the GRAS determination of "whey protein isolate" for use in high-energy food and beverage products, such as yogurt, pudding, ice cream, margarine, and mayonnaise. Similarly, in GRN000037, FDA had no questions regarding the GRAS determination of "dairy product solids" for use in a variety of foods and in the production of alcohol and organic chemicals, galactose and glucose syrups, and sugar and corn syrup replacers. Both whey protein isolate and dairy product solids are manufactured using

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[11] *See, e.g.*, Institute of Food Technologists, Food Chemistry Experiments, IFT Experiments in Food Science Series, at page 3-1, available at http://www.ift.org/~/media/Knowledge%20Center/Learn%20Food%20Science/Experiments/TeacherGuidePROTEINS.pdf (last accessed on November 28, 2017).

[12] Bonlac Foods Limited, GRN000011 (regarding Calcium casein peptone-calcium phosphate), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm264465.pdf (last accessed on November 28, 2017), and January 29, 1999 FDA Response to GRN000011, available at http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm154906.htm (last accessed on November 28, 2017).

physical separation techniques involving the application of membrane filtration systems and optional dialysis to process whey.[13]

In GRN000052, FDA had no questions regarding the GRAS determination of "whey mineral concentrate" for use as a source of calcium in fortified beverages, fortified foods, and enriched dairy products. Whey mineral concentrate is produced by subjecting pasteurized fluid whey to a precipitation and membrane separation process, followed by purification and drying. The resulting concentrate is a free-flowing white powder that is soluble at acid pH.[14]

In GRN000196, FDA did not object to the determination that "bovine milk basic protein fraction (BMBPF)" is GRAS for use in cottage cheese, imitation milk (including rice and soy milk), juice, meal replacement bars and drinks, milk, processed cheese, salad dressing, and yogurt at levels of up to 40% in some of the applications. BMBPF is produced from pasteurized bovine skim milk that is applied to a cation exchange chromatographic column, removing acid milk proteins and lactose. The basic proteins remaining on the column are eluted from the resin using sodium chloride. The resulting eluate is concentrated and dialyzed to produce BMBPF solids. These BMBPF solids are then crushed and packaged.[15]

In GRN000504, FDA had no questions regarding the GRAS determination related to "milk protein concentrate" containing 42 to 85% protein and "milk protein isolate" containing greater than 90% protein produced by ultrafiltration of skim milk.[16] The concentrated milk proteins described in GRN000504 were determined to be GRAS for use as ingredients in: meal replacements and meal supplements, milk products including milk drinks, yogurt, fermented milks, spreads, dips; non-standardized cheese products; dairy product analogs; frozen dairy desserts and mixes; desserts and mousses; confections and frostings; snack foods; coatings and fillings; salad dressings; soups and soup mixes; and sauces.

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[13] American Dairy Products Institute, GRN000037 (regarding whey protein isolate and dairy product solids), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm266140.pdf (last accessed on November 28, 2017), and April 21, 2000 FDA Response to GRN000037, available at http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm154133.htm (last accessed on November 28, 2017).

[14] Glanbia Ingredients, GRN000052 (regarding whey mineral concentrate), available at https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=52 (last accessed on November 28, 2017), and January 30, 2991 FDA Response to GRN000052, available at http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm153729.htm (last accessed on November 28, 2017).

[15] Snow Brand Milk Products, GRN000196 (regarding bovine milk basic protein fraction), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm263904.pdf (last accessed on November 28, 2017), and September 1, 2006 FDA Response to GRN000196, available at http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm154673.htm (last accessed on November 28, 2017).

[16] American Dairy Products Institute, GRN000504 (regarding whey proteins), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm400536.pdf (last accessed on November 28, 2017), and November 21, 2014 FDA Response to GRN000504, available at https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm427497.htm (last accessed on November 28, 2017).

Finally, in GRN000633, FDA had no questions regarding the GRAS determination related to "whey protein" consisting of concentrated milk protein with a ratio of ≥60 whey to 40 casein for use as an emulsifier, flavoring agent, formulation aid, humectant, stabilizer, thickener, texturizer, and protein source in meal replacements and meal supplements; powdered nutritional beverages; nutritional bars; acidified sports beverages; milk products; yogurt and fermented milk products; non-standardized cheese products; spreads, dips and cream substitutes; frozen dairy desserts and mixes; desserts and mousses; confections; snack foods; coatings and fillings; salad dressings; soups, soup mixes, and sauces.[17]

The GRAS Affirmations and Notices above exhibit FDA's confidence in the safety of these milk-derived ingredients. Similar to the milk-derived ingredients above, alpha-lactalbumin is produced using physical processes that do not present any safety concerns that have not already been addressed in the existing, favorably-reviewed GRAS Affirmations and Notices discussed above.

A. Safety Overview

Due to the substantial similarities between alpha-lactalbumin and traditional whey protein, as well as the substantial similarities between these products and the concentrated milk proteins that are the subject of GRN000504, the safety discussion related to the latter group of products is directly applicable to establishing the safety and GRAS status of alpha-lactalbumin. We incorporate the safety overview provided in GRN000504 by reference and highlight the relevance of those safety data to our assessment of alpha-lactalbumin below.

1. Human Consumption of Milk Protein

The raw material used in the manufacture of alpha-lactalbumin is milk or skim milk. Milk and products derived from milk, such as whey, have a long history of safe consumption by humans at all ages in the form of fluid milk, in dried form (*i.e.*, milk powder), or as milk-derived ingredients.

2. Purification of Alpha-Lactalbumin

Alpha-lactalbumin is manufactured using safe and well-characterized physical separation techniques that are analogous to the processes employed in the manufacture of the whey protein concentrate and whey protein isolate products described above. Such physical separation processes do not cause substantive alterations to the chemical character and safety-related properties of the constituents. The food additives that may be utilized in the manufacturing process for alpha-lactalbumin are all either approved food additives or GRAS food ingredients for these applications and are used in accordance with food GMPs. The manufacturing process does not generate, concentrate, or introduce any potential toxicants. As a result, the alpha-lactalbumin is as safe as milk itself.

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[17] Leprino Foods, GRN000633 (regarding concentrated milk proteins), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm505250.pdf (last accessed on November 28, 2017), and September 21, 2016 FDA Response to GRN000633, available at https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm528187.htm (last accessed on November 28, 2017).

3. Safety Studies on Alpha-Lactalbumin

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, whom we address immediately below – 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.

