ORIGINAL SUBMISSION

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Land of Innovative Solutions

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1611
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GRN 000611

November 12, 2015

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for Vitalarmor® GF-100, a Basic Whey Protein Isolate

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], Armor Protéines S.A.S. [19 bis, rue de la Libération, 35460 Saint-Brice-en-Coglès, France] (the notifier) is hereby submitting two (2) GRAS Notices of the determination, on the basis of scientific procedures, that Vitalarmor® GF-100, a basic whey protein isolate, produced by Armor Protéines, as defined in the enclosed documents, is GRAS for use under specified conditions in term infant and toddler formulas and in meal replacement beverages and medical foods, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act.*

Enclosed you will find one GRAS Notice for the determination of Vitalarmor® GF-100 as GRAS for use as an ingredient in infant and toddler formulas ("GRAS Exemption Claim for Vitalarmor® GF-100, a Basic Whey Protein Isolate, for use as an Ingredient in Term Infant Formulas and Toddler Formulas"), and a second GRAS Notice for the determination of Vitalarmor® GF-100 as GRAS for use as an ingredient in meal replacement beverages and medical foods ("GRAS Exemption Claim for Vitalarmor® GF-100, a Basic Whey Protein Isolate, for use as an Ingredient in Meal Replacement Beverages and Medical Foods").

One (1) hard copy and one (1) electronic copy (enclosed CDs) of each GRAS Notification are included herewith. The enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using McAfee VirusScan 8.8.

The information setting forth the basis for the GRAS determinations as presented to the agency for review in the enclosed GRAS Notifications includes detailed information on the notified substance and a summary of the basis for the GRAS determinations, as well as corresponding consensus opinion statements of two independent Panels of Experts in support of the safety of Vitalarmor® GF-100 for use in infant and toddler formulas and in meal replacement beverages and medical foods, respectively.

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5100 Paint Branch Parkway
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Should you have any questions or concerns regarding the GRAS Notices, please do not hesitate to contact me at any point during the review process. Armor Protéines would greatly appreciate the opportunity to respond as necessary in a timely manner to any requests for further information in support of the determination of Vitalarmor® GF-100 as GRAS.

We would like to thank you in advance for your time and consideration of the GRAS Exemption Claims for Vitalarmor® GF-100.

Sincerely,

(b) (6)

Emmanuel TREUIL
Food Law Director
Legal Division of SAVENCIA Group

Enclosures

GRAS Exemption Claim for Vitalarmor® GF-100, a Basic Whey Protein Isolate, for use as an Ingredient in Meal Replacement Beverages and Medical Foods

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
USA 20740-3835

Submitted by:

Armor Protéines
19 bis, rue de la Libération
35460 Saint-Brice-en-Coglès
France

October 26, 2015

GRAS Exemption Claim for Vitalarmor® GF-100, a Basic Whey Protein Isolate, for use as an Ingredient in Meal Replacement Beverages and Medical Foods

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I. GRAS EXEMPTION CLAIM

I.A Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

Armor Protéines (hereafter Armor) hereby claims that the use of Vitalarmor® GF-100, a basic whey protein isolate, in meal replacement beverages and medical foods, as described in Section I.D below, is exempt from the requirement of premarket approval of the *Federal Food, Drug, and Cosmetic Act* because we have determined that such uses are Generally Recognized as Safe (GRAS).

Signed,
(b) (6)

10/30/2015

Emmanuel TREUIL
Food Law Director
Legal Division of SAVENCIA Group

Date

I.B Name and Address of Notifier

Armor Protéines S.A.S.
19 bis, rue de la Libération
35460 Saint-Brice-en-Coglès
France

Emmanuel TREUIL
Food Law Director
Legal Division of SAVENCIA Group
Email: emmanuel.treuil@lalliance.com
Phone: +33 1 34 58 67 91

I.C Common Name of the Notified Substance

Basic whey protein isolate

I.D Conditions of Intended Use

Vitalarmor® GF-100 is intended for use in meal replacement beverages and medical foods intended for consumption by adults as a source of specific whey proteins. As an ingredient of meal replacement beverages Vitalarmor® GF-100 is proposed for addition at use-levels of up to 100 mg per serving (240 mL). For use as an ingredient of medical foods, Vitalarmor® GF-100 would be added at a level that will provide, under recommended conditions of use of the medical food, up to 610 mg Vitalarmor® GF-100 per day.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2015a), Vitalarmor® GF-100 has been determined by Armor to be GRAS through scientific procedures.

I.F Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the United States (U.S.) Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Armor Protéines S.A.S.
19 bis, rue de la Libération
35460 Saint-Brice-en-Coglès
France

Should the FDA have any questions or additional information requests regarding this notification, Armor will supply these data and information.

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

II.A Identity

II.A.1 Chemical Identity

Common Name: Basic whey protein isolate

Trade Name: Vitalarmor® GF-100

II.A.2 Chemical and Physical Characteristics and Product Composition

Vitalarmor® GF-100 is an isolate of basic whey proteins obtained from skimmed cow's milk by chromatographic fractionation. Approximately 3% of cow's milk composition is accounted for by protein, which can be broadly divided into 2 main categories: caseins (micellar proteins; approximately 80% of cow's milk protein) and whey proteins (soluble proteins; approximately 20% of cow's milk protein) (Fox, 2003; Cornell University, 2014). The fractionation process used to produce Armor's whey protein isolate removes the acidic proteins, including casein and major whey proteins (β-lactoglobulin and α-lactalbumin), and thus selectively isolates certain minor basic whey proteins, such as lactoferrin and lactoperoxidase. The resulting product is therefore a heterogeneous mixture of basic whey proteins naturally present within cow's milk.

Vitalarmor® GF-100 is a yellowish grey powder, characterized by a protein content of not less than 90% (on an 'as is' basis), which is consistent with the Food Chemicals Codex (FCC) protein content requirement for whey protein isolates (not less than 90% calculated on a dried basis) (FCC, 2014). The protein composition of Vitalarmor® GF-100 has been partially characterized by proteomic analyses, which identified the basic whey proteins, lactoferrin and lactoperoxidase, as the 2 most compositionally prominent proteins of the isolate. High-performance liquid chromatography (HPLC) analysis of production material samples obtained before terminal pasteurization (see Section II.B.2 for description of the production process) indicated that lactoferrin and lactoperoxidase comprise approximately 73% of the ingredient's composition (average levels of 47 and 26% in powdered ingredient, respectively). In addition to lactoferrin and lactoperoxidase, the remainder of the protein fraction is composed of minor whey proteins that are naturally present in cow's milk. Transforming growth factor (TGF)-β_2 is one such compositionally minor protein that is present in Vitalarmor® GF-100. Since target levels of TGF-β_2 in the final food product will in certain applications of Vitalarmor® GF-100 be used to establish the use-level of the ingredient (*i.e.*, for use in infant formula – please refer to separate GRAS Notice for the use Vitalarmor® GF-100 in infant and toddler formulas), additional quantitative estimation using the enzyme-linked immunosorbent assay (ELISA) was conducted

to determine levels of TGF-β_2 in Vitalarmor® GF-100. Average concentrations of 0.015% [155 ± 18 µg/g (n=9)] of TGF-β_2 were identified in Vitalarmor® GF-100.

Vitalarmor® GF-100 may be considered a sub-category of whey and whey protein concentrates which are affirmed as GRAS under 21 CFR §§ 184.1979 and 184.1979c (U.S. FDA, 1979, 1981, 2015b). The manufacturing process and compositional identity of Vitalarmor® GF-100 also is similar to other whey protein isolates that have been previously determined to be GRAS for general food uses [*e.g.*, whey protein isolate (GRAS Notice Nos. GRN 000037 - U.S. FDA, 2000) and bovine milk basic protein fraction (GRAS Notice No. GRN 000196 - U.S. FDA, 2006)]. High-purity bovine lactoferrin, one of the main protein constituents of Vitalarmor® GF-100, accounting for approximately 47% of the ingredient's composition, also has been already determined as GRAS for use in a number of foods (GRAS Notice Nos. GRN 000464 and 000465 - U.S. FDA, 2014a,b).

II.B Method of Manufacture

II.B.1 Raw Materials and Processing Aids

The starting material for the production of Vitalarmor® GF-100 is unpasteurized cow's milk. The milk is of European Union (EU) origin and conforms to all EU legislation pertinent to the safety of milk for human consumption. The milk used in the production of Vitalarmor® GF-100 is derived from European suppliers producing milk products to standards consistent with the quality standards of the Grade "A" Pasteurized Milk Ordinance of 2009 (U.S. FDA, 2009). Results of analysis for potential environmental contaminants of the milk are presented in Section II.C.3. Milk that is used as the starting material for Vitalarmor® GF-100 is deemed to be free of bovine tuberculosis (the milk is collected in France and as such is subject to French sanitary regulations; with regard to bovine tuberculosis specifically, the risk for the disease is monitored by the French authorities).

Isolation of the basic whey proteins from the milk is carried out through physical separation methods, and apart from food-grade sodium chloride (NaCl) and hydrochloric acid (HCl), no other processing aids are used in the manufacture of the final product. The chromatographic column (the cation-exchange column is subject of an effective FDA Food Contact Substance Notification) and all filters used in the manufacture of Vitalarmor® GF-100 satisfy requirements for food-contact use. The materials used in the manufacture of Vitalarmor® GF-100 are listed in Table II.B.1-1.

Table II.B.1-1 Raw Material and Processing Aids Used in the Manufacture of Vitalarmor® GF-100

Material	Function
Raw Materials	
Cow's milk	Source raw material
Processing Aids	
Sodium chloride (NaCl) solution	Eluent
Hydrochloric acid (HCl) solution	pH adjuster
Chromatographic Resin and Filters	
Cation-exchange resin	Isolation of specific whey proteins
Felt bag filter	Removal of insoluble materials
Ultrafiltration membrane	Protein concentration and demineralization
Microfiltration membrane	Microbial load reduction

II.B.2 Manufacturing Process

Vitalarmor® GF-100 is manufactured in compliance with current Good Manufacturing Practice (cGMP). Additionally, the production plant where Vitalarmor® GF-100 for food application is produced is certified under ISO 9001 and ISO 22000.

The production method used to obtain the protein isolate can be broadly divided into 2 stages, 1) isolation of basic whey protein fraction and 2) purification and drying of the isolated basic whey proteins, which are described briefly in Sections II.B.2.1 and II.B.2.2, respectively. The manufacturing process employs only physical separation techniques that result in the selective isolation of specific whey proteins from the source material (cow's milk) to yield a protein isolate comprising a select composition of basic whey proteins. No chemical processes or other synthesis steps that could introduce new constituents or chemically alter the natural constituents of cow's milk are part of the manufacturing of Vitalarmor® GF-100. The manufacturing processes (*i.e.*, filtration, ion-exchange separation) used for the production of the ingredient are already well-established and widely used in the dairy industry for production of other milk ingredients and are simply optimized to selectively isolate the desired basic whey proteins. The selective isolation of certain proteins yields some minor differences in protein ratios in the ingredient *versus* those naturally found in cow's milk, with the magnitude in the difference varying depending on the particular proteins. Additionally, the isolated proteins are subject to partial denaturation during the pasteurization process; however, the denaturation level of the protein constituents of Vitalarmor® GF-100 is similar to that found in pasteurized cow's milk.

II.B.2.1 Manufacturing Stage 1: Isolation

Stage 1 of the manufacturing process consists of isolating the proteins of interest. This involves passing the cow's milk, which has been previously defatted *via* centrifugal separation, through

an ion-exchange resin to isolate the basic whey protein fraction. The run-off from the chromatographic column contains casein, the major whey proteins (β-lactoglobulin, α-lactalbumin, *etc.*), lactose, and minerals. The basic whey proteins bound to the cationic resin are eluted with NaCl solution, such that the eluate contains the selective isolate of basic whey proteins.

II.B.2.2 Manufacturing Stage 2: Purification and Drying

Stage 2 of the manufacturing process consists of additional purification and drying of the isolated basic whey proteins obtained from Stage 1. The NaCl-eluate is pre-concentrated using ultrafiltration and then microfiltrated to reduce the microbial load. The NaCl content of the eluate is removed at the ultrafiltration stage. The basic whey protein fraction is further subjected to pasteurization. The remaining water is removed from the purified protein fraction by spray-drying. The resulting powdered product (Vitalarmor® GF-100) is packaged in a cardboard box with a double polyethylene liner bag, which is food contact-compliant.

II.B.3 Quality Control

Vitalarmor® GF-100 is produced in accordance with cGMP and the principles of Hazard Analysis and Critical Control Points (HACCP). The production process comprises a number of in-process critical control points. Ultimately, the specifications for the final product and specifically adherence to the specifications (demonstrated *via* routine batch testing) are the final controls ensuring a final food-grade product of high quality.

II.C Specifications for Food Grade Material and Product Analysis

Specifications for Vitalarmor® GF-100 and results of confirmatory batch analyses on several representative lots of the ingredient are presented in Sections II.C.1 and II.C.2, respectively. Results of additional analysis on the cow's milk starting material for potential environmental contaminants are presented in Section II.C.3.

II.C.1 Specifications for Food Grade Material

Food-grade product specifications have been established for Vitalarmor® GF-100 (Table II.C.1-1). All methods of analysis are nationally or internationally recognized or have been validated. The specifications for Vitalarmor® GF-100 are similar to those of other whey protein ingredients that are currently marketed in food and infant formula products in the U.S. (***e.g.***, bovine milk basic protein fraction, as presented in GRAS Notice No. GRN 000196) (U.S. FDA, 2006), and are largely consistent with the whey protein isolate specifications laid out in the FCC (2014).

In addition to the determination of total protein content, Vitalarmor® GF-100 also is characterized by its lactoferrin and lactoperoxidase content, and the levels of these proteins are measured for quality control purposes to ensure consistency of the finished product. As lactoferrin and lactoperoxidase are partially denatured during the production process, the levels of these proteins are measured using HPLC based methods on interim material sampled prior to terminal pasteurization (see Section II.B.2.2 – Manufacturing Stage 2). While down-stream processing may be associated with additional protein denaturation, no changes in protein composition per se will occur at this stage. The specification for Vitalarmor® GF-100 also includes a range for TGF-β_2 content (0.012 to 0.018% w/w), which is used for establishing the use-level of Vitalarmor® GF-100 for use in other food applications (*i.e.*, for addition to infant and toddler formulas).

Table II.C.1-1 Product Specifications for Vitalarmor® GF-100, a Basic Whey Protein Isolate

Parameter	Specification	Method
Appearance	Yellowish gray powder	Visual Inspection
Foreign matter (Scorched particles)	Absent (Disc B or better[a])	Visual inspection in 25 g (ADMI chart, solubilized with 0.15 M NaCl solution)
pH (5% solution w/v)	5.5 to 7.6	5% (w/v) solution, pH meter
Total Protein	Not less than 90%	Kjeldahl method (IDF20/ISO 8968) [N x 6.38]
Lactoferrin	25 to 75%	HPLC[b]
Lactoperoxidase	10 to 40%	HPLC[b]
TGF-β_2	12 to 18 mg/100 g	ELISA (Quantikine human TGF-β2, R&D Systems)
Moisture	Not more than 6.0%	ISO 5550
Lactose	Not more than 3.0%	Enzymatic method (Lactose/D-Galactose kit, Boehringer Mannheim/R-Biopharm)
Fat	Not more than 4.5%	AFNOR Chimie II 3B 1986
Ash (Residue on Ignition)	Not more than 3.5%	AFNOR NF V04-208
Iron	≤25 mg/100 g	AAS
Heavy Metals		
Lead	<0.1 mg/kg	ICP-MS
Cadmium	<0.2 mg/kg	ICP-MS
Mercury	<0.6 mg/kg	ICP-MS
Microbiological Parameters		
Aerobic mesophilic count	Not more than 10,000 CFU/g	ISO 4833
Enterobacteriaceae	Not more than 10 CFU/g	ISO 21528-1
Yeasts	Not more than 50 CFU/g	ISO 6611 | IDF 94:2004
Molds	Not more than 50 CFU/g	ISO 6611 | IDF 94:2004
Escherichia coli	Negative (in 1 gram)	ISO 16649-2
Coagulase positive *Staphylococci*	Negative (in 1 gram)	ISO 6888-3
Salmonella	Negative (in 25 grams)	VIDAS Easy *Salmonella* method (equivalent to ISO6579)

Table II.C.1-1 Product Specifications for Vitalarmor® GF-100, a Basic Whey Protein Isolate

Parameter	Specification	Method
Listeria	Negative (in 25 grams)	VIDAS LIS method (equivalent to ISO 11290-1/A1:2004)
Cronobacter spp.	Negative (in 25 grams))	ISO/TS 22964:2006

AAS = atomic absorption spectrometer; ADMI = American Dry Milk Institute; CFU = colony-forming unit; ELISA = enzyme-linked immunosorbent assay; HPLC = High-performance liquid chromatography; ICP-MS = Inductively coupled plasma mass spectrometry; TGF-β = transforming growth factor.
[a] American Dry Milk Institute (ADMI) standard discs (Discs A, B, C, or D) representing the following amounts of scorched particles: 7.5, 15.0, 22.5, or 32.5 mg, respectively.
[b] HPLC conducted on in-process samples prior to terminal pasteurization. To determine levels of each protein in the final product, total protein content is multiplied by % protein in the in-process sample.

