ORIGINAL SUBMISSION

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Land of Innovative Solutions

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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 Term Infant Formulas and Toddler Formulas

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 Term Infant Formulas and Toddler Formulas

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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 term infant and toddler formulas, 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 term infant formulas – excluding exempt formulas as defined in Title 21 § 107.3 of the *Code of Federal Regulations* (CFR) – and toddler formulas at a maximum use-level of 30 mg/100 g (formula powder) (U.S. FDA, 2015a). Specifically, Vitalarmor® GF-100 is intended for addition to formulas that are low or deficient in transforming growth factor-β_2 (TGF-β_2) (*e.g.*, partially hydrolyzed formula). TGF-β_2 is a cytokine that is present in milk from lactating women, and the use-level of Vitalarmor® GF-100 in infant formula products will be adjusted on a case-by-case basis, up to 30 mg Vitalarmor® GF-100/100 g formula, such that following reconstitution, levels of total TGF-β_2 (from Vitalarmor® GF-100 and from normal background occurrence in the base formula) in a particular infant formula preparation will be comparable to levels of TGF-β_2 in human milk. Assuming the maximum inclusion rate of 30 mg/100 g of powder and a reconstitution ratio of 130 g of formula powder/L, TGF-β_2 from Vitalarmor® GF-100 would be present at a concentration of up to 7.0 µg/L. The addition of Vitalarmor® GF-100 to certain infant formulas is consistent with efforts to produce formulas that are compositionally similar to human milk.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30 (U.S. FDA, 2015b), 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, including TGF-β_2. Since the inclusion levels of Vitalarmor® GF-100 for use in infant formula will be determined based on TGF-β_2 levels (as discussed above), quantitative measures of the TGF-β_2 content of the ingredient were obtained using a validated enzyme-linked immunosorbent assay (ELISA). In Vitalarmor® GF-100, TGF-β_2 was identified at average concentrations of 0.015% [155 ± 18 µg/g (n=9)].

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, 2015c). 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, including infant formula [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), and the production plant is certified compliant with ISO 9001 quality management and ISO 22000 food safety management standards.

The production method 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 (up to 30 mg/100 g) in a particular infant formula preparation.

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), proteins that are currently present within whey based infant formulas (see Section IV.B.1.2). 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. Vitalarmor® GF-100 contains concentrated levels of intact TGF-β_2 and is intended for use as a source of TGF-β_2 to restore quantities that are lost during the processing of formulas or whey proteins for use in formulas that are used as protein sources for infant formula (*e.g.*, partially hydrolyzed infant formulas). Vitalarmor® GF-100 is intended for addition to infant formula at levels providing up to 7 µg TGF-β_2/100 mL of reconstituted formula, concentrations that are within levels that have been measured in human milk samples from lactating women (see Section IV.B.1.1). Therefore, addition of Vitalarmor® GF-100 to infant formula is consistent with efforts to produce formulas that are compositionally similar to human milk.

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 of infants. 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 infants. 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, including for use in infant formula. 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 Uses in Infant and Toddler Formulas

Estimates for the daily mean and 90th percentile intakes of Vitalarmor® GF-100 from its use in term infant formulas and toddler formulas in 0- to 3-year-olds were calculated based on a maximum use level of 30 mg per 100 g of infant formula in conjunction with infant formula 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). The intake estimates for toddlers (1 to 3 years) also considered the proposed use of the ingredient in meal replacement beverages (up to 100 mg Vitalarmor® GF-100/240 mL in meal replacement beverages)[2]. The population groups consuming infant formulas consisted of infants (0 to 6 and 7 to 12 month age groups) and toddlers (1 to 3 years), with infants ages 0 to 6 months identified as the largest consumer group. In toddlers, the combined intake of formula and meal replacement beverages was limited to only 3.5% of the population.

Estimates for the daily intake of Vitalarmor® GF-100 from its use in infant and toddler formulas 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.

In infants aged 0 to 6 months, the mean and 90th percentile all-person intake estimates for Vitalarmor® GF-100 based on the proposed use in formula were estimated to be 24.6 and 44.5 mg/day, respectively. When the assessment was limited to identified consumers of formula specifically (all-users), the mean and 90th percentile intakes were estimated to be 32.9 and 47.2 mg/day, respectively, in the 0- to 6-month old infants. Within infants between 7 and 12 months of age, the all-person mean and 90th percentile intakes were estimated to be 20.7 and 40.5 mg/day, respectively, while the all-user intakes were estimated to be 27.5 and 42.7 mg/day, respectively. The lowest level of intake of Vitalarmor® GF-100 was observed to occur in

[2] Armor also intends to use Vitalarmor® GF-100 in meal replacements beverages and medical foods. Vitalarmor® GF-100 for use in meal replacement beverages and medical foods 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 Meal Replacement Beverages and Medical Foods"*). While Vitalarmor® GF-100 is proposed for use only in meal replacement beverages and medical foods intended for adults, a limited number of toddlers were identified as consumers of meal replacement beverages.

toddlers, with an all-person mean intake of 0.5 mg/day and all-user mean and 90th percentile intakes of 18.9 and 27.7 mg/day, respectively, resulting from the consumption of both formula and meal replacement beverages.