4. Allergenicity of Milk Protein

An allergy to milk is among the eight most common food allergies.[18] Because the substances are chemically identical, milk and isolated milk proteins will produce a milk protein allergy when consumed. All concentrated milk protein ingredients, including alpha-lactalbumin, will clearly indicate that the product is derived from milk protein and will inform those consumers who are allergic to milk and satisfy food allergen labeling requirements.

For any food containing alpha-lactalbumin, the label will bear a statement indicating that the product has been derived from a milk source to satisfy allergen labeling requirements.

5. Lactose Intolerance

Lactose intolerance is the inability or insufficient ability to digest lactose, a sugar found in milk and milk products. Lactose intolerance is caused by a deficiency of the enzyme lactase, which is produced by the cells lining the small intestine. Lactase breaks down lactose into two simpler forms of sugar called glucose and galactose, which are then absorbed into the bloodstream. People with lactose intolerance may feel uncomfortable 30 minutes to 2 hours after consuming milk and milk products. Symptoms range from mild to severe based on the amount of lactose consumed and the amount a person can tolerate. Common symptoms include abdominal pain, abdominal bloating, gas, diarrhea, and nausea.[19]

Research indicates that most people with lactose intolerance are able to consume the amount of lactose in up to two cups of milk a day if taken with meals, one at breakfast and the other at dinner. Other dairy foods, such as aged cheese and yogurts are also well-tolerated because lactose is converted to lactic acid by select microorganisms during the making of the products.[20]

The percentage of lactose is inversely related to the protein content of the concentrated milk protein. The percentage of lactose in alpha-lactalbumin products is <0.2%. This level is

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[18] "Eight major foods or food groups – milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans– account for 90% of food allergies." Pub. L. 108-282, title II § 202(1)(2)(A) (Aug. 2, 2004).

[19] Lactose Intolerance, National Digestive Diseases Information Clearinghouse (NDDIC), available at http://digestive.niddk.nih.gov/ddiseases/pubs/lactoseintolerance/ (last accessed on November 28, 2017).

[20] National Dairy Council, Handbook of Dairy Foods and Nutrition 6 (3rd ed. 2006).

order of magnitude lower than the level of lactose in Nonfat Dry Milk, which is around 49.0-52.3%.[21] Therefore, we do not anticipate any unique impact on lactose sensitive populations.[22]

B. Summary of Basis for GRAS Determination

Agropur has determined that alpha-lactalbumin is Generally Recognized as Safe (GRAS) based on the following:

- The fact that alpha-lactalbumin is manufactured under current good manufacturing practices (cGMP) for food (21 C.F.R. Part 110) and meets appropriate food grade specifications;
- That potential contaminants, such as pesticides and heavy metals, are either absent (not detected) or below toxicological and regulatory limits;
- The digestibility and nutritional quality of alpha-lactalbumin;
- The intended uses and the estimated consumption of alpha-lactalbumin;
- The proper labeling of the products;
- Supportive evidence from the long history of safe use of milk and milk protein as food; and
- Supportive evidence from the successful GRAS Notice for concentrated milk proteins, GRN000504.

VII. List of supporting data and information in your GRAS Notice (21 C.F.R. § 170.255)

1. American Dairy Products Institute, GRN000037 (regarding whey protein isolate and dairy product solids), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm266140.pdf (last accessed on November 28, 2017), and April 21, 2000 FDA Response to GRN000037, available at http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm154133.htm (last accessed on November 28, 2017).
2. American Dairy Products Institute, GRN000504 (regarding whey proteins), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm400536.pdf (last accessed on November 28, 2017), and November 21, 2014 FDA Response to GRN000504, available at https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm427497.htm (last accessed on November 28, 2017).

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[21] The Really Big List of Lactose Percentages, available at http://www.stevecarper.com/li/list_of_lactose_percentages.htm (last accessed on November 28, 2017).

[22] Additional information on lactose intolerance can be found in the scientific status report from the National Dairy Council, Science Summary: Dairy & Lactose Intolerance (February 23, 2016), available at https://www.nationaldairycouncil.org/content/2015/science-summary-dairy-and-lactose-intolerance (last accessed on November 28, 2017).

3. Bonlac Foods Limited, GRN000011 (regarding Calcium casein peptone-calcium phosphate), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm264465.pdf (last accessed on November 28, 2017), and January 29, 1999 FDA Response to GRN000011, available at http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm154906.htm (last accessed on November 28, 2017).
4. Bos C, Gaudichon C, Tome D. Nutritional and physiological criteria in the assessment of milk protein quality for humans. J Am Coll Nutr 2000;19(suppl):191S–205S.
5. Bos C, Mahe S, Gaudichon C, et al. Assessment of net postprandial protein utilization of 15N-labelled milk nitrogen in human subjects. Br J Nutr 1999;81:221–6.
6. Bos C, Metges CC, Gaudichon C, et al. Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. J Nutr 2003;133:1308–15.
7. Cook BB, Morgan AF, Singer B, Parker J. The effect of heat treatment on the nutritive value of milk proteins. II. Rat growth studies with casein and lactalbumin and their lactose derivatives. J Nutr 1951;44:63–81.
8. Dietary protein quality evaluation in human nutrition: Report of an FAO Expert Consultation, FAO Food and Nutrition Paper 92 (FAO, 2013).
9. Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Total Water and Macronutrients. Food and Nutrition Board, Institute of Medicine, National Academies, available at https://www.ncbi.nlm.nih.gov/books/NBK56068/table/summarytables.t4/?report=objectonly (last accessed on November 28, 2017).
10. FDA, Grade "A" Pasteurized Milk Ordinance (2013 Revision), at Appendix N ("Drug Residue Testing and Farm Surveillance – Industry Responsibilities – Monitoring and Surveillance").
11. Gaudichon C, Mahe S, Benamouzig R, et al. Net postprandial utilization of [15N]-labeled milk protein nitrogen is influenced by diet composition in humans. J Nutr 1999;129:890–5.
12. Gausseres N, Mahe S, Benamouzig R, et al. [15N]-labeled pea flour protein nitrogen exhibits good ileal digestibility and postprandial retention in humans. J Nutr 1997;127:1160–5.
13. Gilani GS, Sepehr E. Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. J Nutr 2003;133:220–5.
14. Glanbia Ingredients, GRN000052 (regarding whey mineral concentrate), available at https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=52 (last accessed on November 28, 2017), and January 30, 1991 FDA Response to GRN000052, available at http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm153729.htm (last accessed on November 28, 2017).