II.C.2 Product Analysis

Batch analyses for 5 non-consecutive commercial batches of Vitalarmor® GF-100 are presented in Table II.C.2-1. The batch analysis confirms that the manufacturing process as described in Section II.B.2 produces a consistent product that conforms with the product specifications laid out in Section II.C.1.

Table II.C.2-1 Batch Analyses Demonstrating Compliance with Specifications for Vitalarmor® GF-100

Specification Parameter	Limit	Batch Nos.				
		111106	111217	130830	131114	140724
Appearance	Yellowish gray powder	Pass	Pass	Pass	Pass	Pass
Foreign matter	Absent (Disc B or better)	Absent	Absent	Absent	Absent	Absent
pH (5% solution w/v)	5.5 to 7.6	6.2	6.4	6.3	6.1	6.7
Total Protein (%)	≥90	91.0	93.0	94.3	96.0	94.5
Lactoferrin (%)	25 to 75	32	51	50	63	70
Lactoperoxidase (%)	10 to 40	32	24	25	19	17
TGF-β_2 (mg/100 g)	12 to 18	14.6	13.6	16.2	17.4	14.0
Moisture (%)	≤6.0	4.6	4.1	4.8	3.7	4.2
Lactose (%)	≤3.0	<0.09	<0.09	<0.09	<0.09	<0.09
Fat (%)	≤4.5	2.5	2.8	2.0	0.5	2.0
Ash (Residue on Ignition) (%)	≤3.5	1.7	1.3	1.0	0.5	0.5
Iron (mg/100 g)	≤25	18.8	19.2	17.4	16.7	16.9
Heavy Metals						
Lead (mg/kg)	<0.1	<0.02	<0.02	<0.02	<0.02	<0.02
Cadmium (mg/kg)	<0.2	<0.005	<0.005	<0.005	<0.005	<0.005
Mercury (mg/kg)	<0.6	<0.005	<0.005	<0.005	<0.005	<0.005

Table II.C.2-1 Batch Analyses Demonstrating Compliance with Specifications for Vitalarmor® GF-100

Specification Parameter	Limit	Batch Nos.				
		111106	111217	130830	131114	140724
Microbiology						
Aerobic mesophilic count (CFU/g)	≤10,000	50	50	330	20	10
Enterobacteriaceae (CFU/g)	≤10	0	0	0	0	0
Yeasts (CFU/g)	≤50	0	0	0	0	0
Molds (CFU/g)	≤50	0	0	0	0	0
Escherichia coli (in 1 gram)	Negative	Negative	Negative	Negative	Negative	Negative
Coagulase positive *Staphylococci* (in 1 gram)	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella (in 25 grams)	Negative	Negative	Negative	Negative	Negative	Negative
Listeria (in 25 grams)	Negative	Negative	Negative	Negative	Negative	Negative
Cronobacter spp. (in 25 grams)	Negative	Negative	Negative	Negative	Negative	Negative

CFU = colony-forming unit; TGF = transforming growth factor.

II.C.3 Additional Analysis

The starting material (unpasteurized cow's milk) is subjected to quality control testing and is analyzed for the following environmental contaminants:

- Antibiotics: tetracyclines, aminoglycosides, quinolones, macrolides, and chloramphenicol
- Heavy metals: lead, cadmium, and mercury
- Radioactivity: Cesium-134, Cesium-137, and Strontium-90
- Mycotoxin: Aflatoxin M_1
- Pesticides: organochlorine and organophosphate pesticides, and pyrethroids
- Dioxins, furans, dioxin-like PCBs.

Analytical data from 3 samples of cow's milk used as the starting material for Vitalarmor® GF-100 are presented in Table II.C.3-1. Analyses for pesticide and mycotoxins were consistently below the limit of quantification. Where maximum permitted levels ("action levels") for potential contaminants of milk intended for human consumption have been established by the FDA (aflatoxin M_1 and several pesticides[1]), the analysis revealed no measurable levels based on limits of quantification which in all cases were below the set action levels.

[1] aflatoxin M_1: 0.5 ppb; aldrin and dieldrin: 0.3 ppm; benzene hexachloride (BHC): 0.3 ppm; DDT, DDE, and TDE (DDD): 1.25 ppm; ethylene dibromide: 0.1 ppb; heptachlor and heptachlor epoxide: 0.1 ppm; and lindane: 0.3 ppm.

Table II.C.3-1 Summary of Analytical Data for Environmental Contaminants in Unpasteurized Cow's Milk Used as the Starting Material for Vitalarmor® GF-100

Specification Parameter	Specification Limit	Date of Analysis of Milk Samples			
		May 2011	May 2012	June 2013	June 2014
Radioactivity (bq/kg)					
Strontium-90	<125	<0.12	0.2	0.23	<0.12
Cesium-134, Cesium-137	<1,000	<5	<3	<7.9	<3
Pesticides					
Pesticides *(mg/kg)*	<MRL	<LOQ	<LOQ	<LOQ	<LOQ
Antibiotics (µg/kg)					
Chloramphenicol	<0.3	<0.1	<0.1	<0.1	<0.1
Macrolites					
Erythromycin	<40	--	<20	<20	<20
Tylosin	<50		<20	<20	<20
Tilmicosin	<50		<20	<20	<20
Lincomycin	<150		<50	<50	<50
Spiramycin	<200		<50	<50	<50
Quinolones	<30	<10	<10	<10	<10
Streptomycin	<200	<50	<50	<50	--
Tetracyclin	<100	<10	<10	<10	<10
Mycotoxin (µg/L)					
Aflatoxin M_1	<0.05	<0.02	<0.02	<0.02	<0.01
Dioxin and PCB					
Dioxin and furan *(pg/g fat)* [a]	<2.5	0.257	0.338	0.479	0.377
Dioxin, Furan, PCB *(pg/g fat)* [b]	<5.5	0.55	0.54	0.712	0.739
PCB indicators *(ng/g fat)* [c]	<40	--	1.98	2.27	3.77
Heavy Metals (mg/kg)					
Lead	<0.02	<0.02	<0.02	<0.02	<0.02
Cadmium	<0.005	<0.005	<0.005	<0.005	<0.005
Mercury	<0.01	<0.005	<0.005	<0.005	<0.005

-- = not tested LOQ = limit of quantification (not more than MRL); MRL = maximum residue limit; PCB = Polychlorinated biphenyls.

[a] Sum of dioxins (WHO-PCDD/F-TEQ) [Commission Regulation (EC) No 1881/2006] (EC, 2006).

[b] Sum of dioxins and dioxin-like PCBS (WHOPCDD/F-PCBTEQ) [Commission Regulation (EC) No 1881/2006] (EC, 2006).

[c] Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6) [Commission Regulation (EC) No 1881/2006] (EC, 2006).

II.D Stability

Vitalarmor® GF-100 is packaged in a cardboard box with a double polyethylene liner bag which is food contact-compliant (no migration into the product). The bulk stability of Vitalarmor® GF-100 was evaluated under ambient storage conditions (20°C and relative humidity between 40 and 50%) over a 36-month period after the date of production. The analytical data are presented in Table II.D-1. Although an increase in moisture levels above the specification limit was apparent after 6 months of storage, the increase in moisture was not accompanied by any evidence of microbial growth.

Table II.D-1 Storage Test Data for Vitalarmor® GF-100 (stored at 20°C and RH 40 to 50%)

Parameter	Specification Limit	Time Point						
		0	6 months	12 months	18 months	24 months	30 months	36 months
Appearance	Yellowish, grey powder	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant	Compliant
Moisture (%)	≤6%	4.1	5.2	6.3	7.0	8.0	7.9	7.7
TGF-β_2 (mg/100 g)	12-18 mg/100 g	13.6	13.2	13.6	12.3	12.0	11.7	12.2
Aerobic mesophilic microorganisms (CFU/g)	≤10,000 CFU/g	50	--	40	--	30	--	80
Enterobacteriaceae (CFU/g)	≤10 CFU/g	0	--	0	--	0	--	0
Salmonella (in 25 g)	Negative	Negative	--	Negative	--	Negative	--	Negative
Coagulase-positive *Staphylococci* (in 1 gram)	Negative	Negative	--	Negative	--	Negative	--	Negative
Yeasts (CFU/g)	≤50 CFU/g	<5	--	<5	--	<5	--	<5
Moulds (CFU/g)	≤50 CFU/g	<5	--	10	--	<5	--	<5

-- = not tested; CFU = colony-forming unit; NA = not applicable; RH = relative humidity; TGF-β2 = transforming growth factor-β_2.

III. SELF-LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with Vitalarmor® GF-100.

IV. BASIS FOR GRAS DETERMINATION

Vitalarmor® GF-100 is a basic whey protein isolate obtained from cow's milk. The protein composition of the ingredient is largely accounted for by partially denatured lactoferrin and lactoperoxidase (combined these 2 proteins comprise approximately 73% of the ingredient's composition). The remainder of the protein fraction comprises numerous other proteins that occur naturally in cow's milk, including proteins that do not contribute significantly to the overall composition of the ingredient, but are known to possess biological properties (*e.g.*, TGF-β_2). As an ingredient of meal replacement beverages and medical foods, Vitalarmor® GF-100 is intended for use as a source of specific whey proteins. Although the manufacturing process produces a selective isolate of minor whey proteins, the whey proteins are isolated in roughly equivalent ratios to their respective levels in cow's milk. Furthermore, the production process does not introduce any novel compounds or involve any chemical reactions. As such, significant exposure to the constituents of the ingredient already exists from the background diet, including from the consumption of cow's milk, dairy products, as well as food products supplemented with whey proteins. These sources provide exposure to both native, as well as denatured forms of the proteins found in Vitalarmor® GF-100. Furthermore, the main protein constituents of Vitalarmor® GF-100 and other quantitatively minor proteins (*e.g.*, TGF-β_2) also are produced endogenously by humans and are present in human milk. In many cases, the human and bovine forms of these proteins have been found to be highly homologous. In nursing infants, human milk therefore provides an alternate source of exposure to the proteins. The protein constituents of Vitalarmor® GF-100 are therefore normal nutritive components of human and cow's milk and may be ingested throughout life.

Safety studies of Vitalarmor® GF-100 were conducted in mature and juvenile rat models. Subchronic toxicity was evaluated in mature Sprague-Dawley rats using current Good Laboratory Practice (cGLP), and in accordance with OECD guideline No. 408. Vitalarmor® GF-100 was administered to groups of weaned rats *via* gavage at doses of 600, 1,200, and 2,000 mg/kg body weight for 13 weeks. No test article related findings were observed in the study and the highest dose of 2,000 mg/kg body weight was determined as the no-observed-adverse-effect level (NOAEL) in adult rats. Vitalarmor® GF-100 also was well-tolerated in juvenile (pre-weaning) Sprague-Dawley rats when administered *via* gavage at a dose of 600 mg/kg body weight, the highest permissible dose in this model, starting on Postnatal Day (PND) 7 (neonatal animals), over a 6-week period. The results of these studies are discussed in further detail in Section IV.B.3.1. Additionally, Vitalarmor® GF-100 also was shown to be non-genotoxic and non-mutagenic in a bacterial reversion assay and in an *in vitro* mammalian micronucleus assay. Furthermore, Vitalarmor® GF-100 is derived from a common food source

and as discussed all components of the ingredient are normal constituents of the diet. Accordingly, no mutagenicity or genotoxicity risk was identified for the ingredient.

Vitalarmor® GF-100 is produced using manufacturing methods that are consistent with traditional isolation methods applied during milk processing and the ingredient is therefore qualitatively similar to other whey protein-based ingredients that have been previously evaluated for safety for general food uses (including ingredients that have been determined as GRAS). Although there are minor qualitative and quantitative differences between Vitalarmor® GF-100 and other whey protein isolates, there are no published findings based on the results of animal and human safety studies to suggest whey protein isolates in general would be unsafe or unsuitable for use in food for human consumption. Also, the safety of bovine lactoferrin, one of the main protein constituents of Vitalarmor® GF-100, also has been previously determined as GRAS for use in food. Data in support of the safety of these ingredients are discussed in brief in Section IV.B.3.2.

IV.A Probable Consumption

IV.A.1 Estimated Consumption of Vitalarmor® GF-100 from Use in Meal Replacement Beverages

Estimates for the daily mean and 90th percentile intakes of Vitalarmor® GF-100 from its use in meal replacement beverages were calculated based on a maximum use level of 100 mg per 240 mL of meal replacement beverages in conjunction with meal replacement beverage consumption data obtained from the U.S. National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2011; USDA, 2012). While Vitalarmor® GF-100 is proposed for use only in meal replacement beverages intended for adults and no consumers of meal replacement beverages were identified within the 0- to 12-month age categories, toddlers and children (4 to 11 years of age) were identified as consumers of meal replacement beverages. These age groups, particularly toddlers, also comprise users of infant and toddler formulas, which present an additional use for Vitalarmor® GF-100[2]. Therefore, the intake estimates for toddlers (1 to 3 years of age) and children (4 to 11 years of age) also considered the proposed use of the ingredient in toddler formula (up to 30 mg Vitalarmor® GF-100/100 g formula). In population groups 12 years of age and older, the intake estimates for Vitalarmor® GF-100 are reflective of use of the ingredient in meal replacement beverages only (*i.e.,* no users of toddler formula were identified in individuals in the older age groups). At 4.5% (% users), male adults (≥20 years of age) represented the largest consumer group of meal replacement beverages.

[2] Armor also intends to use Vitalarmor® GF-100 in infant and toddler formulas. Vitalarmor® GF-100 for use in infant and toddler formulas also was determined to be GRAS and a separate GRAS Notification has been filed with the FDA ("*GRAS Exemption Claim for Vitalarmor® GF-100, a Basic Whey Protein Isolate, for use as an Ingredient in Term Infant Formulas and Toddler Formulas"*).

Estimates for the daily intake of Vitalarmor® GF-100 from its use in meal replacement beverages are summarized in Table IV.A.1-1 on a per person basis by population group. The data are presented on a per kilogram body weight basis in Table IV.A.1-2.

Within the total population, the mean all-person intake of Vitalarmor® GF-100 from all proposed food uses was estimated to be 1.4 mg/day (an insufficient number of consumers were identified within the total population to generate a 90th percentile intake estimate), while the mean and 90th percentile all-user intake estimates were 47.1 and 88.4 mg/day, respectively. The greatest mean all-person intake estimates for Vitalarmor® GF-100 based on the proposed uses were observed in male adults at 2.5 mg/day. Male adults also displayed the largest statistically reliable estimates for the all-user mean and 90th percentile intakes of Vitalarmor® GF-100 at 56.1 and 229.2 mg/day, respectively. The lowest statistically reliable level of intake of Vitalarmor® GF-100 was observed to occur in female adults with an all-person mean intake of 1.2 mg/day and all-user mean and 90th percentile intakes of 36.0 and 77.5 mg/day, respectively.

Table IV.A.1-1 Summary of the Estimated Daily Intake of Vitalarmor® GF-100 Resulting from the Intended Use in Meal Replacement Beverages in the United States (2009-2010 NHANES Data)

Population Group	% Users	n	All-Person Intake (mg/day)		All-User Intake (mg/day)	
			Mean	90th Percentile	Mean	90th Percentile
Toddlers 13 to 36 months[a]	3.5	18[b]	0.5*	na	18.9*	27.7*
Children 4 to 11 years[a]	1.0	16[c]	0.3*	na	25.5*	29.5*
Female Teenagers 12 to 19 years	0.2	1	0.7*	na	361.7*	361.7*
Male Teenagers 12 to 19 years	1.9	12	0.3*	na	13.2*	31.6*
Female Adults 20 and older	3.3	70	1.2	na	36.0	77.5
Male Adults 20 and older	4.5	61	2.5	na	56.1	229.2
Total Population, all ages	3.0	152	1.4	na	47.1	88.4

n = number of users identified; na = not available.
* Indicates a result that is considered not statistically reliable on the basis that fewer than 30 consumers were identified.
[a] Also includes use of Vitalarmor® GF-100 in toddler formula.
[b] 1 consumer of meal replacement beverages and 17 consumers of infant and toddler formulas.
[c] 9 consumers of toddler formulas and 8 consumers of meal replacement beverages.