Table IV.A.1-1 Summary of the Estimated Daily Intake of Vitalarmor® GF-100 in Infants and Toddlers Resulting from the Intended Use in Infant and Toddler Formula 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
Infants 0 to 6 months	80.5	161	24.6	44.5	32.9	47.2
Infants 7 to 12 months	80.1	129	20.7	40.5	27.5	42.7
Toddlers 13 to 36 months[a]	3.5	18	0.5*	na	18.9*	27.7*

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 meal replacement beverages.

On a body weight basis, the highest estimated intake of Vitalarmor® GF-100 also was observed to occur in infants between 0 and 6 months of age. The mean and 90th percentile all-person intakes of Vitalarmor® GF-100 in this age group were estimated to be 4.0 and 7.8 mg/kg body weight/day, respectively, while the all-user intakes were estimated to be 5.4 and 8.7 mg/kg body weight/day, respectively. Within infants between 7 and 12 months of age, the all-person mean and 90th percentile intakes of Vitalarmor® GF-100 were estimated to be 2.3 and 4.5 mg/kg body weight/day, respectively. When non-consumers of infant formula were removed from the assessment, the mean and 90th percentile intakes (all-user) were estimated to be 3.1 and 5.0 mg/kg body weight/day, respectively. The lowest intakes on a body weight basis were observed in toddlers (all-user mean and 90th percentile intakes of 1.7 and 2.5 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 in Infants and Toddlers Resulting from the Intended Use in Infant and Toddler Formula 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
Infants 0 to 6 months	80.5	161	4.0	7.8	5.4	8.7
Infants 7 to 12 months	80.1	129	2.3	4.5	3.1	5.0
Toddlers 13 to 36 months[a]	3.5	18	<0.1*	na	1.7*	2.5*

bw = body weight; 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 meal replacement beverages.

IV.A.2 Estimated Consumption of the Main Protein Constituents of Vitalarmor® GF-100 in Infants and Toddlers

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.2-1 and IV.A.2-2 on a per person and per kilogram body weight basis, respectively.

For lactoferrin and lactoperoxidase, the intakes were greatest in infants aged 0 to 6 months. The mean all-user intakes of lactoferrin and lactoperoxidase in 0- to 6-month old infants were calculated to be 15.5 and 8.6 mg/person/day, respectively, equivalent to 2.5 and 1.4 mg/kg body weight/day, respectively. At the 90th percentile level, intakes of lactoferrin and lactoperoxidase were calculated to be 22.2 and 12.3 mg/person/day, respectively, equivalent to 4.1 and 2.3 mg/kg body weight/day, respectively, in infants aged 0 to 6 months.

Table IV.A.2-1 Summary of the Estimated Daily All-User Intakes of the Main Constituents of Vitalarmor® GF-100 In Infants and Toddlers Resulting from the Intended Use of Vitalarmor® GF-100 in Infant and Toddler Formula in the United States (2009-2010 NHANES Data)

Population Group	Lactoferrin (mg/day)		Lactoperoxidase (mg/day)	
	Mean	90th Percentile	Mean	90th Percentile
Infants 0 to 6 months	15.5	22.2	8.6	12.3
Infants 7 to 12 months	12.9	20.1	7.2	11.1
Toddlers 13 to 36 months[a]	8.8*	12.9*	4.9*	7.1*

* 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 meal replacement beverages.

Table IV.A.2-2 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 Infant and Toddler Formula 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
Infants 0 to 6 months	2.5	4.1	1.4	2.3
Infants 7 to 12 months	1.5	2.4	0.81	1.3
Toddlers 13 to 36 months[a]	0.8*	1.2*	0.4*	0.6*

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 meal replacement beverages.

IV.A.3 Estimated Consumption of TGF-β_2 from Vitalarmor® GF-100 Added to Formula

Since Vitalarmor® GF-100 is intended for addition to formula at use-levels (up to 30 mg/100 g) such that levels of total TGF-β_2 in reconstituted formula will mimic those identified in human milk, intakes of TGF-β_2 from Vitalarmor® GF-100 in supplemented formula also were calculated (see Table IV.A.3-1). Intakes of TGF-β_2 from Vitalarmor® GF-100 were based on TGF-β_2 concentrations of 0.012 to 0.018% in the basic whey protein isolate. Highest mean and 90th percentile intakes of 0.97 and 1.6 µg TGF-β_2/kg body weight/day, respectively, were observed in 0- to 6-month old infants.

Table IV.A.3-1 Summary of the Estimated Daily All-User Intakes of TGF-β_2 Resulting from the Intended Use of Vitalarmor® GF-100 in Infant and Toddler Formula in the United States (2009-2010 NHANES Data)

Population Group	µg/day		µg/kg body weight/day	
	Mean	90th Percentile	Mean	90th Percentile
Infants 0 to 6 months	3.9-5.9	5.7-8.5	0.65-0.97	1.0-1.6
Infants 7 to 12 months	3.3-5.0	5.1-7.7	0.37-0.56	0.6-0.9
Toddlers 13 to 36 months[a]	2.3-3.4	3.3-5.0	0.20-0.31	0.3-0.45

* 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 meal replacement beverages.

IV.B Information to Support the Safety of Vitalarmor® GF-100 for Use in Infant and Toddler Formulas

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

The protein constituents of Vitalarmor® GF-100 are existing components of the infant and toddler diet as a result of their occurrence in cow's milk and related dairy-products, including infant formulas formulated with cow's milk-derived whey protein isolates and concentrates. These sources provide exposure to both native, as well as denatured forms of the proteins. Furthermore, the main protein constituents of Vitalarmor® GF-100 and other quantitatively minor proteins (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.