15. Institute of Food Technologists, Food Chemistry Experiments, IFT Experiments in Food Science Series, at page 3-1, available at http://www.ift.org/~/media/Knowledge%20Center/Learn%20Food%20Science/Experiments/TeacherGuidePROTEINS.pdf (last accessed on November 28, 2017).

16. Joint FFAO/WHO Food Standards Programme Codex Committee on Nutrition and Foods for Special Dietary Uses, Thirty-Eighth Session (Hamburg, Germany, 5 – 9 December 2016), Review Of The Standard For Follow-Up Formula (Codex Stan 156-1987), Physical Working Group Report, at http://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-720-38%252FCRDs%252FCRD_13.pdf (last accessed November 28, 2017).

17. Lactose Intolerance, National Digestive Diseases Information Clearinghouse (NDDIC), available at http://digestive.niddk.nih.gov/ddiseases/pubs/lactoseintolerance/ (last accessed on November 28, 2017).

18. Leprino Foods Company, GRN000633 regarding concentrated milk proteins, available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm505250.pdf (last accessed November 28, 2017), citing DH Business Consulting and Associates, Micellar casein ingredients business & market analysis (2013).

19. Leprino Foods, GRN000633 (regarding concentrated milk proteins), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm505250.pdf (last accessed on November 28, 2017), and September 21, 2016 FDA Response to GRN000633, available at https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm528187.htm (last accessed on November 28, 2017).

20. Morens C, Bos C, Pueyo ME, et al. Increasing habitual protein intake accentuates differences in postprandial dietary nitrogen utilization between protein sources in humans. J Nutr 2003;133:2733–40.

21. National Dairy Council, Handbook of Dairy Foods and Nutrition 6 (3rd ed. 2006).

22. National Dairy Council, Science Summary: Dairy & Lactose Intolerance (February 23, 2016), available at https://www.nationaldairycouncil.org/content/2015/science-summary-dairy-and-lactose-intolerance (last accessed on November 28, 2017).

23. Pub. L. 108-282, title II § 202(1)(2)(A) (Aug. 2, 2004).

24. Rutherford et al., Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J Nutr. 2015 Feb;145(2):372-9. 2014 Nov 26, summary available at https://www.ncbi.nlm.nih.gov/pubmed/25644361 (last accessed November 28, 2017).

25. Rutherfurd SM, Moughan PJ. The digestible amino acid composition of several milk proteins: application of a new bioassay. J Dairy Sci 1998; 81:909–17.

26. Schaafsma G. The Protein Digestibility-Corrected Amino Acid Score. J Nutr 2000; 130:1865S-1867S (internal citations omitted).

27. Snow Brand Milk Products, GRN000196 (regarding bovine milk basic protein fraction), available at https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm263904.pdf (last accessed November 28, 2017), and September 1, 2006 FDA Response to GRN000196, available at http://www.fda.gov/food/ingredientspackaginglabeling/gras/noticeinventory/ucm154673.htm (last accessed on November 28, 2017).

28. The Really Big List of Lactose Percentages, available at http://www.stevecarper.com/li/list_of_lactose_percentages.htm (last accessed on November 28, 2017).

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Based on the documentation provided in this GRAS Notice, and as discussed above, Agropur Inc. has concluded that alpha-lactalbumin is GRAS based on scientific procedures for use in nutritional products and dairy-based products.

KELLER AND HECKMAN LLP
Serving Business through Law and Science®

1001 G Street, N.W.
Suite 500 West
Washington, D.C. 20001
tel. 202.434.4100
fax 202.434.4646

Writer's Direct Access
Richard F. Mann
(202) 434-4229
mann@khlaw.com

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Via Electronic Mail and Mail

Stephen DiFranco, PhD
Consumer Safety Officer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Petition Review
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740
Stephen.difranco@fda.hhs.gov

Re: Agropur, Inc.'s GRAS Notice No. GRN 000763

Dear Dr. DiFranco:

We are writing to respond to a series of questions posed by FDA with respect to GRAS Notice No. GRN 000763 for α-lactalbumin derived from cow's milk.[1] For ease of reference, we reproduce each question below, followed by the relevant response.

Part 1

1. *In the notice proposes uses of α-lactalbumin (α-lactalbumin or α-La) in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.*

We can confirm that α-La will only be added to foods that are the subject of a standard of identity if the applicable standard of identity would permit for the use of the ingredient.

[1] Letter from S. DiFranco to R. Mann (June 24, 2018).

2. *For premixes (e.g. powdered nutritional beverages), please indicate if the use level is expressed on an as consumed basis.*

For the premixes, the use level is expressed not on an as-consumed basis but rather as a percentage of the powdered beverage on a dried basis. The percentage of α-La in the beverage on an as-consumed basis will vary based on the beverage mix reconstitution instructions.

Part 2

1. *Please provide a statement that the ingredient meets the specifications of α-lactalbumin published in the Food Chemicals Codex (FCC 11).*

We can confirm that Agropur's α-La meets the specifications for α-La under FCC 11.

2. *Please provide information about the molecular weight, isoelectric point, structure and stability (e.g., to acid, heat) of α-lactalbumin and whether it is present in the apo- and/or holo-form in the final product described in the notice. We note that the literature describes multiple methods of separation of α-lactalbumin from other milk proteins on the basis of the molecular weight, Ca2+ binding, or isoelectric point (Lindahl and Vogel, 1984; Kamau* et al.*, 2010; Neyestani* et al. *2003).*

The molecular weight of Agropur's α-La is approximately 14,000 Daltons. Agropur's is the holo- form of α-La. The iso-electric point is approximately 4.3; it is in the range of 4.2-4.5. Structurally, the molecule is composed of 123 amino acids, the sequence of which is publicly available.[2]

In terms of heat stability, Agropur has determined that α-La is heat stable at typical concentrations of 2-5% in aqueous solutions. The heat stability is impacted by presence of other

[2] Brew, Keith, *et al.*, The Complete Amino Acid Sequence of Bovine α-Lactalbumin, The Journal of Biological Chemistry 245, 4570-4582. September 10, 1970, available at http://www.jbc.org/content/245/17/4570.full.pdf.

molecules and ions that may interact with α-La, as well as the pH.[3] A-lactalbumin is acid-stable and forms a clear solution at pH below 4.0.[4]