On a body weight basis, the all-person mean intake of Vitalarmor® GF-100 from all proposed food uses was estimated to be 0.02 mg/kg body weight/day for the total population. The all-user mean and 90th percentile total population intake estimates were 0.64 and 1.30 mg/kg body weight/day, respectively. The mean all-person intake estimates for Vitalarmor® GF-100 based on the proposed uses were observed to be greatest in male adults at 0.03 mg/kg body weight/day. Male adults also displayed the largest statistically reliable estimates for the all-user mean and 90th percentile intakes of Vitalarmor® GF-100 at 0.67 and 2.74 mg/kg body weight/day, respectively. The lowest statistically reliable level of intake of Vitalarmor® GF-100 was observed to occur in female adults with an all-person mean intake of 0.02 mg/kg body

weight/day and all-user mean and 90th percentile intakes of 0.53 and 1.09 mg/kg body weight/day, respectively.

Table IV.A.1-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Vitalarmor® GF-100 Resulting from the Intended Use in Meal Replacement Beverages in the United States (2009-2010 NHANES Data)

Population Group	% Users	n	All-Person Intake (mg/kg bw/day)		All-User Intake (mg/kg bw/day)	
			Mean	90th Percentile	Mean	90th Percentile
Toddlers 13 to 36 months[a]	3.5	18	0.01*	na	1.68*	2.49*
Children 4 to 11 years[a]	1.0	16	0.01*	na	1.07*	1.45
Female Teenagers 12 to 19 years	0.2	1	0.01*	na	6.96*	6.96*
Male Teenagers 12 to 19 years	1.9	12	<0.01*	na	0.20*	0.39*
Female Adults 20 and older	3.3	70	0.02	na	0.53	1.09
Male Adults 20 and older	4.5	61	0.03	na	0.67	2.74
Total Population, all ages	3.0	152	0.02	na	0.64	1.30

n = number of users identified; na = not available.
* Indicates a result that is considered not statistically reliable on the basis that fewer than 30 consumers were identified.
[a] Also includes use of Vitalarmor® GF-100 in toddler formula.

Calculation of the intakes of the main constituents of Vitalarmor® GF-100 (*i.e.*, lactoferrin and lactoperoxidase) was based on the anticipated all-user mean and 90th percentile intakes generated for the ingredient as a whole, in addition to the average concentrations of lactoferrin and lactoperoxidase in Vitalarmor® GF-100 (47 and 26%, respectively). The results of the assessment of the anticipated intake of the main constituents of Vitalarmor® GF-100 are presented in Tables IV.A.1-3 and IV.A.1-4 on a per person and per kilogram body weight basis, respectively.

Within the total population, the mean and 90th percentile all-user intake estimates for lactoferrin were 22.1 and 41.5 mg/day (0.30 and 0.61 mg/kg body weight/day), respectively, and 12.2 and 23.0 mg/day (0.17 and 0.34 mg/kg body weight/day), respectively for lactoperoxidase. In male adults, the largest (statistically reliable) consumers of Vitalarmor® GF-100, the associated mean and 90th percentile all-user intakes of lactoferrin were 26.4 and 107.7 mg/day, respectively. The mean and 90th percentile all-user intakes of lactoperoxidase were equivalent to 14.6 and 59.6 mg/day, respectively, in male adults. On a body weight basis, male adults also presented with the highest statistically reliable intakes. The mean and 90th percentile all-user intakes of lactoferrin resulting from the proposed use Vitalarmor® GF-100 were 0.31 and 1.29 mg/kg body weight/day, respectively. The mean and 90th percentile all-user intakes of lactoperoxidase were equivalent to 0.17 and 0.71 mg/kg body weight/day, respectively.

Table IV.A.1-3 Summary of the Estimated Daily All-User Intakes of the Main Constituents of Vitalarmor® GF-100 Resulting from the Intended Use of Vitalarmor® GF-100 in Meal Replacement Beverages in the United States (2009-2010 NHANES Data)

Population Group	Lactoferrin (mg/day)		Lactoperoxidase (mg/day)	
	Mean	90th Percentile	Mean	90th Percentile
Toddlers 13 to 36 months[a]	8.8*	12.9*	4.9*	7.1*
Children 4 to 11 years[a]	12.0*	13.9*	6.6*	7.7*
Female Teenagers 12 to 19 years	170.0*	170.0*	94.0*	94.0*
Male Teenagers 12 to 19 years	6.2*	14.9*	3.4*	8.2*
Female Adults 20 and older	16.9	36.4	9.4	20.2
Male Adults 20 and older	26.4	107.7	14.6	59.6
Total Population, all ages	22.1	41.5	12.2	23.0

* Indicates a result that is considered not statistically significant on the basis that fewer than 30 consumers were identified.
[a] Also includes use of Vitalarmor® GF-100 in toddler formula.

Table IV.A.1-4 Summary of the Estimated per Kilogram Body Weight Daily All-User Intakes of the Main Constituents of Vitalarmor® GF-100 Resulting from the Intended Use of Vitalarmor® GF-100 in Meal Replacement Beverages in the United States (2009-2010 NHANES Data)

Population Group	Lactoferrin (mg/kg bw/day)		Lactoperoxidase (mg/kg bw/day)	
	Mean	90th Percentile	Mean	90th Percentile
Toddlers 13 to 36 months[a]	0.74*	1.12*	0.41*	0.62*
Children 4 to 11 years[a]	0.50*	0.68*	0.28*	0.38*
Female Teenagers 12 to 19 years	3.27*	3.27*	1.81*	1.81*
Male Teenagers 12 to 19 years	0.09*	0.18*	0.05*	0.10*
Female Adults 20 and older	0.25	0.51	0.14	0.28
Male Adults 20 and older	0.31	1.29	0.17	0.71
Total Population, all ages	0.30	0.61	0.17	0.34

bw = body weight
* Indicates a result that is considered not statistically significant on the basis that fewer than 30 consumers were identified.
[a] Also includes use of Vitalarmor® GF-100 in toddler formula.

IV.A.2 Estimated Consumption of Vitalarmor® GF-100 from Use in Medical Foods

In medical foods, the inclusion level will be adjusted on a case-by-case basis such that the maximum intake of Vitalarmor® GF-100 from the consumption of medical food products supplemented with Vitalarmor® GF-100 under the recommend conditions of use of the food, will be not more than 610 mg/day (10 mg/kg body weight/day in the case of a 60-kg adult). Use of a medical food supplemented with Vitalarmor® GF-100 under conditions such that the total intake of Vitalarmor® GF-100 does not exceed 610 mg per day, will result in daily exposures of not more than 287 and 156 mg lactoferrin and lactoperoxidase, respectively. Daily maximum intake

of Vitalarmor® GF-100 and the corresponding intakes of lactoferrin and lactoperoxidase from the use of Vitalarmor® GF-100 in medical foods are presented in Table IV.A.2-1.

Table IV.A.2-1 Daily Maximum Intake of Vitalarmor® GF-100 and Corresponding Intakes of Lactoferrin and Lactoperoxidase from the Use of Vitalarmor® GF-100 in Medical Foods

Ingredient/Main Constituents	Intake	
	mg per day	mg per kg body weight[a]
Vitalarmor® GF-100	610	10.2
Main Constituents		
Lactoferrin (470 mg per 1 g Vitalarmor® GF-100)	287	4.8
Lactoperoxidase (256 mg per 1 g Vitalarmor® GF-100)	156	2.6

[a] assuming 60-kg individual.

IV.B Information to Support the Safety of Vitalarmor® GF-100 for Use in Meal Replacement Beverages and Medical Foods

IV.B.1 Occurrence in the Background Diet and Background Dietary Consumption

The protein constituents of Vitalarmor® GF-100 are existing components of the human diet resulting from their natural presence in cow's milk and foods of cow's milk origin (*e.g.*, dairy products and cow's milk protein-based infant formula), as well as from the use of cow's milk-derived protein-based ingredients (*e.g.*, whey protein concentrates, whey protein isolates) added intentionally to foods based on existing authorizations. Additionally, nursing infants and toddlers also may be exposed to some proteins of Vitalarmor® GF-100 *via* consumption of human milk.

Although Vitalarmor® GF-100 is not intended as a direct source of lactoferrin or lactoperoxidase, lactoferrin and lactoperoxidase, in partially denatured form, account for the majority of Vitalarmor® GF-100's composition. Lactoferrin and lactoperoxidase have a history of safe consumption as a result of their natural presence in the background diet. Information on the exposure to lactoferrin and lactoperoxidase from the background diet is discussed in Section IV.B.1.1. Additional exposure to these proteins from their existing uses in food is discussed in Section IV.B.1.2.

Since the protein constituents of Vitalarmor® GF 100 are normal nutritive components of human and cow's milk, history of safe consumption resulting from the consumption of the protein constituents as part of the normal human diet at all stages of life corroborates the safety of Vitalarmor® GF-100.

IV.B.1.1 Natural Occurrence in the Diet and Estimates of Exposure

Lactoferrin and lactoperoxidase are present in cow's milk at concentrations of 100 to 150 mg/L (Ferenc Levay and Viljoen, 1995; Korhonen and Pihlanto, 2003) and 30 mg/L (Korhonen and Pihlanto, 2003; Indyk *et al*., 2006; FAO, 2015), respectively. Exposure to the main proteins of Vitalarmor® GF-100 will occur through consumption of cow's milk, as well as cow's milk-based (dairy) products (*e.g*., yoghurts).

In the U.S., annual *per capita* consumption of milk was estimated to be approximately 72 L (approximately 197 mL/day) (Government of Canada, 2015). Assuming the identified concentrations of lactoferrin and lactoperoxidase in cow's milk, crude daily intakes of approximately 20 to 30 mg/person of lactoferrin and 6 mg/person of lactoperoxidase from milk consumption can be estimated. Slightly higher intakes of the proteins were obtained based on average daily consumption of dairy foods (milk, cheese, and yogurt) as reported by the National Dairy Council (2011) for the U.S. population. The intake estimates of dairy and the corresponding intakes of each of the main protein constituents of Vitalarmor® GF-100 are presented in Table IV.B.1.1-1.

Table IV.B.1.1-1 Daily Average Servings of Dairy in the United States (National Dairy Council, 2011)[a] and Corresponding Daily Intakes of Lactoferrin and Lactoperoxidase

Age Group (years)	Total Dairy Servings in 'cup equivalent servings'/day[b] (in mL[c])	Intakes (mg/day)	
		Lactoferrin[d]	Lactoperoxidase[e]
2 to 3	2.4 (600)	60 to 90	18
4 to 8	2.0 (500)	50 to 75	15
9 to 18	2.1 (525)	52 to 79	16
≥19	1.5 (375)	38 to 56	11
19 to 50	1.6 (400)	40 to 60	12
≥50	1.4 (350)	35 to 52	11

[a] Based on NHANES 2007-2008 data.
[b] Cup equivalent servings: 1 cup of milk or yogurt, 1.5 oz natural cheese or 2 oz processed cheese. Includes dairy in food mixtures made with dairy (*e.g*., smoothies, sauces, sandwiches, pizza).
[c] Assuming 1 cup (250 mL) of milk or yogurt.
[d] Assuming concentrations of 100 to 150 mg/L milk.
[e] Assuming concentration of 30 mg/L milk.

No published data regarding high-end consumers of milk and dairy products in the U.S. were identified. However, both average and high-end lactoferrin consumption from the background diet was estimated by Morinaga Milk Industry Co., Ltd. (Morinaga) (GRN Nos. 000464 - U.S. FDA, 2014a) based on mean and 90th percentile consumption of cow's milk and dairy products by the U.S. population (NHANES 2007-2008) and concentrations of lactoferrin in the relevant foods [from 0.05 mg/g in cheese soups, up to 0.75 mg/g in dry milk (milk powder) and powdered mixtures with dry milk] (see Table IV.B.1.1-2).

Table IV.B.1.1-2 All-User Daily Background Intake Estimates of Cow's Milk-Derived Lactoferrin (from Cow's Milk and Cow's Milk Protein-Based Products[a]) in the United States (U.S. FDA, 2014a,b)[b]

Age Group (years)	Mean Intake		90th Percentile Intake	
	mg/day	mg/kg bw/day	mg/day	mg/kg bw/day
Toddlers (12 to 35 months)	59	4.8	105	8.6
Children (3 to 11 years)	45	1.8	79	3.5
Teens (12 to 19 years)	44	0.7	96	1.5
Adults (≥20 years)	36	0.5	74	1.0
Total population	39	0.8	80	1.9

bw = body weight
[a] Yogurt, flavored milk/milk drinks, milk-based meal replacements, dry milk,, milk desserts, sauces, and gravies, cheeses, cheese mixtures, and cheese sauces.
[b] Based on NHANES 2007-2008 data.

Although GRAS Notice No. 000464 does not directly present the mean and 90th percentile intakes of milk and milk-based products (only the resulting lactoferrin intakes were provided), comparison of the mean and 90th percentile lactoferrin intakes as estimated by Morinaga suggests that there is a 1.8- to 2.2-fold difference between intakes of average and high-end consumers (U.S. FDA, 2014a). Representative estimates of 90th percentile lactoperoxidase consumption (see Table IV.B.1.1-3) were therefore generated by applying these factors to the average intakes of lactoperoxidase (as estimated based on average consumption of dairy foods presented by the National Dairy Council, 2011 – Table IV.B.1.1-1).

Table IV.B.1.1-3 Mean and 90th Percentile Estimates of Background Lactoperoxidase Exposure

Age Group (years)	Lactoperoxidase Intakes (mg/day)	
	Mean[a]	90th Percentile[b]
2 to 3	18	32
4 to 8	15	27
9 to 18	16	35
≥19	11	24
19 to 50	12	25
≥50	11	23

[a] Based on intakes of dairy reported by National Dairy Council (2011) – see Table IV.B.1.1-1.
[b] Using factors of 1.8 for the 2 to 3 and 4 to 8 age groups, 2.2 for the 9 to 18 age group, and 2.1 for the 19 and older age group based on mean and 90th percentile intakes presented for lactoferrin consumption in GRN No. 000464 (U.S. FDA, 2014a).

While infants do not typically consume significant amounts of cow's milk, exposure to the protein constituents of Vitalarmor® GF-100 in this younger population group can occur as a result of consumption of cow's milk protein-based formula. Additionally, the main constituents of Vitalarmor® GF-100, lactoferrin and lactoperoxidase, are produced endogenously and are present in human milk. Lactoferrin and lactoperoxidase of human and bovine origin are closely

related. The homology between bovine and human lactoferrin has been determined to be approximately 69% (Wal, 2004), although Manzoni *et al*. (2010) have suggested an even higher degree of amino acid identity (77%). Similarly, sequence identity of bovine and human lactoperoxidase has been shown to be 83% (BLAST analysis). Levels of lactoferrin range from approximately 1,000 to 3,200 mg/L in mature human milk (Pamblanco *et al*., 1986; Prentice *et al*., 1987; Hirai *et al*., 1990; Hennart *et al*., 1991; Rudloff and Kunz, 1997; Mastromarino *et al*., 2014). The mean concentration of lactoperoxidase in mature human whey (soluble phase of human milk) (1 to 5 months postpartum) was reported to be 0.77±0.38 mg/L (as determined using a sandwich ELISA by using antibodies raised against recombinant human lactoperoxidase) (Shin *et al.,* 2001). Therefore, in nursing infants, exposure to these proteins also occurs from the ingestion of mother's (human) milk. Based on an intake of approximately 1,000 mL of human milk in infants (U.S. EPA, 2011), infants may consume daily 1,000 to 3,200 mg lactoferrin and 0.77 mg lactoperoxidase from human milk (164 to 525 and 0.13 mg/kg body weight/day, respectively, assuming an infant body weight of 6.1-kg). As such, exposure to the proteins of Vitalarmor® GF-100 can occur at all stages of life.

In addition to lactoferrin and lactoperoxidase, Vitalarmor® GF-100 comprises numerous other proteins at lower levels. Although as discussed in Section II.A.2, detailed quantitative analysis of the non-lactoferrin/non-lactoperoxidase protein fraction of Vitalarmor® GF-100 (approximately 20% of the ingredient) was not conducted, given the source of Vitalarmor® GF-100 (cow's milk), exposure to the proteins present in Vitalarmor® GF-100 at lower concentrations also is expected to occur through the consumption of cow's milk and products made thereof. Similarly, proteins other than lactoferrin and lactoperoxidase that are present in Vitalarmor® GF-100 at lower concentrations also have been identified in human milk. For example, TGF-β_2, one of the minor proteins identified in Vitalarmor® GF-100, also occurs naturally in human milk. In mature human milk, the average concentrations vary from approximately 0.5 to 5.6 µg/L (up to 57 µg/L at upper-end of concentrations) (Srivastava *et al*., 1996; Hawkes *et al*., 1999; Kalliomäki *et al*., 1999; Böttcher *et al.,* 2000; McPherson and Wagner, 2001; Rautava *et al*., 2002; Laiho *et al*., 2003; Rosales *et al.,* 2009). The consumption of TGF-β_2 from human milk is therefore estimated to be as high as 57 µg/day or 9.3 µg/kg body weight/day.