Vitalarmor® GF-100 is intended for addition to infant formula as a source of TGF-β_2, and studies characterizing the TGF-β_2 content of infant formula products and human milk from lactating women are presented in Section IV.B.1.1 below. Although Vitalarmor® GF-100 is not intended specifically 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 by the infant and

information on the background exposure to lactoferrin and lactoperoxidase from human milk, cow's milk, and commercial infant formula products are 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 Background Exposure to TGF-β_2

As previously discussed, the use-levels of Vitalarmor® GF-100 will be adjusted based on levels of TGF-β_2 in the final reconstituted formula, such that the concentration of TGF-β_2 in reconstituted formula with Vitalarmor® GF-100 will be in the range of TGF-β_2 levels that have been identified in human milk.

Mature polypeptide of bovine milk-derived TGF-β_2 shows100% amino acid sequence identity with human milk-derived TGF-β_2 (Derynck *et al*., 1986, 1987; Massague, 1990). Concentrations of TGF-β_2 in human milk are variable. 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). This variability may be attributable to a number of different factors, including the maternal diet, differences in the rates of endogenous production of TGF-β_2 by epithelial cells in the mammary gland as determined by the mother's genetics, and/or by inter-assay differences in analyses methodologies used by the investigators (Oddy and Rosales, 2010).

In addition to its natural presences in human milk, TGF-β_2 also has been identified to occur in existing formulas based on unhydrolyzed cow's milk protein. A study was identified in which the concentrations of TGF-β_1 and TGF-β_2 were determined (ELISA based method) in samples (n=25) of cow's milk protein-based infant formula (Enfamil® LIPIL powder) available in the United States, Mexico, Thailand, China, and the Philippines (Jouni *et al*., 2009). A mean concentration of TGF-β (isoform not specified) of 4,900 pg/mL was reported in the reconstituted formula (range: 2,800 to 9,900 pg/mL).

For comparison, the levels of TGF-β_2 in human milk, cow's milk based formula already on the market, and TGF-β_2 from Vitalarmor® GF-100 in reconstituted formula are summarized in Table IV.B.1.1-1.

Table IV.B.1.1-1 Comparison of the Levels of TGF-β_2 from Vitalarmor® GF-100 in Reconstituted Formula to those Identified in Human Milk and Standard Infant Formula Already on the Market

Source of TGF-β_2	Mean Concentration (µg/L) [range]	Reference
Reconstituted Formula with Vitalarmor® GF-100	5.9[a] [4.7 to 7.0]	Not applicable
Human Milk	0.5 to 5.6 [0.2 to 57] (n=8 studies)	See footnote 'b'
Standard Cow's Milk Protein Formula (Enfamil LIPIL)[c]	4.9 [2.8 to 9.9] (n=25 samples of formula)	Jouni *et al*. (2009)

[a] Based on TGF-β_2 concentration of 12 to 18 mg per 100 g of Vitalarmor® GF-100, the maximum use-level of 30 mg Vitalarmor® GF-100/100 g formula and a reconstitution ratio of 130 g formula/L.
[b] 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).
[c] Isoform of TGF-β not identified in the study.

Assuming infant consumption of approximately 1,000 mL of mature human milk per day (U.S. EPA, 2011) and average concentrations of TGF-β_2 identified in human milk, nursing babies are exposed to approximately 0.5 to 5.6 µg TGF-β_2/day (or 0.08 to 0.9 µg/kg body weight/day for a 6.1-kg baby). At the upper end of the concentrations of TGF-β_2 in human milk, exposures as high as 57 µg/day (9.3 µg/kg body weight/day) are estimated. Likewise, assuming daily consumption of approximately 1,140 mL of liquid formula (CDC, 2011) and the mean concentration of TGF-β measured in an existing brand of cow's milk protein infant formula (Enfamil® LIPIL), infants are estimated to consume approximately 5.6 µg TGF-β per day (range: 3.2 to 11.3 µg TGF-β/day) from available standard formulas. Assuming an infant body weight of 6.1 kg (3-month old infant), ingestion of the cow milk-based formula would result in a mean daily TGF-β intake of 0.92 µg/kg body weight (0.52 to 1.85 µg/kg body weight).

In comparison, under the proposed conditions of use, 0- to 6-month old infants, identified as the highest consumers of Vitalarmor® GF-100, would be consuming 5.7 to 8.5 µg TGF-β_2/day (or 1.0 to 1.6 µg TGF-β_2/kg body weight/day) from Vitalarmor® GF-100 (based on 90th percentile all-user intakes).

IV.B.1.2 Background Exposure to Lactoferrin and Lactoperoxidase

1. Human Milk

Lactoferrin accounts for approximately 47% of the composition of Vitalarmor® GF-100. It is a transferrin family non-heme iron-binding glycoprotein comprising approximately 700 amino acid residues with a molecular weight of approximately 80 kDa (Ferenc Levay and Viljoen, 1995; Lönnerdal, 2009). Lactoferrin is one of the main whey proteins found in mammalian milk (Manzoni *et al*., 2010), with significant amounts present in human milk and in cow's milk (Prentice *et al.,* 1987; Goodman and Schanbacher, 1991; Ferenc Levay and Viljoen, 1995; Möller *et al.,* 2008; Lönnerdal, 2009; Legrand, 2012). Bovine and human lactoferrin 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%).