3. *Please provide a brief description of how α-lactalbumin differs from other whey proteins. It is not clear from the notice why Agropur would isolate α-lactalbumin from other whey proteins for addition to particular foods, particularly if it has a PDCAAS similar to whey, as indicated in the notice. Please provide publicly-available information about the use and functionality (in food) of this ingredient. (We note several references are available, including Layman* et al.*, 2018; Barbana* et al.*, 2011; Chatterton* et al.*, 2006; Ipsen and Otte, 2007).*

There are benefits to using α-lactalbumin in place of whey protein generally because of its heat stability.[5] Although α-lactalbumin accounts for approximately 15 to 20% of total protein in whey protein concentrates, α-lactalbumin possesses enhanced heat stability compared to many of the other protein present in whey, such as lactoglobulin. As a result, and because it is characterized by a PDCAAS similar to whey in general, α-lactalbumin can be used as a replacement for whey protein concentrates in applications in which enhanced heat stability is required. In addition, *a*lpha-lactalbumin is rich in the amino acid tryptophan. Some food formulations that target delivery of higher amounts of tryptophan can use alpha-lactalbumin as a replacement for whey protein isolate.

a.1. Was it your intent to incorporate these method references from the GRN 000504 amendments in GRN 000763? If so, please provide a statement in your response indicating applicable references.

Yes, we incorporate by reference the methods in GRN 000504.

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[3] Permyakov, Eugene A., and Lawrence J. Berliner. "α-Lactalbumin: structure and function." FEBS letters 473.3 (2000): 269-274.

[4] Boye, Joyce I., Inteaz Alli, and Ashraf A. Ismail. "Use of differential scanning calorimetry and infrared spectroscopy in the study of thermal and structural stability of α-lactalbumin." Journal of Agricultural and Food Chemistry 45.4 (1997): 1116-1125.

[5] Layman et al. (2018); Crowley SV, Dowling AP, Caldeo V, Kelly AL, O'Mahony JA, "Impact of α-lactalbumin:β-lactoglobulin ratio on the heat stability of model infant milk formula protein systems." Food Chem. 2016 Mar 1;194:184-90.

a.2. Are both microfiltration and ultrafiltration used in the GRN 000763 method of manufacture? If so, please discuss. The description of membrane filtration is not adequate without discussion of membrane pore size or components of milk that are separated.

Yes, both microfiltration and ultrafiltration are used to individually separate different molecular weight components present in the whey streams. The typical pore size for microfiltration is 100-500 kD, and the typical pore size of the ultrafiltration membrane is 1-50 kD.

a.3. Is electrodialysis used in the GRN 000763 method of manufacture? If so, please discuss.

a.4. Are enzymes used in the GRN 000763 method of manufacture? If so, please discuss.

Neither electrodialysis nor enzymes are utilized in purification of α-lactalbumin by Agropur. Chromatography is utilized to isolate the whey proteins from non-protein components, as a core step in isolating/fractionating α-lactalbumin.

b.1. Was it your intent to incorporate references cited in GRN 000633 and its amendments into GRN 000763? If so, please provide a statement in your response indicating applicable references.

Yes, given the similarity in the identity and production of whey protein addressed in GRN 000633 and α-lactalbumin, we would like to incorporate the references cited in GRN 000633 into GRN 000763.

c.1. Please discuss use of chromatography for α-lactalbumin purification, including published literature, previous GRNs (e.g., GRNs 196, 669), or other publicly available references.

Agropur has chosen to utilize our core expertise in chromatography and isolation technologies to manufacture a higher purity α-lactalbumin. *See* Ye X, Yoshida S, Ng TB. 2000. Isolation of lactoperoxidase, lactoferrin, α-lactalbumin, β-lactoglobulin B and β-lactoglobulin A from bovine rennet whey using ion exchange chromatography. Int J Biochem Cell Biol. 2000 Nov-Dec;32(11-12):1143-50.

d.1. Please cite regulations or effective food contact notifications supporting use of these ion-exchange materials for fluid milk or milk protein products.

The resins used in Agropur's process comply with 21 CFR 173.25 ("Ion-exchange resins"), paragraph (a)(20). These resins comply with related provisions under paragraphs (b)(5) and (d)(2) of Section 173.25.

e.2. Are added salts removed during the method of manufacture?

As part of the ultra- and dia-filtration processes used in purification, sodium chloride is partitioned and removed from the α-lactalbumin stream. Some of the calcium is bound to the proteins and is not removed.[6]

f.1. Please provide a statement that the starting material or subsequent whey protein is pasteurized.

As stated in Section II.A (page 6 of GRN 000763), "To ensure pathogen control and compliance with regulatory requirements, the clarified and separated liquid sweet dairy whey is pasteurized in accordance with Pasteurized Milk Ordinance (PMO) requirements. Specifically, the product is pasteurized using High Temperature Short Time (HTST) at a minimum temperature of 161°F for a minimum of 15 seconds."

f.2. Please identify the organic solvent used, state whether it is food grade, and discuss residual levels of solvent in the finished product.

We clarify that no organic solvents are used in this process. Our primary source for alpha lactalbumin is whey from cheesemaking. Alternatively, milk may also be used as the source. Agropur uses an aqueous salt solution to precipitate proteins other than alpha-lactalbumin.

g.1. There is a statement in the notice that "major whey proteins are fractionated by ion exchange to remove extraneous components, resulting in a purified liquid protein." Referring to the flow diagram on page 7, ion exchange is shown as a processing step used only for whey derived from cheese making Is this correct? If yes, how are extraneous component proteins removed when whey is obtained directly from milk?

Ion exchange is used to remove non-protein extraneous components (lactose, minerals, and some unwanted proteins) from the whey stream. Further membrane processing is needed to separate and concentrate individual proteins.

h.1. *Please provide statement regarding food-grade milk starting material and compliance with 21 CFR 1240.61 and applicable limits for pesticides, veterinary drugs, and PCBs.*

The milk starting material for Agropur's α-lactalbumin is food grade and produced in accordance with good agricultural practices. As such, it meets applicable state and federal regulations,

[6] Calcium Binding by α-Lactalbumin in Human Milk and Bovine Milk, Lonnerdal B, and Glazier C. 1985. The Journal of Nutrition 115:9 pages 1209-1216.

including regulatory limits on veterinary drug residues, polychlorinated biphenyls (PCBs), and pesticides.