IV.B.1.2 Consumption from Direct Addition to Food Based on Existing Authorizations

It is recognized that proteins in cow's milk and cow's milk-based products intended for human consumption will have undergone some denaturation as a result of pasteurization and other technological processes. While the pasteurization stage of the Vitalarmor® GF-100 production process will also result in a certain degree of protein denaturation (similar to the level of protein denaturation occurring in cow's milk as a result of pasteurization), the ingredient will in part retain some level of native proteins. Apart from exposure *via* direct consumption of cow's milk or products derived thereof, exposure to the native forms of the whey proteins comprising

Vitalarmor® GF-100 also may occur as a result of intentional addition of individual high-purity whey proteins or similar whey-derived ingredients to food based on existing authorizations.

In the U.S., whey and whey protein concentrate are already permitted for use in food. The FDA previously affirmed whey and whey protein concentrate (protein content of ≥25%) as GRAS (21CFR§184.1979 and 21CFR§184.1979c, respectively - U.S. FDA, 2015b). The FDA also posed no questions in response to a previous GRAS Notice informing the agency of the GRAS determination of whey protein isolate (protein content ≥90%) (GRN No. 000037 – U.S. FDA, 2000). In the FDA's response to GRN No. 000037 for whey protein isolate, the agency noted that at the time of their review of the notice, the *per capita* consumption of whey products was approximately 3.8 grams/person/day in the U.S. The FDA noted that the incremental intake of 200 mg/person/day resulting from the proposed uses of the whey protein isolate (and dairy product solids) would result in an overall increase in the combined intake of whey products of 5%. Based on more recent data published by the USDA, the *per capita* consumption of whey (dry) in the U.S. was estimated at 1.6 lbs/year for 2013 (USDA-ERS, 2014), equivalent to approximately 2 grams/person/day.

The FDA also had no objections to the determination of a specific bovine milk basic protein fraction (milk basic protein or MBP®)[3] and highly purified bovine lactoferrin as GRAS (U.S. FDA, 2006, 2014a,b). High-purity bovine lactoferrin was determined to be GRAS for addition to various milk-based products (*i.e.*, yoghurts, powdered milk, and milk-based frozen desserts) at levels of up to 400 mg/100 g and chewing gum at a level of 30 mg/g (U.S. FDA, 2014a). Based on the specified conditions of use, all-user total population mean and 90th percentile lactoferrin intakes of 142 mg/day (2.7 mg/kg body weight per day) and 273 mg/day (5.7 mg/kg body weight/day), respectively, were estimated. Bovine lactoferrin also was considered GRAS for use in infant formula (100 mg/per 100 g powdered formulas, 26 mg/100 mL liquid concentrates, and 13 mg/100 mL ready-to-feed formula) (U.S. FDA, 2014b). Under the conditions of use presented in the GRAS Notice, mean and 90th percentile intakes of 87.4 to 100.3 and 129.0 to 145.5 mg/day were estimated for infants under 12 months of age. Bovine milk basic protein fraction or MBP® was determined to be GRAS for use in a number of foods including dairy-type foods, juice, meal replacement products, and salad dressings at levels of 10 to 40 mg/serving (U.S. FDA, 2006). Based on the maximum concentrations of the individual proteins in MBP®, intakes up to 70 and 60 mg/day of lactoferrin and lactoperoxidase may occur from the consumption of foods with added MBP®.

In addition to the conventional food uses, lactoferrin and lactoperoxidase also are available as ingredients of food (dietary) supplements. In the U.S., recommended doses of up to 1,000 mg/day are indicated for lactoferrin from dietary supplement use (NIH, 2015). Likewise,

[3] ≥90% total protein: 40 to 70% lactoferrin, 15 to 60% lactoperoxidase, and ≥0.02% cystatin C, as well as trace amounts of HMG (high-mobility group)-like protein and kininogen fragment 1·2.

dose recommendations of up to 2 mg lactoperoxidase/day were identified for lactoperoxidase-containing dietary supplement products (NIH, 2015).

Bovine lactoferrin also is permitted for addition to a number of foods in other jurisdictions. In the EU, maximum permitted use-levels for conventional foods range from 50 to 3,000 mg bovine lactoferrin/100 g (EU, 2012a,b). Foods for special medical purposes may provide up to 3,000 mg of lactoferrin per day. In infant and toddler (follow-on) formulas, lactoferrin is permitted for use at a maximum use level of 100 mg/100 mL. Lactoferrin also is permitted for use in infant formulas and conventional foods in several East Asia countries including Japan, China, Korea, and Taiwan.

The safety of a lactoperoxidase-containing system (including thiocyanate and hydrogen peroxide in addition to lactoperoxidase) for use in milk preservation was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives and found to be acceptable (JECFA, 1990). However, because the lactoperoxidase system for use in milk does not require addition of exogenous sources of lactoperoxidase, but rather relies on the natural presence of this enzyme in milk, the Committee did not consider it necessary to specifically assess the safety of lactoperoxidase for human consumption from the use of this system in milk. A lactoperoxidase-based antimicrobial system also is approved for use on meat in Australia and New Zealand (FSANZ, 2002). Lactoperoxidase also appears on the '*List of Existing Food Additives in Japan*' (MHLW, 2014).

IV.B.1.3 Summary

Significant levels of background exposure to the proteins of Vitalarmor® GF-100 resulting from the natural occurrence of the proteins in food, as well as from their direct addition to food, are expected at all stages of life, including during infancy. Exposure to the whey proteins comprising Vitalarmor® GF-100 may occur as a result of the consumption of cow's milk and food based on cow's milk, as well as foods which have been supplemented with high-purity proteins or similar whey protein-based ingredients derived from cow's milk. During infancy, exposure also may occur *via* consumption of either human milk or cow's milk protein-based formulas and other formulas supplemented with specific whey proteins.

IV.B.2 Absorption, Distribution, Metabolism, and Excretion

Studies examining the metabolic fate of Vitalarmor® GF-100 specifically are not available. However, Vitalarmor® GF-100 is a whey protein isolate consisting primarily of proteins that are naturally present in cow's milk (≥90% protein matter; 'as is' basis). Therefore, the metabolic fate of the proteins present in Vitalarmor® GF-100 following consumption of products containing the ingredient is expected to be largely similar as that following their consumption from cow's milk or any other food source containing cow's milk-derived protein (*e.g.*, dairy products, powdered milk, whey protein products, *etc*.).

Limited data were identified related to the metabolic fate and possible bioavailability of the main constituents of Vitalarmor® GF-100, lactoferrin and lactoperoxidase, following oral administration.

In a few studies conducted to characterize the metabolic fate of bovine lactoferrin in mice and rats, some resistance to degradation of the protein in the gut was observed (Kuwata *et al.,* 1998a, 2001; Wakabayashi *et al*., 2004). Data regarding systemic uptake of the unhydrolyzed protein or major protein fragments are somewhat inconsistent with some studies suggesting uptake of lactoferrin from the gut (Takeuchi *et al*., 2004; Fischer *et al*., 2007), but not others (Wakabayashi *et al*., 2004; Morishita *et al*., 2013). Studies in piglets appear to also indicate resistance to degradation (Harada *et al*., 1999; Comstock *et al*., 2014; Reznikov *et al*., 2014). In one study, orally administered bovine lactoferrin (direct infusion into the stomach or duodenum at 1 or 3 g/kg body weight) also was shown to be taken up into the circulation of neonatal and weaner piglets and excreted into the bile (Harada *et al*., 1999).

In adults, resistance to proteolytic degradation of lactoferrin was observed (Kuwata *et al.,* 1998b; Troost *et al*., 2001). Khan *et al*. (2000) reported the presence of protein bound radioactivity in the plasma of study participants (male and female adults) following consumption of encapsulated radiolabeled human lactoferrin.

Results from some *in vitro* investigations suggest that lactoperoxidase retains some peroxidase activity following incubation in the presence of infant or adult gastric juices (Gothefors and Marklund, 1975), but is liable to degradation in the presence of pepsin (Kussendrager and van Hooijdonk, 2000; Goodman *et al*., 2007). Resistance of lactoperoxidase to degradation by enzymes of the upper intestine (trypsin and chymotrypin) was reported by Kussendrager and van Hooijdonk (2000). Lactoperoxidase activity was only slightly reduced in the stomach when human colostrum samples were provided to an infant with pyloric stenosis prior to the infant undergoing pyloromyotomy (Gothefors and Marklund, 1975).

Available data suggest at least partial resistance of lactoferrin and lactoperoxidase to digestion in the stomach and small intestine. Although a few animal studies appear to indicate permeation of undegraded lactoferrin, true absorption of intact protein is not expected to occur. The metabolic fate of proteins within Vitalarmor® GF-100 will be largely similar to that following the consumption of these proteins from cow's milk, cow's milk-based dairy products, or other whey protein-supplemented foods.

IV.B.3 Toxicological Studies

IV.B.3.1 Studies with Vitalarmor® GF-100

1. Subchronic Oral Toxicity Study

A subchronic oral toxicity study of Armor's whey protein isolate (Vitalarmor® GF-100; 97% protein content; Batch No. 111217) was conducted with 6-week old male and female Sprague-Dawley rats using cGLP and in accordance with OECD guidelines for the testing of chemicals (OECD Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents) (Forster *et al*., 2014). Groups of rats (10/sex/group) were administered the test material *via* gavage at doses[4] of 0 (control, vehicle [0.15 M NaCl solution]), 600, 1,200, or 2,000 mg/kg body weight/day for a period of 13 weeks. Two additional satellite groups of rats (6/sex/group) at 0 (control) and 2,000 mg/kg body weight/day dose levels were dosed for the 13-week treatment period and then followed by a 4-week recovery period.

Two (2) or 4 animals of the same gender and from the same test group were housed together per cage (polycarbonate cages) and provided laboratory chow (pelleted maintenance chow) and water *ad libitum* for the duration of the study period. The rats were inspected at least once daily for clinical signs of toxicity, morbidity, and mortality. More detailed clinical examinations were performed once before the start of the study and then weekly thereafter and included assessments of posture, skin, eyes, coat, mucus membranes, abnormal secretions, and autonomic activity. Each rat was weighed during the acclimation period, on the first day of treatment and weekly thereafter (except for Week 14). Daily food consumption/animal was calculated on the basis of total weekly food consumption (*i.e*., quantity of food consumed by the animals in each cage per week). Ophthalmological examinations were performed on both eyes of all rats before the beginning of the treatment period and on surviving animals treated with 0 and 2,000 mg/kg body weight/day at completion of the treatment period. A Functional Observational Battery (FOB) was performed for all main group animals on Week 12. Standard hematological, blood biochemistry, and urinalysis parameters were measured at the end of the 13-week treatment period (or at the time of sacrifice in the case of animals killed prematurely) and following the 4-week recovery period for any parameters exhibiting test article-related changes. All animals surviving until the completion of the treatment and recovery periods were euthanized, necropsied, and subjected to full macroscopic evaluations. Animals were weighed immediately prior to death and the adrenals, brain, epididymides, heart, kidneys, ovaries, spleen, testes, thymus, and uterus were excised and weighed (absolute and relative to body weight). Microscopic examination was performed on all major organ tissues from rats treated at the 0 or 2,000 mg/kg body weight/day doses and on any macroscopic lesions identified in low- (600 mg/kg body weight/day) and mid- (1,200 mg/kg body weight/day) dosed rats euthanized at

[4] The highest dose was considered to be the maximum feasible dose based on dosage volume (10 mL/kg body weight/day) and viscosity of the formulation (Forster *et al*., 2014).

the end of the treatment period. Any animals terminated prematurely also were subjected to gross and histopathological examination.

The results reported did not include findings in the satellite groups. A single high-dose (2,000 mg/kg body weight/day) male was sacrificed prematurely on Day 33 due to poor general health conditions and thoracic abscess attributed to esophageal perforation from gavage error. No other deaths occurred and no compound-related clinical signs of toxicity were reported in any of the other animals. Also reported were lacrimation in 1 female and piloerection in 1 male treated with 600 and 1,200 mg/kg body weight/day, respectively, but these findings were not dose-related and not attributed to treatment. No evidence of neurotoxicity from FOB testing at the end of the treatment was reported.

There were no significant effects of the test article on food consumption or mean body weight.

Hematological analyses conducted at the end of the study period showed a few statistically significant variations between control and test group animals; however, given the small amplitude of these changes and the fact that differences were only observed in one sex and lacked a dose–response, the hematological changes were considered incidental and biologically insignificant. A few blood biochemistry parameters were statistically significant, but all were considered incidental and biologically insignificant (small amplitude of change, no dose-response, seen in only 1 sex). Urinalysis revealed significantly lower urine volume in high-dosed females only and was accompanied by significantly lower urine pH and significantly increased urine specific gravity. Notably, similar effects have been reported with other protein materials, including a 13-week rat study with a compositionally similar milk-protein based material (*e.g.*, bovine milk basic protein fraction) and may be simply related to the handling of increased amounts of protein by the renal system (Kruger *et al.*, 2007 – see Section IV.B.3.2). The changes in urinary parameters observed in the study with Vitalarmor® GF-100 were not accompanied by any clinical chemistry or histopathological evidence that suggested a nephrotoxic effect. Since none of the changes in hematological, biochemistry, or urinalysis parameters were considered to be related to the administration of the compound, analyses were not conducted at the end of the recovery period in the satellite group.

End-of treatment investigations revealed slightly (less than 5%), but statistically significant lower absolute brain weights in females receiving 600 mg/kg body weight/day compared to controls. As this difference was limited to females, was not dose-dependent, and was of low magnitude, it was not attributed to the administration of the test material. Furthermore, no statistically significant variability was reported in relative brain weights. No other differences in organ weights were apparent between treatment and control groups at the end of the treatment period and at the end of the recovery period. No gross pathological or histopathological findings related to the test material were identified at the end of the treatment period.

Based on the lack of any adverse effects reported in the study at any of the doses tested, the NOAEL for Armor's Vitalarmor® GF-100 was established as 2,000 mg/kg body weight/day, the highest dose tested (Forster *et al*., 2014).

2. 6-Week Juvenile Rat Oral Toxicity Study

The toxicity of Vitalarmor® GF-100 was evaluated in a juvenile rat study (Forster *et al*., 2014). The study was conducted under cGLP and in consideration of the U.S. FDA's 2006 Guidance for Industry: *Nonclinical Evaluation of Pediatric Drug Products*. Sprague-Dawley rats (10/sex/group) were randomized to one of two treatment groups provided Vitalarmor® GF-100 *via* gavage at doses providing 0 or 600[5] mg/kg body weight. Dosing was initiated on PND 7[6] and animals were gavaged daily for 6 weeks until approximately the onset of sexual maturation (PND 49) (Campion *et al*., 2013; Sengupta, 2013; Boston University, 2011). Additional groups of rats (6/sex/group) were administered 0 (control, vehicle [0.15 M NaCl solution]) or 600 mg/kg body weight/day from PND 7 to PND 49 inclusive, which was followed by a 4-week recovery period. Dams remained with their offspring until weaning on PND 21. Until weaning, dams were housed individually with their pups. Thereafter (PND 21), pups were housed in groups of 3 to 4 animals/cage.

The rats were inspected twice daily for signs of morbidity and mortality. Once daily, both dams and pups were observed for clinical signs of toxicity. Pup body weight was recorded once before group allocation, on the first day of treatment and weekly for the rest of the treatment period for all animals and twice weekly during the recovery period. Food consumption was recorded twice weekly from weaning until euthanasia (food consumption was calculated per animal based on total food consumption in cages divided by the number of animals in the cage). The length of the tibia (long bone growth) was recorded every 2 days from the beginning of treatment up to weaning and then weekly thereafter to the end of the treatment period. Animals were assessed daily for developmental landmarks and neurological development (including pre-weaning development, FOB, motor activities, learning and memory). Reproductive maturity was monitored starting from PND 40 and 28 in males and females until preputial separation and vaginal opening were observed, respectively. Ophthalmological examinations were performed on one occasion after weaning (PND 25) and at the end of the treatment period. Standard hematological, blood biochemistry, and urinalysis parameters were measured at the end of the treatment period and recovery period (in the case of satellite animals). At the end of the treatment or recovery periods, animals were euthanized and the adrenals, brain, epididymides, kidneys, liver, spleen, testes, thymus, thyroids with parathyroids, and uterus were weighed. Tissues were preserved and microscopic examination was performed on all major organ tissues

[5] The dose was selected based on the viscosity of the formulation and the dosage volume for pre-weaning pups of 2 mL/kg body weight/day (Forster *et al*., 2014). The dose selected for the 6-week juvenile rat study corresponded to the lowest dose tested in the standard 90-day multi-dose study, in which the highest dose tested (2,000 mg/kg body weight/day) was determined to be the NOAEL (Forster *et al*., 2014).
[6] "*Neonates are defined as mouse or rat pups up to 10 days of age*." (Boston University, 2011).

from all animals terminated at the end of the treatment period and all macroscopic lesions identified during the gross examination.