Exposure to lactoferrin in the infant appears to occur predominantly from the consumption of the mother's milk (Prentice *et al*., 1987). The level of lactoferrin in human milk is dependent on the general state of maternal nourishment, with lower levels in the milk of malnourished mothers (Ferenc Levay and Viljoen, 1995). The concentrations of lactoferrin in human colostrum and mature human milk have been measured in several studies. Levels of lactoferrin range from approximately 3,000 to 7,000 mg/L in human colostrum (Masson and Heremans, 1971; Hirai *et al.,* 1990; Mathur *et al*., 1990; Mastromarino *et al*., 2014) and from 1,000 to 3,200 mg/L in mature 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).

Lactoperoxidase contributes approximately 26% of the protein composition of Vitalarmor® GF-100. Lactoperoxidase (molecular weight approximately 80 kDa) is a heme-containing enzyme (glycoprotein) with peroxidase activity and is present in human colostrum and other bodily secretions such as saliva and tears (Gothefors and Marklund, 1975; Shin *et al.,* 2001). Lactoperoxidase is present in bovine milk where it is presumed to serve a similar nutritional role in the calf as in human infants (Indyk *et al*., 2006). The sequence identity of bovine and human lactoperoxidase has been shown to be 83% (BLAST analysis).

Total peroxidase activity is the highest in precolostrum and colostrum (3.28, 0.5, and 0.34 ΔA_{400}/min·mL in precolostrum, 1-week post-delivery colostrum, and 9-week post-delivery colostrum, respectively, as determined by incubating milk serum samples with hydrogen peroxide) and activity declines rapidly within the first few days of milk production (Gothefors and Marklund, 1975). 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).

Considering exposure estimates of approximately 1,000 mL of human milk for upper percentile infants up to 12 months of age (U.S. EPA, 2011) and the concentrations of lactoferrin and lactoperoxidase identified in mature human milk as presented above, the daily exposure to each protein from human milk was estimated to be 1,000 to 3,200 mg/day for lactoferrin and 0.77 mg/day for lactoperoxidase. These estimates are equivalent to 163 to 525 mg/kg body weight/day and 0.13 mg/kg body weight/day, respectively.

2. Infant Formula

The protein constituents of Vitalarmor® GF-100 are expected to be already present in infant formula preparations based on cow's milk protein. Some existing infant formulas are specifically

fortified with whey and/or individual whey proteins in efforts to produce formulas that are similar in composition to human milk. De-mineralized whey and whey protein concentrates are already widely used in infant formula (Lloyd, 2002; U.S. FDA, 2003).

Bovine lactoferrin has been determined to be GRAS for addition to term infant formulas at use levels of up to 100 mg/100 g of powdered formula or 13 mg/100 mL ready-to-feed formula [GRN No. 000465 (U.S. FDA, 2014b)]. Intake estimates of lactoferrin from the consumption of commercially available cow's milk-based formulas (*i.e.*, Similac®, Enfamil®) have been estimated to be as high as 137.4 and 199.4 mg/day for average and high-end consumers, respectively, with the highest intakes observed to occur in newborns (0 to 4 months of age) [GRN No. 000465 (U.S. FDA, 2014b)]. The GRAS Notice for the use of bovine lactoferrin in infant formulas also discusses availability of a bovine lactoferrin-supplemented formula (added at 80 mg/100 g) in Japan (named "Hagukumi"), and several other countries.

In Europe, bovine lactoferrin also is permitted for use in infant formula (ready-to-drink) at levels of up to 100 mg/100 mL (EU, 2012a,b). As part of their safety assessment of bovine lactoferrin, EFSA considered worst-case intake estimates of up to 1.2 g bovine lactoferrin per day in infants from consumption of formula with lactoferrin added at the proposed levels of use (or approximately 210 mg/kg body weight/day) (EFSA, 2012a,b).

No literature sources have been identified which report existing levels of lactoperoxidase in infant formulas currently available on the market. Nevertheless, the potential concentration of total (intact and denatured) lactoperoxidase in reconstituted cow's milk-based infant formula can be estimated to be approximately 40 mg/L based on first principles. Specifically, lactoperoxidase is present in cow's milk at a concentration of 30 mg/L. It is also known that whey protein makes up approximately 20% of the protein composition of cow's milk (33 g protein/L milk). Therefore, lactoperoxidase can be estimated to comprise 0.45% of cow's milk whey protein [30 mg lactoperoxidase/L milk x 1 L milk/(33 g x 20%) g whey protein = 4.5 mg lactoperoxidase/g whey protein]. Assuming a protein concentration of 14 g/L in formula and a typical casein/whey ratio of standard infant formula of 40/60, the total lactoperoxidase concentration in formula can be estimated to be 38 mg/L. Based on the estimated level of total lactoperoxidase in standard cow's milk protein-based formulas (38 mg/L), infants provided formula [assuming daily consumption of approximately 1,140 mL of liquid formula (CDC, 2011)] would consume approximately 43.3 mg lactoperoxidase per day (or approximately 7.1 mg/kg body weight/day assuming a 6.1-kg infant).

