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**Notice to US Food and Drug Administration of the
Conclusion that Inactivated *Bacillus coagulans*
GBI-30, 6086 is Generally Recognized as Safe for
use in Non-exempt Term Infant Formula**

Submitted by the Notifier:

Ganeden Biotech, Inc.
5800 Landerbrook Drive, Suite 300
Mayfield Heights, Ohio 44124

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc
2800 E. Madison, Suite 202
Seattle WA 98112

August 8, 2017



August 8, 2017

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

Dear Dr. Carlson:

In accordance with proposed regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of Geneden Biotech, Inc. (the notifier), the undersigned, Timothy Murbach, submits, for FDA review, the enclosed notice that Inactivated *Bacillus coagulans* GBI-30, 6086 is GRAS for use in non-exempt term infant formula.

Should you have any questions or concerns regarding this notice, please contact me at 253-286-2888 or tim@aibmr.com.

Sincerely,

(b) (6)



Timothy S. Murbach, ND, DABT (agent of the notifier)
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc. ("AIBMR")

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Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

Ganeden Biotech, Inc. (the notifier) is submitting a new GRAS notice in accordance with 21 CFR part 170, subpart E, regarding the conclusion that Inactivated *Bacillus coagulans* GBI-30, 6086 is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

1.2 Name and Address of the Notifier and Agent of the Notifier

Notifier

David Keller, DPM, MBA
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Ganeden Biotech, Inc.
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Agent of the Notifier

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1.3 Name of the Substance

Inactivated *Bacillus coagulans* GBI-30, 6086 (STA-IMUNE™)

Synonyms: Thermally killed *Bacillus coagulans* GBI-30, 6086

Heat killed *Bacillus coagulans* GBI-30, 6086

1.4 Intended Conditions of Use

Inactivated *Bacillus coagulans* GBI-30, 6086 is intended to be added to non-exempt term infant formula at levels up to 2×10^8 CFU inactivated cells per 100 mL infant formula as ready for consumption.

*Inactivated *B. coagulans* GBI-30, 6086 is not intended for use in any product that would require additional review by USDA.

1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of Inactivated *Bacillus coagulans* GBI-30, 6086 for its intended conditions use, stated in Part 1.4 of this notice, has been made based on scientific procedures.

1.6 Not Subject to Premarket approval

We have concluded that Inactivated *Bacillus coagulans* GBI-30, 6086 is GRAS for its intended conditions of use, stated in Part 1.4 of this notice, and, therefore, such use of Inactivated *Bacillus coagulans* GBI-30, 6086 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7 Data and Information Availability Statement

The data and the information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of David Keller, DPM, MBA, Vice President of Scientific Operations, Ganeden Biotech, Inc., 5800 Landerbrook Drive, Suite 300, Mayfield Heights, Ohio 44124, Telephone: (440) 229-5204, email: keller@ganedenbiotech.com or will be sent to FDA upon request.

1.8 Exemption from Disclosure under the Freedom of Information Act

None of the information in Parts 2 through 7 of this GRAS notice is considered exempt from disclosure under the Freedom of Information Act (FOIA) as trade secret or commercial or financial information that is privileged or confidential.

1.9 Certification of Completion

We hereby certify that, to the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of Inactivated *Bacillus coagulans* GBI-30, 6086.

(b) (6)



August 8 2017

David Keller, DPM, MBA
Vice President of Scientific Operations
Notifier

Date

Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification

Bacillus coagulans was first described in 1915 at the Iowa Agricultural Experiment Station, with regard to the coagulation of canned evaporated milk.¹ GenedenBC³⁰ is the trade name for a proprietary preparation of a *B. coagulans* strain designated as *B. coagulans* GBI-30, 6086. *B. coagulans* GBI-30, 6086 is a patented probiotic organism. It is L+ lactic acid producing, non-toxicogenic, and non-pathogenic. The organism is a gram-positive spore-forming rod that is aerobic to microaerophilic in nature. The spore size is 0.9 µm x 3.0 µm x 5.0 µm. *B. coagulans* GBI-30, 6086 is manufactured as a pure cell mass consisting solely of *B. coagulans*. This GRAS Notice is for inactivated, thermally killed cells of this identical strain; STA-IMUNE™ is the trade name of the proprietary inactivated preparation. The culture conditions in the manufacturing process are not optimized to induce sporulation although, due to the nature of the product and technical limitations, we cannot exclude with 100% certainty the possibility that the product contains absolutely no spores.