With the exception of one control group male pup found cannibalized on Study Day 3, all animals survived until the end of the treatment and recovery periods. No clinical signs of toxicity were reported in the test group animals. Mean body weights in both males and females in the treatment group were significantly lower compared to controls until Study Day 19, resulting from significantly lower body weight gains during the first 15 days of the testing period. Thereafter, body weight gains of test animals were comparable to controls and by the end of the treatment period, body weights of test animals were only slightly lower (approximately 5%) compared to controls and the differences were no longer statistically significant. Throughout the recovery period, test animals gained comparable amounts of weight or slightly more weight than controls, with no significant differences in mean body weights reported at study end. No differences in tibia lengths were observed between groups. While the authors did note that the changes in body weights were likely the result of changes in the nutritional benefits of the dams' milk (possibly resulting from the viscosity of the test substance, physical obstruction, or pup satiety), given the magnitude of the change (less than 10% difference) and the reversibility, the small body weight reductions of treated pups were not considered adverse. There were no effects indicative of toxicity to reproductive development based on mean age of vaginal opening or cleavage of the balanopreputial gland. No adverse developmental or neurological effects on motor activity and pre-weaning development and no impairment of learning or memory were associated with the administration of the test article. In addition, there were no test article-related ophthalmological findings reported.

There were no consistent, treatment-related, statistically significant adverse effects reported on the hematological and biochemical parameters evaluated. Eosinophil levels of male and female test group animals were lower in the treatment animals relative to controls (0.12 *versus* 0.07 G/L in males and 0.16 *versus* 0.09 G/L in females). However, eosinophil levels were within the laboratory's historical control values, and no differences in eosinophil levels were observed between the satellite recovery test and control groups. The authors noted that eosinophil levels are highly variable, and as such the reduction may have been a fortuitous finding; however, given the magnitude of the change and occurrence of the finding in both genders, the possibility that the difference in eosinophil levels between the test and control animals was related to the administration of Vitalarmor® GF-100 could not be excluded. Furthermore, the authors also reported that *in vitro* TGF-β_2 has been shown to possess modulating properties on eosinophil development. However, since the changes in eosinophil values observed in this study were not associated with any clinical consequences and levels remained within historical control limits, the effects were not considered adverse. Moreover, similar effects on eosinophil counts were not observed in the subchronic toxicity study at equivalent and higher dose levels in either sex.

A decrease in urine volume was accompanied by an increase in urine specific gravity (also seen in the 90-day study) at the end of the treatment period, but only in the females (compared to controls). At the end of the recovery period, the inverse was apparent (increase in urine volume and decrease in specific gravity).

A statistically significant increase in the relative thymus weights of females and in the absolute brain weights of males compared to controls were reported at the end of the treatment period. For animals euthanized at the end of the recovery period, the following statistically significant variations were observed: an increase in male absolute and relative liver weights, a decrease in male absolute and relative epididymides weights, and a decrease in female absolute thyroid weights. None of these changes had been reported during the treatment period, and the changes only occurred in a single gender and were not accompanied by any relevant clinical chemistry variations or macroscopic lesions. Therefore, they were regarded as not related to the administration of the test material. Additionally, at the end of the recovery period, thymus weights (absolute and relative) of treated males were significantly greater (approximately 1.4 times) than those of controls. In the control male group, an expected decrease in the size of the thymus gland (consistent with thymus involution following birth) was apparent during the 4-week recovery period (from approximately 0.67 grams at the end of the treatment period to 0.50 grams at the end of the recovery period). However, in test males, a similar decrease in thymus weights was not observed during the 4 weeks following treatment (approximately 0.70 grams at the end of the treatment period *versus* 0.68 grams at the end of the recovery period). Similar differences were not reported in the females at the end of the recovery period.

Gross examination of all groups at necropsy was unremarkable and histopathological evaluations did not identify any treatment-related effects. Microscopic examination of the thymus (conducted as a result of the weight differences at the end of the recovery period between test and control males) also did not reveal any changes in cellularity or structure of the thymus between groups. The apparent differences in thymus weights between Vitalarmor® GF-100-treated animals and controls at the end of the recovery period were not consistent between sexes and also were not consistent with findings at the end of the treatment period (while at the end of the treatment period, relative thymus weights of treated females were slightly higher than controls, both absolute and relative thymus weights of females were comparable at the end of the recovery period). The observed differences in thymus weights between groups at various time-points are therefore likely a function of random temporal differences in ontogenic development between animals within the test groups. In addition, thymus involution (age-related regression in the size of the thymus) is a continual process occurring throughout the life of all mammals (Gui *et al*., 2012). In rats, re-modeling of the thymus leading to the organ's involution is apparent throughout the first 12 months of life (Quaglino *et al*., 1998). As such, treatment-related inhibition or delay in thymus involution is not likely to be specific to the neonatal stage, and therefore would be expected to also occur in the 13-week subchronic study (animals at the start of the 13-week study were 6 weeks old). The

absence of any effects on thymus weights or thymus histopathology in the 13-week subchronic toxicity study therefore further support the conclusion that differences in thymus weights were due to random biological variation.

Based on the lack of toxicologically significant effects observed in the study, the NOAEL for the whey protein isolate in juvenile rats was established to be 600 mg/kg body weight/day, the only dose level tested.

3. Short-Term Genotoxicity Studies

The potential genotoxicity of Vitalarmor® GF-100 was investigated *in vitro* in bacterial and mammalian test systems (Forster *et al*., 2014; see Table IV.B.3.1-1).

Evaluated in a standard battery (OECD Test No. 471-compliant) of *Salmonella* Typhimurium strains at concentrations of up to 5,000 µg/plate, with and without metabolic activation, Vitalarmor® GF-100 did not induce an increase in mutant colonies relative to the vehicle control. In the micronucleus assay (OECD Test No. 487-compliant), exposure of L5178Y $TK^{+/-}$ cells to Vitalarmor® GF-100 concentrations of 39, 78, 156.3, 312.5, or 625 µg/mL or 1,250 µg/mL (3- and 24-hour exposure treatments) was not associated with a significant increase in the frequency of micronuclei.

Table IV.B.3.1-1 Summary of *In vitro* Genotoxicity Studies on Vitalarmor® GF-100

Test System	Type	Metabolic Activation	Concentration(s) Tested	Result	Reference (Study No.)
Salmonella Typhimurium TA98, TA100, TA102, TA1535, and TA1537	Mut	+/-S9	156.3 to 5,000 µg/plate	Negative	Forster *et al*. (2014)
L5178Y/TK mouse lymphoma cells	MN	+/-S9	39 to 2,500 µg/mL*	Negative[a]	Forster *et al*. (2014)

Mut = mutation test; MN = micronucleus test.
* Precipitate formed at concentrations of ≥625 µg/mL [with 3-hour treatments (with and without S9) and ≥1,250 µg/mL with 24-hour treatment (without S9)].
[a] The concentrations selected for micronucleus analysis were: 156.3, 312.5, and 625 µg/mL for the 3-hour treatments [with (experiment 1 and 2) and without (experiment 1) S9], and 312.5, 625, and 1,250 µg/mL for the 24-hour treatment [without S9 (experiment 2)].

4. Summary of Product-Specific Studies

The potential toxicity of Vitalarmor® GF-100 was investigated in several ingredient-specific studies, including a standard 90-day oral rat toxicity study, a special 6-week rat toxicity study with juvenile animals that at the start of the study were 7 days old (neonatal stage), and *in vitro* mutagenicity and genotoxicity assays. A NOAEL of 2,000 mg/kg body weight/day (the highest dose tested) was established for Vitalarmor® GF-100 in rats on the basis of a 90-day oral toxicity study. While some statistically significant differences were observed between groups of test animals and the controls, none were considered to be related to the administration of the test

compound. In another study involving oral administration of Vitalarmor® GF-100 to male and female juvenile rats at a dose of 600 mg/kg body weight/day (only dose tested) for a period of 6 weeks, no biologically significant adverse effects were reported. Based on the results of this study, 600 mg/kg body weight/day (the only dose level tested) was determined to be the NOAEL for Vitalarmor® GF-100 in juvenile rats. Additionally, Vitalarmor® GF-100 was reported to be non-mutagenic when examined in the Ames assay and non-genotoxic when tested in the *in vitro* micronucleus assays (at concentrations of up to 5,000 µg/plate and 2,500 µg/L, respectively).

IV.B.3.2 Studies on Compositionally Related Ingredients and on the Major Constituents of Vitalarmor® GF-100

Results of toxicological studies with highly purified bovine lactoferrin and other compositionally related whey protein-based ingredients manufactured using similar production processes as the method used to obtain to Vitalarmor® GF-100 [*i.e.*, bovine milk basic protein fraction (milk basic protein or MBP®) and a proprietary whey extract protein (Lactermin®)] also were considered as part of the GRAS determination of Vitalarmor® GF-100. High-purity bovine lactoferrin and MBP® were previously determined as GRAS and the FDA was notified of both GRAS determinations (GRAS Notice GRN Nos. 000464 and 000465 and GRAS Notice GRN No. 000196, respectively - U.S. FDA, 2006, 2014a,b). The results of the studies included in the previous GRAS Notices, as well as results of any new studies which have become available since the GRAS determinations were notified, are briefly summarized below. Likewise, results of studies conducted with Lactermin® also are presented below.

1. Bovine Lactoferrin

The short- and long-term oral toxicity of bovine lactoferrin was evaluated by Yamauchi *et al*. (2000a) and Tamano *et al*. (2008). These studies were the subject of comprehensive reviews during the GRAS determination of bovine lactoferrin for use in infant formula and other food uses and are therefore incorporated by reference to GRAS Notice Nos. GRN 000464 and 000465 (U.S. FDA, 2014a,b).

In a standard 13-week study, Sprague-Dawley rats were provided bovine lactoferrin by gavage at doses of 0 (control), 200, 600, or 2,000 mg/kg body weight/day (Yamauchi *et al*., 2000a). Some variability was observed in parameters related to urinalysis, thyroid weights (decreased) of high-dose females, and microscopic examination of the pancreas in males; however, none of the changes were considered to be directly related to the administration of lactoferrin. The authors determined a NOAEL of 2,000 mg/kg body weight/day for bovine lactoferrin.

In a further investigation involving longer periods of dosing, F344/DuCrj rats were provided bovine lactoferrin at dietary levels of (i) 0 (control) or 0.2% (approximately 100 mg/kg body weight/day) for 40 weeks or (ii) 0 (control), 0.02, 0.2, 2.0, or 5.0% (equivalent to up to approximately 2,500 mg/kg body weight/day for 60 (males) or 65 (females) weeks (Tamano *et*

al., 2008). In the 40-week study, significant decreases in relative liver weights and levels of AST, ALT, and ALP were observed. The authors considered these hepatic changes as being possibly indicative of improved liver function in the aging rats treated with bovine lactoferrin. Likewise, a decrease in blood urea nitrogen in the 40-week test animals was considered to be possibly associated with a lactoferrin-related protective effect on kidney function. In the 60/65-week study, clinical chemistry measurements were not included; however, body weights, food and water consumption, and organ weights of test and control animals were comparable. Furthermore, macroscopic examination and histopathology were unremarkable. The authors concluded that "the results indicated that the NOAEL for bovine lactoferrin with 60 or 65 weeks dietary treatment is at least 5.0% (2,500 mg/kg body weight/day) for both sexes" (Tamano *et al*., 2008).

Bovine lactoferrin also was shown to be non-mutagenic when tested *in vitro* under the conditions of the Ames assay in *Salmonella* (TA98, TA100, TA1535, and TA1537) and *E. coli* Wp2 *uvr*A with and without metabolic activation at concentrations of up to 5,000 µg/plate (Yamauchi *et al.,* 2000b).

In addition to the studies included in the previous GRAS determination Notices for bovine lactoferrin, a few more recent animal studies with bovine lactoferrin were identified (Comstock *et al*., 2014; Shumake *et al*., 2014; Somm *et al*., 2014; Reznikov *et al*., 2014; Chen *et al*., 2015).

Comstock *et al*. (2014) evaluated the effects of bovine lactoferrin on immune development in piglets. Formula supplemented with bovine lactoferrin was administered to colostrum-deprived piglets starting at birth for a period of 7 or 14 days (piglets were provided sow serum at birth, and at 8, 22, and 36 hours thereafter to provide passive immunity). Piglets in the control group were exposed to a lactoferrin dose of 130 mg/kg body weight/day whereas test animals received either 367 or 1,300 mg lactoferrin/kg body weight/day. Comparison of all 3 groups indicated that the diet tended to have an effect on survival ($p=0.08$), such that piglets in the high-dose lactoferrin group were more likely to survive than the control group animals. Assessment of several parameters related to immune function revealed that dietary administration of lactoferrin had either no effect or in some cases elicited a positive effect on the immune system: higher IgG concentrations; increase in IFN-γ production in splenocytes from high-dose lactoferrin piglets in response to PHA induction; an increase in IL-6 and IL-10 production and in IFN-γ production in LPS-stimulated mesenteric lymph node cells and splenocytes, respectively, from lactoferrin-treated piglets; and an increase in IL-10 and TNF-α production in splenocytes from high-dose lactoferrin piglets in response to LPS induction. In a related publication of the same study (Reznikov *et al*., 2014), the authors further reported that high dose lactoferrin piglets exhibited significantly greater intestinal weights compared to the controls. Furthermore, lactoferrin-fed piglets also had increased crypt depth and area, as well as crypt cell proliferation, suggesting that dietary lactoferrin may contribute to gut maturation during the neonatal period.

In a study conducted to evaluate the potential effects of bovine lactoferrin on neurodevelopment and cognition in postnatal domestic male piglets, lactoferrin administration appeared to facilitate cognition and learning as evidenced by better outcomes in a learning assessment (8-arm radial maze) in piglets provided a higher dose of lactoferrin in the diet (155 mg lactoferrin/kg body weight/day from PND 3 until 38) relative to a group provided a lower lactoferrin dose (15 mg/kg body weight/day) (Chen *et al*., 2015). Body weight gains of piglets receiving either 15 or 155 mg lactoferrin/kg body weight/day were reported to be comparable throughout the treatment period.

A few additional non-standard studies also were conducted with bovine lactoferrin in which parameters related to intrauterine and postnatal rat development were assessed (Shumake *et al*., 2014; Somm *et al*., 2014). Daily intakes of up to 1,300 mg lactoferrin/kg body weight (provided as 0.85% in the diet) by pregnant or lactating Sprague-Dawley OFA rats beginning on Gestation Day 0 until PND 21 was not associated with any adverse effects on dams' health (weight, food intake, food utilization efficiency, hematological analysis), litter size, or pup weight (Somm *et al*., 2014). Oral administration of up to 2,000 mg lactoferrin/kg body weight/day to male and female Holtzman albino rat pups starting on PND 16 until PND 34/36, was not associated with any adverse effects on rats' general motor activity, behavior, and/or learning (Shumake *et al*., 2014). For some parameters related to behavior and/or learning in pups, exposure to lactoferrin appeared to be associated with better outcomes.

2. *Milk Basic Protein (MBP®)*

A series of toxicological studies were conducted with a specific bovine milk basic protein fraction [milk basic protein (MBP®)] (Kruger *et al*., 2007). MBP® consists of lactoferrin and lactoperoxidase, which are present in MBP® at concentrations of approximately 54 and 41%, respectively. MBP has been the subject of a previous GRAS determination for use in various food and beverage products at levels ranging from 10 to 40 mg per serving [GRN No. 000196 (U.S. FDA, 2006)].

A medial lethal dose (LD_{50}) of greater than 2,000 mg/kg body weight/day was determined for MBP® in Crj:CD (SD) IGS rats (Kruger *et al*., 2007). .

In a 13-week study, no compound-related adverse effects were reported following oral gavage administration of MBP® to male and female Crj:CD (SD) IGS rats at doses of 200 or 2,000 mg/kg body weight/day (Kruger *et al*., 2007). Differences observed between control and test animals in body weight, body weight gain, food consumption, and food utilization efficiency were not consistent over time, were not seen in both sexes, were not dose-dependent, and were within historical ranges and as such were not considered to be related to the compound. Furthermore, statistically significant differences between control and test animals were observed in parameters related to clinical chemistry and urinalysis; none of these variabilities were considered by the study authors as being related to the administration of the compound. No

statistically significant variations were reported in organ weights between test and control animals. Gross examination of animals at necropsy and histological examination of major tissues were unremarkable. A NOAEL of 2,000 mg/kg body weight/day was derived for MBP® in rats on the basis of the 90-day toxicity study.

The gavage administration of MBP® at a dose of 2,000 mg/kg body weight/day to pregnant female Crj:CD (SD) IGS rats on Days 7 through 17 of gestation also did not produce any adverse reproductive or developmental effects (Kruger *et al*., 2007). Tested *in vitro*, in the Ames assay in *Salmonella* Typhimurium TA98 and TA100, with and without metabolic activation, MBP® tested negative at concentrations of up to 5,000 µg/plate (Kruger *et al*., 2007).