For comparison, a similar calculation of lactoferrin content in cow's milk-based formula also was performed. Assuming a concentration of 100 to 150 mg lactoferrin/L cow's milk, lactoferrin levels of 127 to 191 mg/L would be expected in standard cow's milk-based formula based on first principles.

3. Cow's Milk

Lactoferrin is present at lower concentrations in cow's milk than in human milk, with average levels ranging from approximately 100 to 150 mg/L (Ferenc Levay and Viljoen, 1995; Korhonen and Pihlanto, 2003). Lactoperoxidase is reported to occur at a concentration of 30 mg/L in cow's milk (Korhonen and Pihlanto, 2003; Indyk *et al*., 2006; FAO, 2015). In babies 1 year of age and older and toddlers (non-consumers of formula), mean and 90th percentile intakes of milk were reported to be approximately 460 and 919 mL/day, respectively (CDC, 2011). Toddlers can therefore be expected to ingest approximately 46 to 69 mg lactoferrin (equivalent to approximately 3.8 to 5.8 mg/kg body weight/day for a 12-kg child) and approximately 14 mg/day lactoperoxidase (equivalent to approximately 1.2 mg/kg body weight/day for a 12-kg child) per day from cow's milk.

4. Summary

In infants, exposure to the protein constituents of Vitalarmor® GF-100 will occur *via* consumption of human milk or cow's milk-based formula. In older babies and toddlers, exposure to the proteins will occur as a results of consumption of cow's milk and products derived thereof. A summary of the exposure estimates in infants and toddlers from background dietary sources to the main proteins comprising Vitalarmor® GF-100, lactoferrin and lactoperoxidase, are provided in Table IV.B.1.2-1.

Table IV.B.1.2-1 Estimates of Intakes of Lactoferrin and Lactoperoxidase in Infants and Toddlers from the Background Diet (Human Milk, Cow's Milk Based Formulas, and Cow's Milk)

Potential Source of Exposure (Infants and Toddlers)	Lactoferrin		Lactoperoxidase	
	Concentration (mg/L)	Intake (mg/kg bw/day)	Concentration (mg/L)	Intake (mg/kg bw/day)
Human milk	1,000-3,200	163-525[a]	0.77	0.13[a]
Standard cow's milk protein formula	127-1,000	26.4[b] to 210[c]	38	7.1[d]
Cow's milk	100-150	3.8-5.8[5]	30	1.2[e]
Vitalarmor® GF-100 added to formula	18[f]	1.2-4.1[g]	10[h]	0.6-2.3[g]

[a] Calculated based on concentrations of proteins in human milk and assuming daily intake of 1,000 mL of human milk (U.S. EPA, 2011) and infant body weight of 6.1-kg.
[b] GRAS Notice GRN No. 000465 (U.S. FDA, 2014b).
[c] EFSA, 2012a,b.
[d] Calculated based on the concentration of lactoperoxidase in cow's milk protein-based formula and assuming daily intake of 1,140 mL of formula (CDC, 2011) and infant body weight of 6.1-kg.
[e] Calculated based on concentrations of proteins in cow's milk and assuming mean daily intake of 460 mL of cow's milk and toddler body weight of 12-kg.
[f] Assuming use-level of 30 mg Vitalarmor® GF-100/100 g formula, a reconstitution factor of approximately 130 g/L, and a concentration of 47% lactoferrin in Vitalarmor® GF-100.
[g] Based on all-user 90th percentile intakes of Vitalarmor® GF-100 for 0- to 36-month-olds (see Table IV.A.2-2).
[h] Assuming use-level of 30 mg Vitalarmor® GF-100/100 g formula, a reconstitution factor of approximately 130 g/L, and a concentration of 26% lactoperoxidase in Vitalarmor® GF-100.

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 exclusively breast-fed infants (Davidson and Lönnerdal, 1987; Prentice *et al*., 1987), fecal excretion of intact lactoferrin further suggests that lactoferrin may be resistant to protein degradation in the infant gastrointestinal tract. In pre-term infants provided mother's milk by enteral feeding, excretion of intact lactoferrin also was observed in the urine (Hutchens *et al*., 1991). The authors therefore considered that in the gut of a pre-term infant, lactoferrin may not only be resistant to degradation, but may also be taken up intact. Resistance to proteolytic degradation of lactoferrin also has been reported in adults (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 unhydrolyzed cow's milk-based formula.

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[3] 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

[3] 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).

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 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[4] mg/kg body weight. Dosing was initiated on PND 7[5] and animals were gavaged daily for 6 weeks until approximately the onset of sexual maturation (PND 49) (Boston University, 2011; Campion *et al*., 2013; Sengupta, 2013). 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 two 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-

[4] 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).
[5] "*Neonates are defined as mouse or rat pups up to 10 days of age*." (Boston University, 2011).

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 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 (U.S. FDA, 2014a,b) and GRAS Notice GRN No. 000196 (U.S. FDA, 2006), respectively]. 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; Reznikov *et al*., 2014; Somm *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 induction with phytohaemagglutinin; an increase in

IL-6 and IL-10 production and in IFN-γ production in lipopolysaccharide (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 including studies in infant populations with high-purity bovine lactoferrin were available for review. Many of the studies also were 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 these constituents, and only a few of the studies included measures related to safety. A series of investigations was conducted to assess the potential effects of bovine lactoferrin on the incidence of sepsis and necrotizing enterocolitis in low-birth-weight babies (Manzoni *et al*., 2009, 2012, 2014). Other studies in infants provided bovine lactoferrin-supplemented formula were conducted to assess the effects of lactoferrin on mineral status, iron bioavailability, fecal flora, and the general well-being of infants (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).