Part 3

1. *Please revise your exposure estimate to include discussion of a comparable milk protein or use intake data from a recent U.S. food survey of individuals to estimate intake of the GRN 000763 ingredient.*

2. *In your exposure discussion, please address background intakes of α-lactalbumin from consumption of whey ingredients and milk-based foods. What is the total estimated intake from background levels plus proposed use of α-lactalbumin ingredient?*

As a conservative numeric estimate for background consumer exposures to α-lactalbumin, we have used data concerning the consumption of dry whey and whey protein concentrates, of which α-lactalbumin is a significant component,[7] as well as the consumption of milk.[8] In this regard, the per capita dry whey and whey protein concentrates consumption is estimated to be approximately 2.9 g/person/day. Approximate intakes for high-end users (typically considered to be 90th percentile users) could be twice this number – 5.8 g/person/day based on an FDA approximation for widely used additives.[9] As stated in Section II of GRN 000763, α-lactalbumin is one of the four major whey proteins present in cow's milk and constitutes 20-30% of the overall protein content contributed by the whey proteins present in milk. Accordingly, the 90th percentile users are expected to consume 1.2–1.7 g α-lactalbumin/person/day. According to the USDA's ERS, the per capita fluid milk consumption is estimated to be 154 lbs/person/year,

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[7] Whey Protein Concentrate – Agricultural Marketing Service USDA - https://www.ams.usda.gov/sites/default/files/media/Whey%20Protein%20Concentrate%20TR.pdf.

[8] USDA data on per U.S. annual per capita consumption of dairy products from July 27, 2018 indicates a whey consumption of 2.3 lbs/person/year. (The spreadsheet with the relevant data is *available at* https://www.ers.usda.gov/data-products/dairy-data/ (last accessed July 27, 2018).) This corresponds to a daily consumption of 2.9 g/person/day, calculated as follows:

2.3 lbs/person/year x 453.59 g/lb ÷ 365 days/year = 2.9 g/person/day

[9] *See* Guidance for Industry: Estimating Dietary Intake of Substances in Food (Aug. 2006), available at http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/foodingredientsandpackaging/ucm074725.htm (last accessed July 27, 2018).

which corresponds to 191 g/person/day.[10] The 90th percentile consumer of milk is expected to consume twice this amount, or 382 g/person/day. As whey proteins comprise approximately 0.6% of liquid milk, the 90th percentile consumption of whey proteins as a component of liquid milk is estimated to be 2.3 g/person/day. The consumption of α-lactalbumin from liquid milk is expected to be 0.5–0.7 g/person/day.

The total consumption (90th percentile) of whey proteins from both dry whey/whey protein concentrates and liquid milk is 8.1 g/person/day, of which 1.7 – 2.4 g/person/day is α-lactalbumin. As the subject of this notification, α-lactalbumin, will simply serve as a replacement for dry whey and whey protein concentrates, it is not expected to significantly increase consumer exposures to such substances. When used as a replacement for dry whey and whey protein concentrates, the 90th percentile consumer of α-lactalbumin is expected to consume approximately 5.8 g/person/day; although this does represent an increase compared to background levels of exposure to α-lactalbumin, the overall exposure to whey proteins, generally, is expected to remain the same.

3. *Please restate your conclusion of general recognition of safety α-lactalbumin for its intended use based on more representative estimates of intake of this ingredient.*

FDA has established a Daily Reference Value (DRV) of 50 g/day for protein for adults and children aged 4 or older.[11] The Institute of Medicine has established a Recommended Dietary Allowance (RDA) for protein of 56 g/day for adult males and 46 g/day for adult females.[12] The estimated daily protein intake for α-lactalbumin (*i.e.*, 2.9 g/person/day for average users and 5.8

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[10] *See* USDA, ERS Dairy Products: Per capita consumption, United States (Annual), at https://www.ers.usda.gov/data-products/dairy-data/ (last accessed July 27, 2018). Our calculation is as follows:

154 lbs/person/year x 453.59 g/lb ÷ 365 days/year = 191 g/person/day

[11] FDA Guidance for Industry: Nutrition Labeling Manual – A Guide for Developing and Using DataBases, at https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm063113.htm (last accessed July 27, 2018).

[12] Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Total Water and Macronutrients. Food and Nutrition Board, Institute of Medicine, National Academies, at http://www.nationalacademies.org/hmd/~/media/Files/Activity%20Files/Nutrition/DRI-Tables/3_RDA%20AI%20AMDR%20Values_Total%20Water%20and%20Macronutr.pdf?la=en (last accessed July 27, 2018).

g/person/day for 90th percentile users) is a fraction of the recommended protein intake. As noted above, because α-lactalbumin will serve as a replacement for competing protein sources on the market such as dry whey and whey protein concentrates, both of which contain significant quantities of α-lactalbumin, we do not realistically expect that the actual consumption of lactalbumin will increase significantly, nor do we expect that the actual consumption of foods containing such protein products will contribute to a significant portion of total protein intake. More specifically, because the α-lactalbumin will replace the consumption of whey proteins generally, the 5.8 g/person/day exposure estimated for α-lactalbumin is expected to result in no net increase in exposures to whey and/or other milk proteins in the diet overall.

The GRAS conclusion is provided in Section VI.B of GRN 000763. To the list of the bases of our GRAS conclusion, we add:

- The intended use of α-lactalbumin is as a replacement for dry whey and whey protein concentrates; at an exposure of up to 5.8 g/person/day, this represents a small fraction of the total daily protein intake of 50 g and is expected to result in no net increase in the consumption of whey and/or other milk proteins.

* * *

We trust that we have fully responded to your questions, but we stand ready to provide you and your colleagues with responses to any additional questions or requests for information.

Sincerely,

[redacted]

Richard F. Mann
Natalie E. Rainer
Counsel to Agropur, Inc

Name	Batch No.	Date of Manuf.	Calcium, mg/100g	mg/100g	Lead, PPM	Beta-lactoglobulin*, %
FCC Specification		As stated =>	NMT 3.5%	NMT 700 ug/g	NMT 0.5 mg/Kg	NMT 6.5%
FCC Specification		Converted =>	NMT 3,500 mg/100g	NMT 70 mg/100g	NMT 0.5 PPM	
Alpha-Lactalbumin	JE 023-7-414	25-May-17	225	65	None Detected	5.9
Alpha-Lactalbumin	JE 027-7-414	31-May-17	185	45	None Detected	6.0
Alpha-Lactalbumin	JE 046-18-414	26-Jun-18	214	43	None Detected	4.2
Alpha-Lactalbumin	JE 047-18-414	26-Jun-18	85	40	None Detected	4.0
Alpha-Lactalbumin	JE 048-18-414	1-Jul-18	220	52	None Detected	6.0
Alpha-Lactalbumin	JE 051-18-414	1-Jul-18	238	56	None Detected	3.5

**** Beta-lactoglobulin % (protein basis), as tested by Reverse Phase HPLC***

From:	Edwards, Alison
To:	DiFranco, Stephen
Subject:	FW: GRN 000763 remaining chemistry questions
Date:	Wednesday, December 05, 2018 6:20:34 PM
Attachments:	image001.png

For the record…

From: Mann, Richard F. <Mann@khlaw.com>
Sent: Monday, December 03, 2018 4:12 PM
To: Edwards, Alison <Alison.Edwards@fda.hhs.gov>
Cc: Rainer, Natalie <rainer@khlaw.com>
Subject: RE: GRN 000763 remaining chemistry questions

Dear Dr. Edwards,

Thank you for sending us the questions on the subject GRAS Notice, for which you require some additional clarification. We have repeated the questions below, along with our response to each.