3. Proprietary Whey Extract (Lactermin®)

Studies also were conducted with a proprietary whey extract, Lactermin®, produced by cation-exchange chromatography and characterized by a lactoferrin and lactoperoxidase composition of at least 50% of the total protein content, as well as smaller amounts of several milk-protein growth factors (IGF-I, IGF-II, PDGF, FGF, TGF-β, and betacellulin) (Dyer *et al*., 2008). In a 13-week oral toxicity study, no adverse effects related to the administration of the extract, provided at target dose levels of 0 (control), 300, 1,000, or 3,000 mg/kg body weight/day, were reported in male and female Sprague-Dawley rats (Dyer *et al*., 2008). A few statistically significant differences were observed between control and test animals in body weights, food consumption, clinical chemistry and urinalysis parameters, and organ weights. These changes however were either not dose-dependent, observed only in one gender, or within historical control ranges and thus were not considered to be related to the administration of the whey extract. No histological variations were observed in the study. The authors concluded the NOAEL for the whey extract to be 3,000 mg/kg body weight/day, the highest dose tested, in rats.

Lactermin® was not associated with any evidence of genotoxicity when examined *in vitro* in the Ames assay (*Salmonella* Typhimurium TA98, TA100, TA1535, and TA1537; *Escherichia coli* Wp2 *uvr*A) or the thymidine kinase (L5178Y/TK mouse lymphoma cells) assay at concentrations of up to 5,000 µg/plate, both the presence and absence of metabolic activation (Dyer *et al.,* 2008). The whey extract also was not genotoxic in an *in vivo* micronucleus assay when ICR mice were dosed at levels of up to 2,000 mg/kg body weight by gavage (Dyer *et al.,* 2008).

4. Summary of Studies Conducted with Compositionally Related Ingredients and Major Constituents of Vitalarmor® GF-100

Overall, no biologically significant adverse effects were observed in subchronic oral rat studies in which animals were administered up to 2,500 mg/kg body weight/day of bovine lactoferrin. Likewise, administration of compositionally related milk protein-based ingredients (MBP®, Lactermin®) to rats at doses of up to 3,000 mg/kg body weight/day for a period of 13 weeks also

was not associated with any adverse effects. No adverse effects also were reported in a few studies evaluating end-points related to in utero and post-natal development in animals, including a standard toxicity study, following exposure to lactoferrin or a compositionally related whey extract (MBP®) at doses of up to 2,000 mg/kg body weight.

IV.B.4 Human Studies

The safety of Vitalarmor® GF-100 has not been evaluated in studies involving humans. However, human studies involving consumption of bovine lactoferrin, one of the main protein components of Vitalarmor® GF-100, were available for review. Many of the studies also were previously included in the 2 GRAS Notices for bovine lactoferrin [GRAS Notice Nos. GRN 000464 and GRN 000465 (U.S. FDA, 2014a,b)]. The clinical studies were primarily designed to assess parameters related to potentially beneficial effects related to the consumption of this protein in healthy and diseased infant, child, adolescent, and adult populations; however, a few measures related to safety also were included in some study protocols.

In studies conducted with healthy adults, bovine lactoferrin was well-tolerated with no associated adverse events when ingested at doses of up to 1,800 mg/day (Koikawa *et al.*, 2008; Mulder *et al.*, 2008; Bharadwaj *et al.*, 2009, 2010[7]; Mueller *et al*., 2011; Vitetta *et al*., 2013). Treatment duration in these studies ranged from 3 weeks to 6 months. All other studies involving consumption of bovine lactoferrin by adults were conducted with subjects presenting specific diseases (*i.e.*, *Helicobacter pylori* infection, chronic hepatitis C, iron deficiency anemia, periodontitis, colorectal polyps, *tinea pedis*) or other undesirable health conditions (*i.e.*, acne, obesity) (Tanaka *et al.*, 1999; Yamauchi *et al.*, 2000c; Iwasa *et al.*, 2002; Okada *et al.*, 2002; Di Mario *et al.*, 2003, 2006; Okuda *et al.*, 2005; Zullo *et al.*, 2005; Konishi *et al.*, 2006; Paesano *et al.*, 2006, 2009, 2010; Ueno *et al.*, 2006; Tursi *et al.*, 2007; Kondo *et al.*, 2008; Kozu *et al.*, 2009; Nappi *et al.*, 2009; Ishikado *et al.*, 2010; Ono *et al.*, 2010). While the majority of these studies focused on exploring the possible beneficial effects of lactoferrin on the specific diseases/conditions, some safety-related parameters also were investigated to substantiate the safety of lactoferrin administration in these studies. Daily consumption of up to 7,200 mg of lactoferrin in studies ranging in duration from 1 week to 12 months (7,200 mg lactoferrin was administered in an 8-week study, and up to 3,000 mg lactoferrin/day was consumed in the 1-year study) was well-tolerated.

Five (5) additional studies were identified which also involved the oral administration of bovine lactoferrin to healthy adults or subjects with underlying health conditions (McMillan *et al.*, 1977; Yamauchi *et al.*, 1998; Troost *et al.*, 2001; Ishii *et al.*, 2003; Shin *et al.*, 2011), but which did not specifically include any assessment of parameters related to safety. In these studies, lactoferrin was administered either once as a dose of 200 or 4,500 mg or over the course of up to 12 months at doses of 100, 600, or 2,000 mg/day. While the study publications did not specifically

[7] Bharadwaj *et al.* (2009) and (2010) are kin publications and were therefore evaluated as one study.

indicate whether any monitoring of adverse events and/or tolerability was conducted, none of these studies included any reports of adverse events or poor tolerability.

In addition to the studies conducted with adult population groups, a few studies were performed in which bovine lactoferrin was ingested by children (up to 10 years of age). Provided at doses of up to 1,000 mg/day for up to 9 months to healthy children or children presenting with *H. pylori*, bovine lactoferrin was well tolerated and not associated with any adverse effects (Okuda *et al.*, 2005; Egashira *et al.*, 2007; Ochoa *et al.*, 2008, 2013).

Although infants are not expected to be consumers of Vitalarmor® GF-100 from its uses in meal replacement beverages and medical foods as indicated herein, Vitalarmor® GF-100 is also intended for use in infant and toddler formulas (separate GRAS Notice). In consideration of the safety of Vitalarmor® GF-100 in infant and toddler formula, numerous studies assessing the safety and/or efficacy of bovine lactoferrin consumption by infants were examined. In brief, the results of the studies demonstrate that lactoferrin provided daily to infants at doses ranging from 100 to 3,000 mg for as long as 1 year was well-tolerated and not associated with any adverse effects (for a more detailed discussion of these studies, please refer to the adjoining GRAS Notice *"GRAS Exemption Claim for Vitalarmor® GF-100, a Basic Whey Protein Isolate, for use as an Ingredient in Term Infant Formulas and Toddler Formulas"*).

Overall, the doses used in studies with human populations far exceed the potential intake of lactoferrin from the use of Vitalarmor® GF-100 under the proposed conditions of use in meal replacement beverages or medical foods. The results of these studies therefore provide corroborative evidence for the safe use of Vitalarmor® GF-100 in meal replacement beverages and medical foods.

In addition to the studies conducted with bovine lactoferrin, results of a number of human studies with compositionally related protein fractions [*i.e.*, other bovine milk-derived protein fractions also produced by Armor and MBP® (GRN No. 000196 - U.S. FDA, 2006)] also were examined.

Two (2) studies (unpublished) involved administration of cow's milk-derived protein fractions similar in composition to that of Vitalarmor® GF-100 and also produced by Armor, to 2 groups of adults: one with psoriasis (Armor Protéines, 2013 [unpublished]) and the other with elevated low density lipoprotein (LDL)-cholesterol levels (Schmitt and Mireaux, 2008 [unpublished]). In the study with individuals afflicted with psoriasis (open, prospective preliminary study), subjects (12 men and 8 women; 19 to 71 years of age) were provided a bovine whey protein isolate that contains approximately 40% lactoperoxidase (TGF-β_2 at a concentration of approximately 100 µg /g powder) (Armor Protéines, 2013 [unpublished]). Study participants consumed daily 1,000 mg of the whey protein isolate (in 2 divided doses), resulting in a daily dose of approximately 400 mg of lactoperoxidase, for a period of 60 days. The study included assessment of number of safety-related outcomes, including hematology and some clinical

chemistry, at baseline and Days 30 and 60. In comparison to baseline values, the study investigators reported no significant effects. In a further study (randomized, placebo-controlled double-blind study), another compositionally related bovine whey protein isolate that contained approximately 30% lactoferrin and approximately 30% lactoperoxidase (TGF-β_2 at a concentration of approximately 150 µg/g powder) was evaluated in individuals with high LDL-cholesterol levels (Schmitt and Mireaux, 2008 [unpublished]). Study participants consumed 0 (11 men and 13 women; 40 to 71 years of age) or 620 mg/day (9 men and 16 women; 36 to 63 years of age) of the whey protein isolate (approximately 180 mg/day of each lactoferrin and lactoperoxidase) for a period of 60 days. Assessment of liver enzyme levels (ALT and AST) was the only safety-related parameter included in the study. Liver enzyme levels of test participants were comparable to the placebo-control group.

A few human studies also were identified with MBP® (54 and 41% lactoferrin and lactoperoxidase, respectively). These studies, which included both unpublished and published reports, also were previously considered by the FDA as part of their review of the GRAS Notice for MBP® (GRN No. 000196 - U.S. FDA, 2006). In the studies which included measures related to safety, male subjects were provided 300 mg MBP® per day (approximately 160 and 120 mg of lactoferrin and lactoperoxidase, respectively) for 17 days and female subjected were provided daily doses of 40 mg MBP® (22 and 16 mg of lactoferrin and lactoperoxidase, respectively) for 6 months (Snow Brand Milk Products, 2001a,b [unpublished]). In both studies, MBP® was well tolerated for the duration of the study periods, and none of the adverse events were considered related to the consumption of the test material. A few minor statistically significant changes were noted in serum parameters; however, none of the variations were considered physiologically relevant. In addition to the unpublished studies designed to specifically assess safety, 6 others studies, 1 with male subjects (Toba *et al*., 2001) and 5 with female subjects (Aoe *et al.,* 2001, 2005; Yamamura *et al*., 2002; Uenishi *et al.,* 2007; Aoyagi *et al.,* 2010), were identified which primarily assessed parameters related to bone metabolism. In the 1 study with male subjects, study participants consumed 300 mg MBP® for 16 days. In the studies with females, the treatment periods were up to 1 year in duration and in each study, participants consumed daily 40 mg of MBP®. All significant variations observed in parameters related specifically to bone health were suggestive of a potentially beneficial effect of MBP® (*i.e*., promotion of bone formation and suppression of bone resorption). No treatment-related adverse symptoms or events were reported in any of the trials.

IV.B.5 Potential Allergenicity

Considering that Vitalarmor® GF-100 is derived from cow's milk, the allergenic potential of the protein isolate was assessed. Although the main protein constituents of Vitalarmor® GF-100 are generally not considered major milk allergens (β-lactoglobulin, α-lactalbumin, and casein are usually associated with cow's milk allergy), some evidence suggests that many other milk

proteins (*e.g.,* lactoferrin) may be responsible for milk-induced allergic reactions (Wal, 2002, 2004; Natale *et al*., 2004).

As part of an assessment of bovine lactoferrin safety for use as an antimicrobial beef carcass spray, Taylor *et al*. (2004) noted the availability of limited data suggesting that bovine lactoferrin may be a minor cow's milk allergen, but the available data are difficult to interpret. The allergenic potential of bovine lactoferrin was also extensively discussed in GRN Nos. 000464 and 000465 (U.S. FDA, 2014a,b). Therein, it is noted that while some data exist showing a limited number of cases of individuals with IgE antibodies only to bovine lactoferrin, there are also reports of infants with antibodies specific to bovine lactoferrin, but who were supplemented with lactoferrin-containing formula for a period of 6 months, without any clinical symptoms of allergy. The potential allergenicity of bovine lactoferrin also was considered by EFSA as part of their review of the safety of lactoferrin for use in food for human consumption (EFSA, 2012a,b). The EFSA Panel concluded that the "risk of allergic reactions [resulting from exposure to bovine lactoferrin] is not dissimilar to other dairy products derived from bovine sources".

The allergenicity of lactoperoxidase was considered by Food Standards Australia New Zealand (FSANZ, 2002) as part of the assessment of the safety of lactoperoxidase for use as a processing aid for meat preservation. In their evaluation of the allergenic potential of lactoperoxidase, FSANZ referenced an expert opinion that "lactoperoxidase is not a known allergen", but that weak evidence suggests that lactoperoxidase may have sensitizing potential (Taylor *et al.,* 2004). Subsequently, as part of the overall determination of the safety of lactoperoxidase-containing MBP® (GRN No. 000196 - U.S. FDA, 2006), the allergenic potential of lactoperoxidase also was considered (Goodman *et al.,* 2007). The amino acid sequence of lactoperoxidase was compared to sequences of known allergens listed on AllergenOnline.com. Lactoperoxidase did not possess any significant sequence homology with any known allergens. The highest scoring match to a recognized antigen was 26% identity, over 109 amino acid (antigen of parasitic scabies).

While there is no conclusive evidence demonstrating that bovine lactoferrin or bovine lactoperoxidase are allergenic or sensitizing in humans, consistent with ingredient labeling regulations, Vitalarmor® GF-100 will be labeled as "derived from cow's milk" on any meal replacement beverage or medical food product.

IV.B.6 Conclusions on the Safety of Vitalarmor® GF-100 for Use in Meal Replacement Beverages and Medical Foods

Vitalarmor® GF-100 is obtained from cow's milk using only physical separation techniques. The manufacturing process does not introduce any novel compounds or involve any chemical reactions or synthesis steps. As such, no new products are formed as a result of the production process. Vitalarmor® GF-100 therefore consists predominantly of proteins, all of which are naturally present in cow's milk and as such have a long history of safe consumption from the

background diet. Existing sources of exposure to the proteins of Vitalarmor® GF-100 from the background diet include cow's milk and cow's milk-based dairy products, as well as products formulated with whey protein-based ingredients already available on the market. The FDA has affirmed whey and whey protein concentrates as GRAS for use in food. Similarly, other compositionally related whey protein isolates and highly purified individual whey proteins also were previously self-determined as GRAS for a number of food uses. In the case of infants, exposure may occur as a result of consuming either human milk, which has been identified to contain some proteins that are also present in Vitalarmor® GF-100, including the major proteins lactoferrin and lactoperoxidase, or cow's milk protein-based infant formulas or formulas specifically supplemented with whey proteins. While the homology between human and bovine proteins is variable, in some cases the proteins may be considered to be identical. The history of safe consumption of the individual protein constituents of the basic whey protein isolate and similar whey derived protein products as part of the normal human diet at all stages of life, including during infancy, corroborates the safety of Vitalarmor® GF-100.

In addition to the safe history of use, safety of Vitalarmor® GF-100 is primarily supported by the results of toxicological studies conducted with the ingredient, and is further corroborated by the results of pre-clinical and clinical studies with compositionally related products. In order to establish safety for the addition of Vitalarmor® GF-100 to food for human consumption, 2 animal studies were performed with Vitalarmor® GF-100: a standard 90-day rat gavage study and a 6-week study conducted in juvenile rats which was designed to assess safety of the ingredient in an animal population that reflected the target population for the additional use of Vitalarmor® GF-100 in infant formula. Test article administration in the juvenile toxicity study commenced with animals still in the neonatal stage of development. In the 13-week oral gavage study, a NOAEL of 2,000 mg/kg body weight per day, the highest dose tested, was determined for Vitalarmor® GF-100 in Sprague-Dawley rats. In juvenile Sprague-Dawley rats, a NOAEL of 600 mg/kg body weight/day, the only dose tested, was determined for Vitalarmor® GF-100 following 6-week exposure period. Vitalarmor® GF-100 also was shown to be non-genotoxic and non-mutagenic as determined in a bacterial reversion assay and in an *in vitro* mammalian micronucleus assay. Further to the ingredient-specific toxicological studies, several pre-clinical and clinical studies on highly-purified bovine lactoferrin, as well as compositionally related whey-based ingredients (*e.g.*, Lactermin®, MBP®) also were available which serve to additionally support the safety of Vitalarmor® GF-100.

Collectively, the available data consistently demonstrate absence of any adverse effects associated with Vitalarmor® GF-100 or its individual main constituents and support the safe use of the ingredient under the intended conditions of use in meal replacement beverages and medical foods. No safety concerns are therefore raised with respect to the conditions of uses of Vitalarmor® GF-100 in meal replacement beverages and medical foods as specified herein.

IV.C Expert Panel Evaluation

Armor Protéines has determined that Vitalarmor® GF-100, a basic whey protein isolate, is GRAS for use in meal replacement beverages and medical foods on the basis of scientific procedures (see Section IV.B). The GRAS determination is based on generally available data available in the public domain that pertain to the safety of Vitalarmor® GF-100, as presented herein, and upon consensus amongst a panel of independent experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of the basic whey protein isolate (Vitalarmor® GF-100) for use as an ingredient of meal replacement beverages and medical foods. The Expert Panel consisted of the following qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), Dr. Paul Pencharz (University of Toronto), and Dr. John A. Thomas (Indiana University School of Medicine).