Although assessment of safety *per se* was not the primary objective of these studies, a few of the studies did include some safety-related endpoints as part of the study design (*e.g.,* body weight, hematology, tolerability) (Lönnerdal and Hernel, 1994; Hernel and Lönnerdal, 2002; King *et al*., 2007; Manzoni *et al.,* 2009, 2012, 2014). In these studies, lactoferrin was provided for 4 weeks to 1 year at doses of 100 mg to approximately 1.5 g of lactoferrin per day. No significant adverse effects were reported. Good tolerability was reported by Hernel and Lönnerdal (2002), King *et al*. (2007), and Manzoni *et al*. (2009, 2012, 2014).

Several further studies were identified that also involved oral administration of bovine lactoferrin to infants (with studies starting as early as birth and continuing for as long as 5 months)

(Kawaguchi *et al*., 1986, 1989; Balmer *et al.,* 1989; Fairweather-Tait *et al*., 1987; Schulz-Lell *et al*., 1991; Chierici *et al*., 1992; Roberts *et al*., 1992), but these studies did not include any assessment of parameters related to safety. With the exception of a single administration study and a 2-week study in which the daily dose of lactoferrin was approximately 3 g (Fairweather-Tait *et al.,* 1987; Balmer *et al*., 1989), lactoferrin was provided to infants at doses up to approximately 1 g per day for periods of up to 150 days. There were no reports of adverse events or poor tolerability.

In addition to the studies with infants, a few studies in which bovine lactoferrin was provided to children (up to 10 years of age), also were identified. Lactoferrin provided at doses up to 1,000 mg/day for up to 9 months to healthy children or children presenting with *Helicobacter pylori*, was well tolerated and not associated with any adverse effects (Okuda *et al.*, 2005; Egashira *et al.*, 2007; Ochoa *et al.*, 2008, 2013).

The results of the above studies which include ingestion of daily gram doses of lactoferrin support the safety of daily, long-term consumption of lactoferrin by infants. The highest level of exposure to lactoferrin from Vitalarmor® GF-100 in infant formula supplemented with the basic whey protein isolate at the proposed use-levels is estimated to be only approximately 22.2 mg/day (in 0- to 6-month old infants; see Section IV.A.2). The results of these studies therefore provide corroborative evidence for the safe use of Vitalarmor® GF-100 in infant and toddler formulas.

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, including infant formula (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 the Food Standards Australia New Zealand (FSANZ) (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 infant or toddler formulas. Furthermore, while Vitalarmor® GF-100 may be added to partially hydrolyzed milk protein based formulas, it is not intended for addition to extensively hydrolyzed formulas indicated for feeding to infants who are allergic to milk or to infants with existing milk allergy symptoms.

IV.B.6 Conclusions on the Safety of Vitalarmor® GF-100 for Use in Infant and Toddler Formulas

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. Whey protein isolates and concentrates are widely used as a source of dietary protein in infant and toddler formulas, and bovine lactoferrin, a basic whey protein, was determined to be GRAS for use in infant formula at levels of up to 130 mg/L of ready-to-feed formula or 100 mg/100 g of powdered formula [GRN No. 000465 (U.S. FDA, 2014b)]. In infants, exposure to the protein constituents of the ingredient occurs from the consumption of cow's milk protein-based formula. Whey derived protein products therefore have an established and generally recognized history of safe consumption from the diet, including from infant formula. Additionally, in nursing infants, exposure also may occur from the ingestion of human milk. In young children, further exposure to the proteins of Vitalarmor® GF-100 also may occur *via* ingestion of cow's milk and cow's milk-based dairy products. 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 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 proposed 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 infant and toddler formula. No safety concerns are therefore raised with respect to the conditions of uses of Vitalarmor® GF-100 in infant and toddler formulas 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 infant and toddler formulas 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 infant and toddler formulas. The Expert Panel consisted of the following qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Ronald Kleinman (Mass General Hospital for Children),

Dr. Robert J. Nicolosi (University of Massachusetts Lowell), 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 infant and toddler formulas 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 term infant and toddler formulas, 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 term infant and toddler formulas 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 Term Infant Formulas and Toddler Formulas

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 Infant and Toddler Formulas