1. Please provide a limit of detection for lead, where it is noted in your analyses provided October 1, 2018 as "none detected".

The analyses provided on October 1, 2018, indicating that lead was not detected, had a limit of detection of <0.02 mg/kg for lead, as well as a <1.0 for heavy metals, as lead.

2. Please clarify the methods used for the analyses provided October 1, 2018. If all values provided in this table were obtained with the methods described in the FCC 11 monograph for a-lactalbumin, please provide a statement confirming adherence to those specified methods.

The a-lactalbumin samples were tested at different labs, including third party labs. It is Agropur's understanding that the methods used for the analyses were indeed those referenced in the FCC 11 monograph.

3. We note that the batch analyses for calcium (0.85-2.25 mg/g) show some lots exceed the limit for calcium stated in the FCC 11 monograph (<1 mg/g). (We also note that the FCC 11 value cited in the October 1, 2018 analyses was the limit for ash (NMT 3.5%) but not calcium per se. For the record, please confirm that, with the exception of calcium, you meet all FCC 11 monograph specifications for a-lactalbumin.

W*ith the exception of calcium, Agropur confirms that all FCC 11 monograph specifications are met.*

Again, we thank you for your continued diligence and assistance on this matter, and we look forward to the Agency's completion of its review.

Best regards,

Richard F. Mann
Natalie E. Rainer
Counsel to Agropur, Inc.

Richard F Mann
Partner
KELLER AND HECKMAN LLP
1001 G Street NW
Washington DC 20001
Tel 202 434 4229
Email mann@khlaw.com

From: Edwards, Alison <Alison.Edwards@fda.hhs.gov>
Sent: Wednesday, November 28, 2018 5:23 PM
To: Mann, Richard F. <Mann@khlaw.com>
Cc: Rainer, Natalie <rainer@khlaw.com>; DiFranco, Stephen <Stephen.DiFranco@fda.hhs.gov>
Subject: RE: GRN 000763 remaining chemistry questions

Dear Mr. Mann,

Regarding the additional information you provided October 1, 2018, we have two points of clarification and a requested statement we hope you can provide for the record within 2 business days (before COB Friday), if possible.

1. Please provide a limit of detection for lead, where it is noted in your analyses provided October 1, 2018 as "none detected".

2. Please clarify the methods used for the analyses provided October 1, 2018. If all values provided in this table were obtained with the methods described in the FCC 11 monograph for a-lactalbumin, please provide a statement confirming adherence to those specified methods.

3. We note that the batch analyses for calcium (0.85-2.25 mg/g) show some lots exceed the limit for calcium stated in the FCC 11 monograph (<1 mg/g). (We also note that the FCC 11 value cited in the October 1, 2018 analyses was the limit for ash (NMT 3.5%) but not calcium per se. For the record, please confirm that, with the exception of calcium, you meet all FCC 11 monograph specifications for a-lactalbumin.

Thank you in advance for your attention to these remaining issues. We look forward to your response.

Sincerely,
Alison J. Edwards, PhD
Review Chemist
DBGNR/CFSAN, Food and Drug Administration
College Park, MD 20740
240-402-1168

From: Mann, Richard F. <Mann@khlaw.com>
Sent: Monday, October 01, 2018 4:55 PM
To: Edwards, Alison <Alison.Edwards@fda.hhs.gov>
Cc: Rainer, Natalie <rainer@khlaw.com>
Subject: GRN 000763 remaining chemistry questions

Dear Dr. Edwards,

On behalf of our client, Agropur, we have responded below to your questions regarding specifications, intended use, and method of manufacture of alpha-lactalbumin (AL) as described in GRAS Notice 763.

Specifications: Per your request, we have attached 6 batch analyses demonstrating the compliance of Agropur's AL with the Food Chemicals Codex AL specifications for lead, calcium, phosphorous, and Beta-lactoglobulin.

Intended use: In our August 21, 2018 response to questions regarding GRAS Notice 763, we provided per capita dietary exposure estimates for AL based on existing whey uses. The per capita dietary exposure estimates were 2.9 g/p/d for the mean and 5.8 g/p/d for 90th percentile consumers. You have informed us that the Office of Food Additive Safety has developed an independent estimate of AL intake based on WWEIA-NHANES 2009-2014 data and has calculated—based on use by 27% of the population—that a users-only estimate of AL consumption is approximately 35.6 g/p/d for average consumers and 74.5 g/p/d for 90th percentile consumers. You further noted that—if calculating exposure based on one of the intended end use applications of interest (e.g., 20% in a sports beverage)—the actual consumption would exceed the per capita exposure estimate. For example, if AL is used at 20% in a 240 ml sports beverage, which is equivalent to 240 g, the exposure would be 48 g (240 g x 20% = 48 g).