The Expert Panel, convened by Armor, independently and critically evaluated all data and information presented herein and concluded that Vitalarmor® GF-100 was GRAS for use in meal replacement beverages and medical foods as described in Section I.D based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of the basic whey protein isolate is presented in Appendix A.

IV.D Conclusion

Based on the above data and the information presented herein, Armor Protéines has concluded that the intended uses of Vitalarmor® GF-100, a basic whey protein isolate, in meal replacement beverages and medical foods, as described in Section I.D, are GRAS based on scientific procedures. General recognition of Armor's GRAS determination is supported by the unanimous consensus rendered by the independent Expert Panel, qualified by experience and scientific training to evaluate the use of Vitalarmor® GF-100 as a component of food, who concluded that the intended use of Vitalarmor® GF-100 in meal replacement beverages and medical foods is GRAS under the conditions of intended use.

Therefore, Vitalarmor® GF-100 may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

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Appendix A

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Use of Vitalarmor® GF-100, a Basic Whey Protein Isolate, in Meal Replacement Beverages and Medical Foods

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Use of Vitalarmor® GF-100, a Basic Whey Protein Isolate, in Meal Replacement Beverages and Medical Foods

February 11, 2015

At the request of Armor Protéines (hereafter Armor), an Expert Panel of independent scientists (the "Expert Panel"), qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information concerning the use of Vitalarmor® GF-100, a basic whey protein isolate, as an ingredient of meal replacement beverages and medical foods intended for consumption by adults. The Expert Panel was asked to determine whether the use of Vitalarmor® GF-100 in meal replacement beverages at up to 100 mg per serving (240 mL) is Generally Recognized as Safe (GRAS), based on scientific procedures. The Expert Panel also considered whether the use of Vitalarmor® GF-100 in medical foods at a level that will provide, under recommended conditions of use of the medical food, up to 610 mg Vitalarmor® GF-100 per day, is GRAS based on scientific procedures. The Expert Panel consisted of the below-signed qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), Dr. Paul Pencharz (University of Toronto), and Dr. John A. Thomas (Indiana University School of Medicine). For purposes of the Expert Panel's evaluation, "safe" or "safety" means that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2014a).

The Expert Panel independently and collectively, critically evaluated a comprehensive package of scientific information and data pertinent to the safety of Vitalarmor® GF-100 compiled from the literature and other published sources through December 2014. The data and information were presented to the Expert Panel by Armor in a dossier, "Documentation Supporting the Evaluation of Vitalarmor® GF-100, a Basic Whey Protein Isolate, as Generally Recognized as Safe (GRAS) for Use in Meal Replacement Beverages and Medical Foods" (final dated: February 10, 2015). In addition, the Expert Panel evaluated other information deemed appropriate, necessary, or pertinent to the safety of the proposed conditions of use of Vitalarmor® GF-100. Technical data, including information on the manufacturing method, product specifications, batch analysis, and stability of Vitalarmor® GF-100 were provided by Armor. Other data evaluated by the Expert Panel included product specifications, analytical results supporting the specifications, the intended use of Vitalarmor® GF-100 in meal replacement beverages and medical foods, dietary intake estimates of Vitalarmor® GF-100 and its constituents resulting from the intended

conditions of use, as well as intake estimates of the quantitatively major constituents of Vitalarmor® GF-100 (lactoferrin and lactoperoxidase) from other potential sources of exposure (*e.g.*, human and cow's milk, dairy products), and a comprehensive assessment of the available scientific literature pertaining to the safety of the intended use of Vitalarmor® GF-100.

Following independent and collaborative, critical evaluation of such data and information, the Expert Panel teleconferenced on December 12th, 2014. Following a review of the data evaluated and discussion, the Expert Panel unanimously concluded that the proposed use in meal replacement beverages at levels up to 100 mg per serving (240 mL) of Vitalarmor® GF-100, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures. The Expert Panel further concluded that the proposed use in medical foods, with Vitalarmor® GF-100 added at a level that will provide, under recommended conditions of use of the medical food, up to 610 mg Vitalarmor® GF-100 per day, also is GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

SUMMARY AND BASIS FOR GRAS DETERMINATION

Armor intends to market a basic whey protein isolate (branded as Vitalarmor® GF-100), as an ingredient of meal replacement beverages and medical foods. The basic whey protein isolate is characterized by a total protein composition of not less than 90%. Lactoferrin and lactoperoxidase account for approximately 47 and 26% of the ingredient's overall composition, respectively. The remainder of the protein fraction (approximately 20%) is composed of an abundance of other whey proteins, including small amounts of other bioactive proteins [*e.g.*, transforming growth factor-β_2 (TGF-β_2) at 0.015%] that are naturally present in cow's milk.

Armor's Vitalarmor® GF-100 is produced from cow's milk, using only physical separation and concentration techniques. Specifically, the selective fractionation to obtain the minor whey protein ingredient is carried out using cation-exchange chromatography, followed by ultrafiltration and microfiltration, pasteurization, and spray-drying. The processes used are consistent with those already routinely employed to produce similar milk-protein isolates and concentrates, as well as highly purified single milk protein isolates (*e.g.*, bovine lactoferrin). The fractionation process employed to produce Armor's basic whey protein isolate, largely removes casein and most of the major whey proteins (β-lactoglobulin and α-lactalbumin), lactose, and minerals, but not other desired proteins (lactoferrin and lactoperoxidase, and other quantitatively minor, but biologically active proteins such as TGF-β_2). The process does not involve any chemical reactions or synthesis steps that would introduce any new compounds beyond the proteins that are normally present in the starting material. Consequently, the production process yields a specific protein fraction consisting of proteins that are all naturally present in cow's milk, but at slightly different relative concentrations (ratios) as a result of the selective concentration. Some protein denaturation is expected to occur during the pasteurization stage

of the production of Vitalarmor® GF-100. The level of protein denaturation has not been assessed and it is generally protein specific, with some proteins being more liable to denaturation than others. The level of protein denaturation during the preparation of Vitalarmor® GF-100 is similar to that which occurs normally as a result of pasteurization of milk.

The starting material for the production of Vitalarmor® GF-100 is cow's milk of European Union origin. The milk is collected in France and is subject to French sanitary regulations. It is deemed to be free of bovine tuberculosis (risk for bovine tuberculosis is monitored by the French authorities) and is tested for the presence of a number of potential environmental contaminants (*e.g.*, antibiotics, aflatoxin M_1, and pesticides). Concentration of the minor whey proteins is carried out through physical separation methods, and food-grade sodium chloride (NaCl) and hydrochloric acid (HCl) are the only processing aids used in the manufacture of Vitalarmor® GF-100. The ion-exchange resin [pursuant to a food contact notification filed for the resin with the United States (U.S.) Food and Drug Administration (FDA)], the felt bag filter (21CFR§177.1520), the ultrafiltration membrane (21CFR§177.2910, 21CFR§177.1630, 21CFR§177.2260, 21CFR§177.1655, 21CFR§177.1520, 21CFR§175.300, 21CFR§175.105), and the microfiltration membrane (21CFR§177.1520) satisfy requirements of the FDA for food-contact use (U.S. FDA, 2014a).

Whey protein isolates are related to whey protein concentrates, which are affirmed as GRAS in the U.S. (21CFR 184.1979c; see below) (U.S. FDA, 2014a) – GRN No. 000037) (U.S. FDA, 2000). In affirming the GRAS status of whey protein concentrates, the FDA noted that it would not object "to the use of newly developed physical separation techniques, if there are no new toxicants introduced as a result of use of these techniques, and if these techniques do not result in a concentration of natural toxicants in whey products".

Analysis of 5 non-consecutive batches of Vitalarmor® GF-100 demonstrates that the established production process produces a consistent product that meets the physical, chemical, and microbiological specifications (see Attachment A for the specifications of Vitalarmor® GF-100). The bulk stability of Vitalarmor® GF-100 was investigated in a study involving storage for a period of up to 36 months at 20 or 4°C and a relative humidity of about 50%. The stability testing included monitoring of one specific protein identified in the ingredient, TGF-β_2. Interim and terminal results demonstrate that levels of TGF-β_2 remain within the acceptable level set out in the ingredient's specifications (12 to 18 mg/100 g). Moisture content of the samples, however, exceeded the limit of 6.0% after 12 and 18 months for samples stored at 20° and 4°C, respectively. The increase in moisture levels was not accompanied by any changes in microbial counts.

As an ingredient of meal replacement beverages and medical foods, Vitalarmor® GF-100 is intended for use as a source of specific whey proteins that may have beneficial effects on various aspects of human health. Vitalarmor® GF-100 is intended to be added to meal replacement beverages at levels up to 100 mg/serving (240 mL). Under the conditions of

intended use in meal replacement beverages, highest statistically reliable all-user mean and 90th percentile intakes of 56.1 and 229.2 mg/day were estimated in male adults, respectively. On a kilogram body weight basis, the highest all-user mean and 90th percentile intake estimates (statistically reliable) also were observed in male adults (0.67 and 2.74 mg/kg body weight/day). In addition to the proposed use of Vitalarmor® GF-100 in meal replacement beverages, Vitalarmor® GF-100 also is proposed for use in medical foods for adults at inclusion levels that will provided no more than 610 mg Vitalarmor® GF-100 per day or 10.2 mg/kg body weight/day for a 60-kg individual.

Considering the percent contribution of lactoferrin and lactoperoxidase to the overall composition of Vitalarmor® GF-100, the highest levels of exposure to lactoferrin and lactoperoxidase on an absolute and per body weight basis would therefore occur in adults from the consumption of Vitalarmor® GF-100-supplemented medical foods: approximately 287 and 159 mg/day of lactoferrin and lactoperoxidase, respectively, (4.8 and 2.8 mg/kg body weight/day, respectively) would occur from the consumption of the proposed use of Vitalarmor® GF-100.

Vitalarmor® GF-100 is a protein isolate which consists mainly of basic whey proteins including lactoferrin and lactoperoxidase and is produced from cow's milk using minimal processing and selective concentration processes. Whey protein concentrates and whey protein isolates have a long-history of safe consumption in the diet, and whey isolates, including lactoferrin and lactoperoxidase, have been the subject of several safety evaluations for food uses by multiple scientific bodies/regulatory authorities. Whey derived protein products have an established and generally recognized history of safe consumption in the diet.

In the U.S., whey and whey protein concentrate are permitted for use in food. The FDA previously affirmed whey and whey protein concentrate (protein content of ≥25%) as GRAS (21CFR§184.1979 and 21CFR§184.1979c, respectively - U.S. FDA, 2014a). The FDA posed no questions in response to a previous GRAS Notice informing the agency of the GRAS determination of whey protein isolate (GRN No. 000037 – U.S. FDA, 2000). Additionally, several related cow's milk-derived basic whey protein based ingredients [*e.g.*, bovine milk basic protein fraction (MBP®)[1] and highly purified bovine lactoferrin (including bovine lactoferrin for addition to infant formula)] have been the subject of GRAS Notices submitted the FDA. The FDA has had no questions with these GRAS Notices.

The safety of lactoferrin, one of the main constituents of Vitalarmor® GF-100, for use in different types of food, including foods for particular nutritional purposes, also was previously assessed by the European Food Safety Authority (EFSA, 2012). For use as an ingredient in foods for special medical purposes, levels of up to 3,000 mg bovine lactoferrin/day are acceptable in the EU (EU, 2012a,b). The safety of lactoperoxidase, the other main constituent of Vitalarmor®

[1] 54% lactoferrin and 41% lactoperoxidase.

GF-100, for use as part of a system for preservation of meats, was previously evaluated by the Food Standards Australia New Zealand (FSANZ, 2002). FSANZ largely considered the natural occurrence of lactoperoxidase in milk and concluded that based on the concentration of lactoperoxidase in milk (approximately 30 mg/L) being similar to or higher than the proposed use-level, lactoperoxidase was considered not to pose any toxicological risk.

Since Vitalarmor® GF-100 is derived from cow's milk by use of only physical separation techniques, its constituents also are naturally present in cow's milk (100 to 150 mg lactoferrin and 30 mg lactoperoxidase/L cow's milk) (Ferenc Levay and Viljoen, 1995; Korhonen and Pihlanto, 2003; Indyk *et al*., 2006; FAO, 2014) and thus exposure also occurs *via* the normal human diet and in particular as a result of cow's milk and dairy consumption. Comparison of daily intakes of lactoferrin and lactoperoxidase resulting from the inclusion of Vitalarmor® GF-100 as an ingredient of food (up to 287 mg and 159 mg, respectively) to background dietary exposure estimates (approximately 74 and 25 mg based on 90th percentile consumption of cow's milk and dairy products; U.S. FDA, 2014b,c), reveals that in adults, intakes of each protein constituent from the use of Vitalarmor® GF-100 in foods would be higher. However, in the case of lactoferrin, which has already been determined to be GRAS for numerous food uses, intakes of lactoferrin from the use of Vitalarmor® GF-100 under proposed conditions of use described herein are estimated to be in a similar range as those resulting from the existing use of lactoferrin in foods (90th percentile lactoferrin intake of 273 mg/day was estimated for lactoferrin based on proposed use in yogurts, powdered milk, and milk-based frozen desserts) (GRAS No. 000464 - U.S. FDA, 2014b). Furthermore, since cow's milk protein-based infant and toddler formulas comprise varying mixtures of casein and whey protein, up to 100% whey protein, the whey basic proteins that comprise Vitalarmor® GF-100 will also occur normally in a cow's milk-based formulas (GRAS No. 000465 – U.S FDA, 2014c).

The protein constituents of Vitalarmor® GF-100 have been identified to be biologically active. In addition to their natural occurrence in cow's milk, the main protein constituents of Vitalarmor® GF-100, lactoferrin and lactoperoxidase, also occur naturally in human milk (1,000 to 3,200 mg lactoferrin and 0.77 mg lactoperoxidase/L human milk) (Pamblanco *et al*., 1986; Prentice *et al*., 1987; Hirai *et al*., 1990; Hennart *et al*., 1991; Rudloff and Kunz, 1997; Shin *et al.,* 2001). The cow's milk proteins are closely related to the proteins in human milk. Bovine lactoferrin and lactoperoxidase demonstrate an amino acid sequence homology of approximately 80% to human counterparts (Wal, 2004; Manzoni *et al*., 2010; BLAST analysis). As such, exposure to the proteins may occur even during infancy. On a per kilogram body weight basis, exposure to lactoferrin in breast-fed babies may be up to 525 mg/kg body weight/day, which is more than 100 times greater than the highest intake on a per kilogram body weight basis of lactoferrin expected under conditions of use specified herein. While not as high, intake of lactoperoxidase from human milk in infants is approximately 0.13 mg/kg body weight/day.

While detailed analysis of the non-lactoferrin/non-lactoperoxidase protein fraction of Vitalarmor® GF-100 was not conducted, given the source of Vitalarmor® GF-100 (cow's milk), exposure to all protein constituents of Vitalarmor® GF-100 occurs through the consumption of cow's milk and products made thereof. Additionally, during infancy, exposure to some proteins also may occur *via* human milk. For example, TGF-β_2, one quantitatively minor protein of Vitalarmor® GF-100, is present in cow's milk at concentrations in the range of 23 to 66 µg/L (Pakkanen, 1998; Elfstrand *et al*., 2002; Chockalingam *et al*., 2005). In human milk, mean concentrations of up to 5.6 and upper-end concentrations of up to 57 µg/L were identified (Srivastava *et al*., 1996; Hawkes *et al*., 1999; Kalliomäki *et al*., 1999; Böttcher *et al.,* 2000; McPherson and Wagner, 2001; Rautava *et al*., 2002; Laiho *et al*., 2003; Rosales *et al.,* 2009).

The safety of Vitalarmor® GF-100 is primarily established through a series of pre-clinical studies (subchronic animal toxicity studies and short-term *in vitro* genotoxicity assays) with Vitalarmor® GF-100 and corroborated by a history of safe consumption and toxicological and clinical studies on related products.

There are no data pertaining to the metabolic fate of Vitalarmor® GF-100 which consists mainly of cow's milk derived protein. The metabolic fate of the individual constituents is expected to be similar to that following consumption of cow's milk or other products based on cow's milk protein. Limited data related to the metabolic fate and possible bioavailability of the main constituents of Vitalarmor® GF-100, lactoferrin and lactoperoxidase, were identified following oral administration. In adults, 62 to 79% of a dose of lactoferrin administered by nasogastric intubation remained intact upon gastric emptying (Troost *et al*., 2001). Khan *et al*. (2000) reported presence of protein bound radioactivity in the plasma of study participants (male and female adults) following consumption of encapsulated radiolabelled human lactoferrin. Results from some *in vitro* investigations suggest that human and bovine lactoperoxidase retain peroxidase activity following incubation in the presence of adult or infant gastric juice (Gothefors and Marklund, 1975). Resistance of lactoperoxidase to enzymes of the upper intestine (trypsin and chymotrypin) was reported by Kussengrager and van Hooijdonk (2000).