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 infant and toddler formulas. The Expert Panel was asked to determine whether the use of Vitalarmor® GF-100 in infant formula at a level of up to 30 mg per 100 g is Generally Recognized as Safe (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. Ronald Kleinman (Massachusetts General Hospital for Children), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), 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 Infant and Toddler Formulas" (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 infant and toddler formulas, dietary intake estimates of Vitalarmor® GF-100 and its constituents resulting from the intended conditions of use, as well as intake estimates of certain protein constituents of Vitalarmor® GF-100 from other potential sources of exposure (*e.g.*, human milk, cow's milk, existing formulas), 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 infant and toddler formulas at levels of up to 30 mg per 100 g (formula powder) of Vitalarmor® GF-100, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice (cGMP), 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 infant formulas (excluding "exempt formulas" as defined in 21 CFR§ Sec. 107.3 in the U.S.) and toddler formulas. 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. The milk 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 are 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 whey protein concentrates as GRAS, 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 non-exempt infant and toddler formulas, Vitalarmor® GF-100 is proposed for addition at use-levels of up to 30 mg per 100 g of powdered formula. Vitalarmor® GF-100 is intended for addition to formula that may be low or deficient (*e.g.*, partially hydrolyzed formula) in TGF-β_2. TGF-β_2 is a physiologically active regulatory cytokine present in human milk and is involved in modulation of the infant host-immune response. Human and bovine TGF-β_2 exhibit ≥98% amino acid sequence identity (100% identity in mature polypeptide). Use-levels of Vitalarmor® GF-100 will be adjusted (up to 30 mg/100 g of powdered formula) such that the level of total TGF-β_2 in reconstituted formula will be similar to levels of TGF-β_2 in human milk. Following reconstitution of a formula supplemented with Vitalarmor® GF-100 (assuming maximum proposed inclusion rate of 30 mg/100 g of powder and a reconstitution ratio of 130 g of formula powder/L), TGF-β_2 from Vitalarmor® GF-100 would be present at a concentration of up to 7.0 µg/L. In comparison, average TGF-β_2 levels of 0.5 to 5.6 µg/L and maximum TGF-β_2

levels of 57 µg/L were identified in human milk (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).

Intake estimates of Vitalarmor® GF-100 under the proposed conditions of use were generated for 0- to 6-month-old infants, 7- to 12-month-old infants, and 13- to 36-month-old toddlers. The intake estimates for toddlers also considered the proposed use of the ingredient in meal replacement beverages[1]. Highest all-user mean and 90th percentile intakes of Vitalarmor® GF-100 were obtained in the 0- to 6-month-olds, at 32.9 and 47.2 mg/day, respectively. On a body weight basis, the highest mean and 90th percentile all-user intakes also were identified in the 0- to 6-month-old infants (*i.e*., 5.4 and 8.7 mg/kg body weight/day, respectively).

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 body weight basis would therefore also occur in 0- to 6-month-old infants from the consumption of Vitalarmor® GF-100-supplemented whey predominant infant formulas: approximately 22.2 and 12.3 mg/day of lactoferrin and lactoperoxidase, respectively (4.1 and 2.3 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 (including infant formula). 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®)[2] and highly purified bovine lactoferrin (including

[1] Armor also is currently in the process of conducting a GRAS determination for the use of Vitalarmor® GF-100 in meal replacements beverages and medical foods ("*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"*). While Vitalarmor® GF-100 is proposed for use only in meal replacement beverages and medical foods intended for adults, a limited number of toddlers were identified as consumers of meal replacement beverages.

[2] 54% lactoferrin and 41% lactoperoxidase.

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 food for human consumption, including use in infant and follow-on formulas, was previously assessed by the European Food Safety Authority (EFSA, 2012). Bovine lactoferrin for use in formula at a level of 100 mg/100 mL was determined to be safe (the intake in 0- to 12-month-old infants was estimated to be 210 mg/kg body weight/day under the proposed conditions of use). The safety of lactoperoxidase, the other main constituent of Vitalarmor® 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.

The main protein constituents of Vitalarmor® GF-100, lactoferrin and lactoperoxidase, also are biologically active and are naturally present in human milk. The cow's milk proteins are closely related to the proteins that occur 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). In comparison to the concentrations of each protein in human milk (up to 3,200 and 0.77 mg/L of lactoferrin and lactoperoxidase in human milk, respectively) (Pamblanco *et al*., 1986; Prentice *et al*., 1987; Hirai *et al.*, 1990; Hennart *et al*., 1991; Rudloff and Kunz, 1997; Shin *et al.,* 2001), concentrations of lactoferrin and lactoperoxidase from Vitalarmor® GF-100, added at the maximum proposed inclusion level of up to 30 mg/100 g, in reconstituted formula will be lower and higher, respectively (18 and 10 mg/L of lactoferrin and lactoperoxidase in Vitalarmor® GF-100-containing formula, respectively). Assuming daily consumption of approximately 1,000 mL of breast milk by an infant (U.S. EPA, 2011), exposure to lactoferrin from human milk is considerably greater than that which would occur from Vitalarmor® GF-100 as a result of consumption of whey predominant infant formula supplemented with the whey protein isolate (up to 525 mg/kg body weight/day *versus* up to 4.1 mg/kg body weight/day). In the case of lactoperoxidase, exposure in infants from human milk would be lower (0.13 mg/kg body weight/day) than that from Vitalarmor® GF-100 added to formula (up to 2.3 mg/kg body weight/day).

The inclusion levels of Vitalarmor® GF-100 will be adjusted such that following formula reconstitution, total levels of TGF-β_2 in the formula will be comparable to levels of TGF-β_2 in human milk, exposure to TGF-β_2 from Vitalarmor® GF-100-supplemented formula (assuming addition at maximum proposed use-level[3]) also was determined. On an absolute and body weight basis, the highest level of exposure to TGF-β_2 from Vitalarmor® GF-100-added to formula would occur in 0- to 6-month-old infants: approximately 7.1 µg/day and 1.3 µg/kg body

[3] For formulas with no background levels of TGF-β_2.

weight/day at the 90th percentile, respectively. Considering mean concentrations of TGF-β_2 in human milk, exposure from human milk in the range of 0.5 to 5.6 µg/day were estimated. At the highest identified concentration of TGF-β_2 in human milk, exposure would be approximately 57 µg/day.