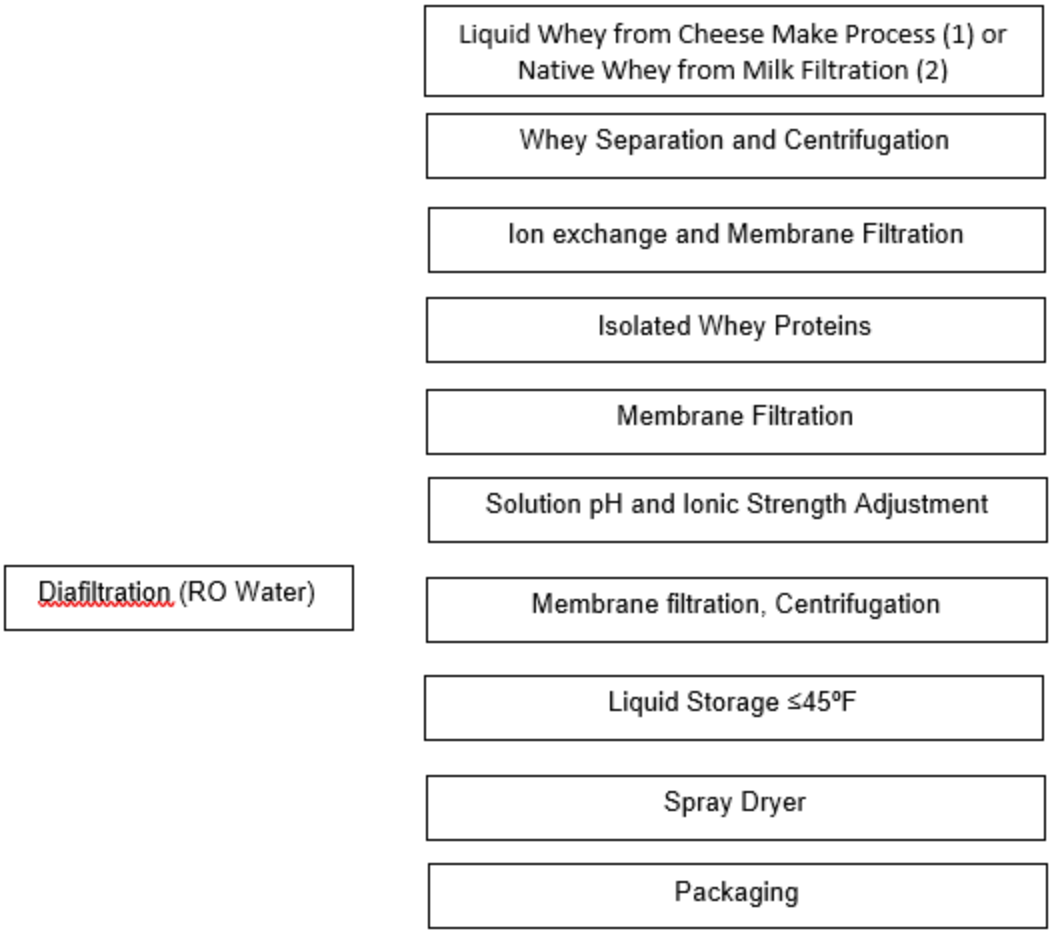
We agree that these higher users-only and use scenario-based consumption estimates do accurately capture the potential exposure scenarios that could to occur from the intended uses of AL. However, even at these higher intakes, we assert that the ingredient is GRAS for its intended uses, based on the nature of the substance – protein – and documented compliance with FCC specifications which also would address any potential concentrations of lead, calcium, phosphorous, and Beta-lactoglobulin.

Method of manufacture: We acknowledge the confusion caused by the flow diagram (i.e., Figure 1)

on page 7 of the GRAS Notice that implied the whey from cheesemaking and native whey undergo different processes. We can confirm that the flow chart should have indicated that the processing is the same, regardless of whether the starting material is whey from cheesemaking or native whey. Agropur has confirmed that the item in the flowchart previously marked as "Purified Whey Protein Solution (2)" was intended to refer not to native whey but to the intermediate material produced in AL manufacturing that is a solution of whey proteins refined from the starting whey material. Accordingly, there is one process for AL, regardless of the type of whey that is used.

A revised process flowchart is provided below:

Figure 1. Manufacturing Process Flow Chart



FDA also posed additional questions regarding the manufacturing process. We summarize FDA's general questions/requests, below, followed by our responses:

1. Question/Request--Indicate the order of use of the filtration membranes and which proteins were removed by these processes.

Response - Various filtration and membrane purification techniques are utilized throughout the process. In general, microfiltration is used to remove the majority of lipid material from proteins and non-protein components. The chromatography process broadly isolates collection of whey proteins from all unwanted materials in the stream. In Agropur's chromatography process,

glycomacropeptide (whey protein) is not included in the isolated proteins. The isolated whey proteins are concentrated and further purified with ultrafiltration/diafiltration techniques.

2. Question/Request --The statement on p. 4 of the amendment (a.4.) "Chromatography is utilized to isolate the whey proteins from non-protein components, as a core step in isolating/fractionating a-lactalbumin" appears to contradict the cited paper (Ye et al. 2000), wherein it describes individual proteins that are separated by chromatography.

Response - Although chromatography may be used to separate individual proteins, such as is described by Ye et al. 2000, such results are heavily dependent on the process conditions used (resin types, pH, ionic strength, temperature, etc.). Process conditions may also be optimized to isolate collections of proteins from mixture such as whey. In this particular case, Agropur uses the chromatography technique to isolate/fractionate a collection of whey proteins for further processing and concentration. Separation of specific proteins is accomplished using other techniques.

3. Question/Request--What is the role of microfiltration in the purification of alpha-lactalbumin and does it vary with the two starting materials?

Response - Microfiltration is used to remove milk lipid components (either fat globules or phospholipoproteins from lipid membranes) from the desirable protein mixtures. It is typically used in processing of cheese whey because of the high relative quantities of lipid material in cheese whey. It may or may not be needed in processing of native whey because microfiltration is used in separating the (native) whey proteins from milk. In essence, microfiltration is used in both processes, but may be used at different stages.

4. Question/Request--For both starting materials, what is the role of the second centrifugation step after 2 or more membrane filtration processes? Please clarify.

Response – As noted in the process flow diagram, the ionic strength of the protein solutions are adjusted between purification steps as necessary. Because the alteration of pH and or ionic strength of protein solutions may result in aggregation of proteins, centrifugal separation is used as an efficient means of separating the components that have aggregated during the process from the desirable products.

Lastly, we can confirm that, regardless of the starting material, the whey input is always pasteurized.

We very much appreciate your feedback and welcome any other questions you may have. We look forward to receiving FDA's feedback on its review of GRAS Notice 763.

Best regards,

Rick Mann and Natalie Rainer
Counsel to Agropur, Inc.

Richard F Mann
Partner
KELLER AND HECKMAN LLP
1001 G Street NW
Washington DC 20001
Tel 202 434 4229

From: Edwards, Alison <Alison.Edwards@fda.hhs.gov>
Sent: Thursday, September 13, 2018 8:31 AM
To: Mann, Richard F. <Mann@khlaw.com>; Rainer, Natalie <rainer@khlaw.com>
Cc: Dinovi, Michael J <Michael.Dinovi@fda.hhs.gov>; DiFranco, Stephen <Stephen.DiFranco@fda.hhs.gov>
Subject: GRN 000763 remaining chemistry questions

Dear Mr. Mann,

We have reviewed Agropur's responses sent August 22, 2018, to our questions pertaining to GRN 000763, sent July 24, 2018. We have a few additional clarification questions, listed below. We'd appreciate your response within 5 business days. Given the short turn-around time, we'd like to arrange a short phone call with you to clarify the remaining questions. If you are unable to complete the response within that time frame, please contact us to discuss further options.