Two (2) repeat-dose toxicology studies were conducted with Vitalarmor® GF-100. In a standard subchronic oral toxicity study, groups of Sprague-Dawley rats (main study: 10/sex/group; recovery group: 6/sex/group) were administered Vitalarmor® GF-100 at doses of 0, 600, 1,200, or 2,000 mg/kg body weight by gavage for 13 weeks (followed by 4-week recovery) (Forster *et al*., 2014). The highest dose tested in this study, 2,000 mg/kg body weight/day, was determined to be the no-observed-adverse-effect level (NOAEL) for Vitalarmor® GF-100. While some statistically significant differences in hematological and clinical chemistry parameters were reported between groups of test animals and the controls, none were considered to be related to the administration of the test compound (lacked a dose-response and occurred in only one sex). Significantly lower absolute brain weights in low-dose females compared to controls were not considered to be related to the administration of Vitalarmor® GF-100. Reductions in urine

volume and pH and an increase in specific gravity were reported in Vitalarmor® GF-100-treated female rats and appeared to be dose-dependent, reaching statistical significance at the high dose only. The changes in urinary parameters of test females were not accompanied by any clinical chemistry or histopathological evidence that suggested a nephrotoxic effect. No significant differences in body weights, body weight gain, and absolute and relative organ weights were reported between the high-dose and control animals at the end of the 4-week recovery period. Gross examination of the high-dose recovery group animals was unremarkable.

When Vitalarmor® GF-100 was administered by gavage to male and female juvenile Sprague-Dawley rats (7 days old) at a dose of 600 mg/kg body weight/day (only dose tested, which was the highest feasible dose based on gavage volume tolerances in these juvenile rats) for 6 weeks (followed by 4-week recovery), no compound-related adverse effects were reported (Forster *et al*., 2014). As described in the 90-day study with the older rats, variations in urinalysis parameters in the female pups in this study also were not accompanied by any relevant clinical biochemistry or histopathological correlates. A statistically significant decrease (approximately 40%) in eosinophil levels was reported in treated males and females at the end of the treatment period, but the values were within the reported normal physiological range and within historical control values and were considered not treatment- related. Absolute and relative thymus weights of test males were reported to be significantly higher than those in the control group. The thymus weights of control males and females were reported to decrease when end-of-treatment and end-of-recovery values were compared and this is consistent with thymic involution as rats age. In treated males only, thymus weights were observed to be approximately the same weight at the end of the test period and recovery period. Histological examination of the male thymus did not reveal any abnormalities. Based on the results of this study, 600 mg/kg body weight/day (the only dose tested) was determined to be the NOAEL for Vitalarmor® GF-100.

Vitalarmor® GF-100 was not mutagenic when examined *in vitro* in both the Ames and mouse micronucleus assays (at concentrations of up to 5,000 µg/plate and 2,500 µg/L, respectively) (Forster *et al*., 2014).

The toxicological studies performed with Vitalarmor® GF-100 are considered pivotal to the determination of the safety of the ingredient for use in meal replacement beverages and medical foods and form the basis of the GRAS determination. Additionally, the safety of Vitalarmor® GF-100 is corroborated by results of a number of subchronic animal toxicity studies and a developmental toxicity study conducted with materials of comparable protein composition [*i.e*., whey extract (Lactermin®[2]) and MBP®], as well as high-purity lactoferrin. An median lethal dose of greater than 2,000 mg/kg body weight was identified for MBP®, indicating the material to be of

[2] A proprietary whey growth factor extract (WGFE) or Lactermin® (over 50% of the total protein content is accounted for by lactoperoxidase and lactoferrin, together with a variety of minor proteins and peptides: IGF-I, IGF-II, PDGF, FGF, TGF-β).

low oral acute toxicity (Kruger *et al*., 2007). No compound-related adverse effects were reported in a 90-day study following gavage administration of MBP® to Crj:CD (SD) IGS young adult male and female rats at doses of 200 or 2,000 mg/kg body weight/day (Kruger *et al*., 2007). A NOAEL of 2,000 mg/kg body weight/day was derived for MBP®. In a 13-week oral toxicity study in which male and female Sprague-Dawley rats were administered by gavage a proprietary whey extract (Lactermin®) at doses of 300, 1,000, or 3,000 mg/kg body weight/day, no adverse effects related to the administration of the extract were identified and a NOAEL of 3,000 mg/kg body weight/day, the highest dose tested, was established (Dyer *et al*., 2008). When the potential oral toxicity of bovine lactoferrin was assessed in a number of repeat-dose rat studies, including studies up to 65 weeks in duration, no compound-related adverse effects were reported at doses of up to 2,500 mg/kg body weight/day (Yamauchi *et al*., 2000a; Tamano *et al*., 2008).

In a developmental toxicity study with MBP® administered to Crj:CD (SD) IGS rats by gavage at 2,000 mg/kg body weight/day, beginning on Day 7 of gestation and continuing until Day 17, no significant differences between control and treated animals in parameters evaluated (uterine contents and maternal toxicity) were reported (Kruger *et al*., 2007).

A few additional non-standard studies also were conducted with bovine lactoferrin in which parameters related to intrauterine and postnatal rat development were assessed (Shumake *et al*., 2014; Somm *et al*., 2014). Oral exposure of lactoferrin to pregnant or lactating Sprague-Dawley OFA rats resulting in daily lactoferrin intakes of up to 1,300 mg/kg body weight was not associated with any adverse effects on dams' health (weight, food intake, food utilization efficiency, hematological analysis), litter size, or pup weight (Somm *et al*., 2014). Oral administration of up to 2,000 mg lactoferrin/kg body weight/day to male and female Holtzman albino rat pups starting on Postnatal Day 16 until 34/36, was not associated with any adverse effects on rats' general motor activity, behavior, and/or learning (Shumake *et al*., 2014).

Lactoferrin and the proprietary whey extract, Lactermin® were reported to be not genotoxic in the Ames and mouse thymidine kinase forward mutation assay both in the presence and absence of metabolic activation (Yamauchi *et al*., 2000b; Dyer *et al*., 2008). Proprietary whey extract was reported to be not genotoxic when administered to groups of male and female ICR mice once by gavage at doses of up to 2,000 mg/kg body weight; there were no significant increases in the incidence of micronucleated polychromatic erythrocytes (PCEs) or in the PCE:total erythrocyte ratio (Dyer *et al.*, 2008).

There were no reported clinical safety studies on Vitalarmor® GF-100. However, clinical trials have been reported with lactoferrin and with compositionally related whey protein fractions. The clinical studies were primarily designed to assess parameters related to potentially beneficial effects related to the consumption of these constituents, and only a few of the studies included measures related to safety. The results of these studies provide corroborative evidence for the safe use of Vitalarmor® GF-100 in humans. A series of studies involved the administration of

bovine lactoferrin to infants, children, and adults. In infants, daily doses of 100 mg to approximately 3,000 mg of lactoferrin for 2 weeks to 1 year were well-tolerated and did not produce any adverse effects (Kawaguchi *et al*., 1986, 1989; Fairweather-Tait *et al*., 1987; Balmer *et al.,* 1989; Schulz-Lell *et al*., 1991; Chierici *et al*., 1992; Roberts *et al*., 1992; Lönnerdal and Hernel, 1994; Hernel and Lönnerdal, 2002; King *et al*., 2007; Manzoni *et al*., 2009, 2012, 2014). In studies involving healthy children or children presenting with *Helicobacter pylori*, lactoferrin, at doses up to 1,000 mg/day for up to 9 months was well-tolerated and not associated with any adverse effects (Okuda *et al.*, 2005; Egashira *et al.*, 2007; Ochoa *et al.*, 2008, 2013). Both the safety and efficacy of lactoferrin also were assessed extensively in healthy and diseased adult populations. As in the younger age groups, daily consumption of up to 7,200 mg of lactoferrin in studies ranging in duration from 1 week to 12 months (7,200 mg lactoferrin was administered in an 8-week study, and up to 3,000 mg lactoferrin/day was consumed in the 1-year study) was well-tolerated (Tanaka *et al.*, 1999; Yamauchi *et al.,* 2000c; Iwasa *et al.*, 2002; Okada *et al.*, 2002; Di Mario *et al.*, 2003, 2006; Okuda *et al.*, 2005; Zullo *et al.*, 2005; Konishi *et al*., 2006; Koikawa *et al.*, 2008; Ueno *et al.*, 2006; Tursi *et al.*, 2007; Kondo *et al.*, 2008 [English summary]; Mulder *et al.*, 2008; Bharadwaj *et al.*, 2009, 2010; Kozu *et al.*, 2009; Nappi *et al.*, 2009; Paesano *et al.*, 2009, 2010; Ishikado *et al.*, 2010; Ono *et al.*, 2010; Mueller *et al*., 2011; Vitetta *et al*., 2013). The doses used in these studies far exceed the potential intake of lactoferrin from the use of Vitalarmor® GF-100 under the proposed conditions of use in medical foods or meal replacement beverages.

Additionally, no adverse effects were observed in studies in which psoriatic patients or subjects with high low-density lipoprotein-cholesterol were provided daily 620[3] or 1,000[4] mg of related cow's milk-derived basic whey protein isolates (also Armor products) for periods of 60 days (Schmitt and Mireaux, 2008 [unpublished]; Armor Protéines, 2013 [unpublished]). Two (2) human studies also were identified with MBP® in which parameters related to safety were assessed. Provided to males for 17 days at a dose of 300 mg (approximately 160 and 120 mg of lactoferrin and lactoperoxidase, respectively) or to females for 6 months at a dose of 50 mg (27 and 21 mg of lactoferrin and lactoperoxidase, respectively), MBP® was well-tolerated (Snow Brand Milk Products, 2001a,b [unpublished]).

The results of the pre-clinical and human studies with bovine lactoferrin and materials compositionally related to Vitalarmor® GF-100 provide additional corroborative evidence of the safety of the proposed uses of Vitalarmor® GF-100.

Since Vitalarmor® GF-100 is a protein derived from cow's milk and cow's milk protein is a recognized protein allergen, the allergenicity of Vitalarmor® GF-100 was carefully considered. Although whole cow's milk and several cow's milk proteins are considered major allergens, the main protein constituents of Vitalarmor® GF-100 (lactoferrin and lactoperoxidase) have not been

[3] Approximately 30% lactoferrin and approximately 30% lactoperoxidase (also TGF-β_2 at a concentration of approximately 150 µg/g powder).
[4] Approximately 40% lactoperoxidase (TGF-β_2 at a concentration of approximately 100 µg/g powder).

identified as major allergens. There is no conclusive evidence demonstrating that either bovine lactoferrin or bovine lactoperoxidase are allergenic or sensitizing in humans. Nevertheless, consistent with ingredient labeling regulations, Vitalarmor® GF-100 will be labeled as "derived from cow's milk" on any meal replacement beverage or medical food containing the ingredient.

The safety of Vitalarmor® GF-100 under the proposed conditions of use in meal replacement beverages and medical foods was established by animal oral toxicity and genotoxicity studies, including a standard 13-week toxicology study in which weaned rats were administered Vitalarmor® GF-100 at doses of up to 2,000 mg/kg body weight/day (the NOAEL) and a 6-week study in which juvenile rats were administered Vitalarmor® GF-100 at a dose of 600 mg/kg body weight/day (the NOAEL). Vitalarmor® GF-100 was non-genotoxic and non-mutagenic in the bacterial reversion assay and in the *in vitro* mammalian micronucleus assay. Further support for safety also is derived from the existing use of whey derived protein products, including whey protein concentrates and whey protein isolates, in foods for human consumption. The safety of the proposed uses of Vitalarmor® GF-100 is corroborated by the fact that the protein constituents of Vitalarmor® GF-100 are normal nutritive components of cow's milk, as well as human milk. Pre-clinical and clinical studies with bovine lactoferrin and related cow's milk-derived protein products also corroborate the safety of the proposed uses of Vitalarmor® GF-100.

CONCLUSION

We, the undersigned independent qualified members of the Expert Panel, have individually and collectively critically evaluated the data and information summarized above, as well as other data and information that we deemed pertinent to the safety of the intended uses of Vitalarmor® GF-100, a basic whey protein isolate, in meal replacement beverages and medical foods. We unanimously conclude that the intended use as an ingredient in meal replacement beverages at a level of up to 100 mg per serving (240 mL) and in medical foods at inclusion levels providing no more than 610 mg/day of Vitalarmor® GF-100, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is safe and suitable.

We further conclude that the intended use as an ingredient in meal replacement beverages at a level of up to 100 mg per serving (240 mL) of Vitalarmor® GF-100, meeting appropriate food-grade specifications and manufactured consistent with Good Manufacturing Practice, is Generally Recognized as Safe (GRAS) based on scientific procedures. We also conclude that the intended use as an ingredient in medical foods at inclusion levels providing no more than 610 mg/day of Vitalarmor® GF-100, meeting appropriate food-grade specifications and manufactured consistent with Good Manufacturing Practice, is GRAS based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

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20 February 2015
Date

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CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
170—Food additives	170.3(i)	Definitions
175—Indirect food additives: adhesives and components of coatings	175.105	Adhesives
	175.300	Resinous and polymeric coatings
177— Indirect food additives: polymers	177.1520	Olefin polymers
	177.1630	Polyethylene phthalate polymers
	177.1655	Polysulfone resins
	177.2260	Filters, resin-bonded
	177.2910	Ultra-filtration membranes
184—Direct food substances affirmed as generally recognized as safe	184.1979	Whey
	184.1979c	Whey protein concentrate

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ATTACHMENT A

Specifications for Vitalarmor® GF-100, a Basic Whey Protein Isolate

Table A-1 Specification for Vitalarmor® GF-100, a Basic Whey Protein Isolate		
Specification Parameter	**Specification Limit**	**Method of Analysis**
Appearance	Yellowish gray powder	Visual Inspection
Foreign matter (Scorched particles)	Absent (Disc B or better[a])	Visual inspection in 25 g (ADMI chart, solubilized with 0.15 M NaCl solution)
pH (5% solution w/v)	5.5 to 7.6	5% (w/v) solution, pH meter
Total Protein	Not less than 90%	Kjeldahl method (IDF20/ISO 8968) [N x 6.38]
Lactoferrin	25 to 75%	HPLC[b]
Lactoperoxidase	10 to 40%	HPLC[b]
TGF-β_2	12 to 18 mg/100 g	ELISA (Quantikine human TGF-β2, R&D Systems)
Moisture	Not more than 6.0%	ISO 5550
Lactose	Not more than 3.0%	Enzymatic method (Lactose/D-Galactose kit, Boehringer Mannheim/R-Biopharm)
Fat	Not more than 4.5%	AFNOR Chimie II 3B 1986
Ash (Residue on Ignition)	Not more than 3.5%	AFNOR NF V04-208
Iron	≤25 mg/100 g	AAS
Heavy Metals		
Lead	<0.1 mg/kg	ICP-MS
Cadmium	<0.2 mg/kg	ICP-MS
Mercury	<0.6 mg/kg	ICP-MS

AAS = atomic absorption spectrometer; ADMI = American Dry Milk Institute; ELISA = enzyme-linked immunosorbent assay; HPLC = High-performance liquid chromatography; ICP-MS = Inductively coupled plasma mass spectrometry; TGF-β = transforming growth factor.
[a] American Dry Milk Institute (ADMI) standard discs (Discs A, B, C, or D) representing the following amounts of scorched particles: 7.5, 15.0, 22.5, or 32.5 mg, respectively.
[b] HPLC conducted on in-process samples prior to terminal pasteurization. To determine levels of each protein in the final product, total protein content is multiplied by % protein in the in-process sample.

Table A-2 Microbiological Specification for Vitalarmor® GF-100, a Basic Whey Protein Isolate		
Specification Parameter	**Specification**	**Analytical Method**
Aerobic mesophilic count	Not more than 10,000 CFU/g	ISO 4833
Enterobacteriaceae	Not more than 10 CFU/g	ISO 21528-1
Yeasts	Not more than 50 CFU/g	ISO 6611 | IDF 94:2004
Molds	Not more than 50 CFU/g	ISO 6611 | IDF 94:2004
Escherichia coli	Negative (in 1 gram)	ISO 16649-2
Coagulase positive *Staphylococci*	Negative (in 1 gram)	ISO 6888-3
Salmonella	Negative (in 25 grams)	VIDAS Easy *Salmonella* method (equivalent to ISO6579)
Listeria	Negative (in 25 grams)	VIDAS LIS method (equivalent to ISO 11290-1/A1:2004)
Cronobacter spp.	Negative (in 25 grams)	ISO/TS 22964:2006

CFU = Colony Forming Unit.

SUBMISSION END