Some currently available formulas are based on cow's milk protein, including whey protein specifically. As such, in formula-fed infants, exposure to the proteins that comprise Vitalarmor® GF-100 may also occur by consumption of cow's milk protein-based formulas. Although published analytical data on lactoferrin levels in presently available formulas were not identified, bovine lactoferrin is permitted for use in the EU in infant formula (ready-to-drink) at levels of up to 100 mg/100 mL (EU, 2012a,b). In the U.S., bovine lactoferrin has been determined to be GRAS for use in infant formula at levels of up to 13 mg/100 mL (U.S. FDA, 2014b). Although no literature sources were identified for lactoperoxidase concentrations in currently available infant formulas, a theoretical estimation[4] of lactoperoxidase in cow's milk protein-based formulas is approximately 40 mg/L. Therefore, in comparison to cow's milk protein-based infant formulas already available on the market, concentrations of lactoferrin and lactoperoxidase from Vitalarmor® GF-100 in reconstituted formula would be lower. Assuming daily consumption of approximately 1,140 mL of formula (CDC, 2011), intakes of lactoferrin from the addition of Vitalarmor® GF-100 to formula would be lower than intakes of lactoferrin from its presence in currently available infant formulas (22.2 *versu*s 137.4 to 1,200 mg/day). Likewise, exposure to lactoperoxidase from the addition of Vitalarmor® GF-100 to formula would be lower than intakes of lactoperoxidase from currently available infant formulas (12.3 *versus* approximately 43 mg/day).

Vitalarmor® GF-100 is derived from cow's milk by use of physical separation techniques only. The introduction of cow's milk and dairy products to the diet will increase exposure to the constituents of Vitalarmor® GF-100. In comparison to concentrations of lactoferrin and lactoperoxidase in cow's milk (100 to 150 mg/L cow's milk and 30 mg/L cow's milk, respectively) (Ferenc Levay and Viljoen, 1995; Korhonen and Pihlanto, 2003; Indyk *et al*., 2006; FAO, 2014), levels of the proteins from the addition of Vitalarmor® GF-100 to formula would be lower (at least about 3-fold lower). Therefore, in the case of toddlers (1- to 3-year-old) consuming cow's milk, exposure to lactoferrin and lactoperoxidase from cow's milk would be higher than intakes of these proteins from Vitalarmor® GF-100 added to follow-on formula (46 to 69 mg lactoferrin and 14 mg lactoperoxidase from cow's milk *versus* 12.9 mg lactoferrin and 7.1 mg lactoperoxidase from Vitalarmor® GF-100 added to formula).

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®

[4] Based on protein composition of cow's milk, level of lactoperoxidase in cow's milk, and typical casein/whey ratio of standard infant formula.

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 the case of lactoferrin, fecal (as a result of biliary elimination) and urinary excretion of intact lactoferrin in exclusively breast-fed infants (Davidson and Lönnerdal, 1987; Prentice *et al*., 1987) and in infants provided mothers' milk by enteral feeding (Hutchens *et al*., 1991), respectively, suggest that lactoferrin may not only be resistant to protein degradation in the gastrointestinal tract, but may also be absorbed. 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 radiolabeled human lactoferrin. Results from some *in vitro* investigations suggest that human and bovine lactoperoxidase retain peroxidase activity following incubation in the presence of infant or adult gastric juice (Gothefors and Marklund, 1975). Resistance of lactoperoxidase to degradation by enzymes of the upper intestine (trypsin and chymotrypin) was reported by Kussengrager 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).

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 infant and toddler formulas 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®,[5]) and MBP®], as well as high-purity lactoferrin. A median lethal dose of greater than 2,000 mg/kg body weight was identified for MBP®, indicating the material to be of 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 by gavage, no adverse

[5] 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).

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 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, a series of clinical trials involving oral administration of bovine lactoferrin to infants and young children was identified. The clinical studies were primarily designed to assess parameters related to potentially beneficial effects related to the consumption of this protein, 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 infants and toddlers. Daily doses of 100 mg to approximately 3,000 mg of lactoferrin for 2 weeks to 1 year were well-tolerated by infants (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). No adverse events/effects were reported in those studies that also included parameters related to safety (*e.g*., mortality, body weight, liver enzymes, hematological indices) (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). The doses used in these studies far exceed the potential intake of lactoferrin from the use of Vitalarmor® GF-100 in infant and toddler formulas (up to approximately 22.2 mg/day in 0- to 6-month-old infants).

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 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 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 infant or toddler formula containing the ingredient. While Vitalarmor® GF-100 may be added to partially hydrolyzed milk protein-based formulas, it is not intended for addition to extensively hydrolyzed formulas indicated for feeding to infants who are allergic to milk or to infants with existing milk allergy symptoms.

The safety of Vitalarmor® GF-100 under the proposed conditions of use in infant and toddler formulas 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 food for human consumption, including in infant formula. Whey forms the basis of whey-based infant formulas, and lactoferrin was determined to be safe for use in infant formula. 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 human and cow's 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 infant and toddler formulas. We unanimously conclude that the intended use as an ingredient in infant and toddler formulas at a level up to 30 mg per 100 g powder formula 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 infant and toddler formulas at a level up to 30 mg per 100 g of powder formula 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.

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

(b) (6)

20 February 2015

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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