The remaining issues are as follows:

Specifications: Agropur states that it meets FCC specifications, but does not include results of batch analyses for lead, calcium, phosphorous, or Beta-lactoglobulin. Agropur provides a statement of "typical values" of Beta-lactoglobulin. For completeness of the record, please provide results of batch analyses for these components.

Intended use: Agropur proposes to substitute a subset of existing uses of whey and WPCs with alpha-lactalbumin but presents an estimate of per capita intake from all existing whey uses. Agropur further assumes that

alpha-lactalbumin will replace existing uses of whey protein and will not result in an overall increase in milk protein consumption. Based on per capita intake of whey ingredients, Agropur estimates mean and 90th (mean x 2 or pseudo-90th) percentile values of 2.9 and 5.8 g/p/d, respectively. Agropur cites daily protein recommendations from the Institute of Medicine, as well as consumption data estimated from ERS/USDA whey poundage data and concludes "The estimated daily protein intake for a-lactalbumin (i.e., 2.9 g/person/day for average users and 5.8 g/person/day for 90th percentile users) is a fraction of the recommended protein intake.

OFAS notes that this approach is useful for estimating per capita background intakes of alpha-lactalbumin in the U.S. diet, but does not provide a good estimate of alpha-lactalbumin from the uses proposed in the notice. OFAS prepared an independent estimate of intake of alpha-lactalbumin using WWEIA-NHANES 2009-2014 data; estimated users-only (27%) intakes are approximately 35.6 g/p/d at the mean and 74.5 g/p/d at the 90th percentile. This large difference between estimates (poundage vs. individual consumption data) is likely a result of a number of factors, including (1) the percent users of products described in the notice is below 30%, while per capita data distributes intakes over the whole population (essentially 100% users); (2) the food categories listed in the notice do not represent all categories where whey is an added ingredient; and (3) the actual levels of use of whey ingredients are commonly well below 20% (w/v).

Further, OFAS notes that, using a scenario estimate, the estimated exposure to a-lactalbumin is also much higher than that obtained assuming per capita intake of whey (background exposure). For example, if an individual consumes a single 8-oz. serving of a sports beverage containing up to 20% alpha-lactalbumin (w/w basis rather than % protein basis), then the estimate is reduced to 0.9 x (purity) x 0.95 (%protein) x 0.20 x 240 g = 41 g alpha-lactalbumin. We note that the Agropur's conclusions, drawn from use of per capita data, describe intake associated with consumption of ~ 1/7 of an 8-oz. sports drink containing alpha-lactalbumin.

Agropur should provide a statement that, even at higher estimates of intake (e.g., those prepared using food consumption data and specified maximum use levels), the ingredient is GRAS for its intended uses.

Method of manufacture: We asked for clarification of the method of manufacture, including supporting references that describe the method. While Agropur indicated the pore size ranges for microfiltration and ultrafiltration membranes, it did not indicate the order of use of these membranes and which proteins were removed by these processes. Agropur cited the method of Ye et al. (2000) for the chromatography method. However, it is unclear if the chromatography method is applied to both cheese whey and native whey (produced by precipitation) starting materials. According to the flow diagram (p. 7 of GRN 000763), native whey starting material is not subject to chromatography. Further, we note that the statement on p. 4 of the amendment (a.4.) "Chromatography is utilized to isolate the whey proteins from non-protein components, as a

core step in isolating/fractionating a-lactalbumin" appears to contradict the cited paper (Ye et al. 2000), wherein it describes individual proteins that are separated by chromatography.

Please clarify how microfiltration and ultrafiltration are used in the production of a-lactalbumin from sweet whey and native whey, including relevant reference if available. While it may be presumed that ultrafiltration/diafiltration is used to concentrate the a-lactalbumin and remove low molecular weight components, it has also been reported to be used to purify a-lactalbumin and ß-lactoglobulin (e.g., per the methods of Espina et al. 2010 or Cheang and Zydney, 2003). At present, the flow diagram and accompanying text suggest multiple approaches that may be used, some of which appear to be redundant. The flow diagram includes 3 steps of membrane filtration when ion exchange chromatography is used as the starting material and 2 steps of membrane filtration when native whey is used and ion exchange chromatography is apparently bypassed.

Please clarify if the intent of the notice is to describe multiple potential methods of manufacture. If so, please provide citations in addition to Ye et al. (2000) to support the public availability of the methods. We note the availability of several relevant review papers in the published literature. If a precipitation approach is used for native whey that does not include ion chromatography, please provide a relevant citation. For example, is the method consistent with that of Gésan-Guiziou et al. (1999)?

Alternatively, if the notice is intended to describe a single method with two potential starting materials, please clarify the flow diagram on p. 7, including supporting discussion. In your answer, please clarify if the same membrane filtration processes used are for the two starting materials. (Figure 1 suggests the same membrane filtration processes are used; however, if ion exchange is only used for sweet whey starting material and not native whey (i.e., whey not produced through cheese making), it would follow that the necessary purification steps would not be the same.

What is the role of microfiltration in the purification of alpha-lactalbumin and does it vary with the two starting materials?

For both starting materials, what is the role of the second centrifugation step after 2 or more membrane filtration processes? Please clarify.

Please provide a statement, that regardless of starting material, the ingredient will be pasteurized or produced from pasteurized milk. We are aware that the notice includes a statement that whey from cheesemaking is pasteurized, but would like clarification for the record that native whey and ingredients derived therefrom will also be pasteurized.

References:

Espina V, Jaffrin MY, Ding L, Cancino B. 2010. Food Res Intl. 43: 1335-46.

Cheang B, Zydney AL. 2003. Biotechnol Bioeng. 83(2):201-9.

Gésan-Guiziou et al. 1999. J. Dairy Res. 66:225-36.

Thank you in advance for your attention to these remaining issues. We

look forward to your response.

Sincerely,
Alison J. Edwards, PhD
Review Chemist
DBGNR/CFSAN, Food and Drug Administration
College Park, MD 20740
240-402-1168