B. coagulans GBI-30, 6086 was identified to the genus level and then confirmed to be a pure strain of *B. coagulans* Hammer as reported in GRN 000399 under the subheading DNA Ribotyping Analysis on page 5, which is incorporated here by reference, where it is also noted that each lot of *B. coagulans* GBI-30, 6086 is subjected to 16S Ribosomal DNA base pair analysis as part of Geneden's ongoing Quality Control (QC) program.

To further characterize *B. coagulans* GBI-30, 6086, whole-genome sequencing was performed using the Illumina GAIIx platform at CRA-Genomics Research Centre (Piacenza, Italy) with a paired-end library.² This sequencing project has been deposited in DDBJ/EMBL/GenBank under the accession number JPSK00000000. The genome consists of 3,458,655 base pairs with a GC% content of 46.38. From the total prediction of 3,373 genes, 3,197 coding sequences (CDS), 18 rRNAs, 82 tRNAs, 79 pseudogenes, 1 ncRNA, and 3 clustered regularly interspaced short palindromic region (CRISPR) arrays were identified. CDS genes were predicted to encode approximately 500 proteins related to energy metabolism and 80 related to dormancy and sporulation. Genes involved in adhesion (i.e., fibronectin- and mucus-binding proteins) and active metabolism (e.g., biotin biosynthesis) were also identified. The complete 16S rRNA gene sequence was searched against the ExTaxon database and was aligned with those of *B. coagulans* DSM 1^T, related taxa, and other representatives of the *Bacillus* genus using Clustal Omega.^{3,4} A phylogenetic tree was constructed using the number of differences algorithm as substitution model and neighbor-joining as tree inference method as implemented in MEGA v.6 software package.⁵ The 16S rRNA analysis

demonstrated 99.92% identity compared to *B. coagulans* DSM 1^T, confirming that *B. coagulans* GBI-30, 6086 be allotted to species *B. coagulans*.

B. coagulans is recognized as a non-pathogenic, non-toxicogenic organism by US FDA (21 CFR 184.1372), Health Canada, and European Food Safety Authority (EFSA). Data and information demonstrating that *B. coagulans* GBI-30, 6086 is not a source of putative virulence factors, genes for known enterotoxins, hemolysins, or other toxins, biogenic amines, or D(-)-lactate, and does not harbor transferable antibiotic resistance genes is summarized in subparts 6.4 and 6.5 of the notice. Potential toxicant and microbial contamination is controlled for in the manufacturing processes and Standard Operating Procedures (SOP) described in subpart 2.3, and checked for compliance through adherence to limits, appropriate for food grade material and the particulars of Inactivated *B. coagulans* GBI-30, 6086, as set in the product specifications, which are provided in subpart 2.4 of this notice. The Inactivated *B. coagulans* GBI-30, 6086 product line contains versions of the ingredient that are produced on culture media containing soy and milk derived proteins as well as a version manufactured on allergen-free culture media. Each version of Inactivated *B. coagulans* GBI-30, 6086 is manufactured according to the processes, standard operating procedures, and specifications described in subparts 2.3 and 2.4 of this notice and labeled as appropriate and required according to the Food Allergen Labeling and Consumer Protection Act.

B. coagulans GBI-30, 6086 was the subject of GRN Nos. 000399 and 000660 submitted to FDA by Ganeden Biotech, Inc. and filed by the Agency on August 23, 2011 and August 16, 2016, respectively.^{6, 7} Inactivated *B. coagulans* GBI-30, 6086 was the subject of GRN 000670 submitted to FDA by Ganeden Biotech, Inc. and filed by the Agency on October 12, 2016.⁸ On July 31, 2012 and March 16, 2017 GRN Nos. 000399 and 000670, respectively, received the Agency's response letters with no questions pertaining to Ganeden's conclusions that *B. coagulans* GBI-30, 6086 and Inactivated *B. coagulans* GBI-30, 6086, respectively, are GRAS for their intended use as an ingredient in baked goods and baking mixes; beverages and beverage bases; breakfast cereals; chewing gum; coffee and tea; condiments and relishes; confections and frostings; dairy product analogs; fruit juices; frozen dairy desserts and mixes; fruit and water ices; gelatins, puddings, and fillings; grain products and pastas; hard candy; herbs, seeds, spices, seasonings, blends, extracts, and flavorings; jams and jellies; milk; milk products; nuts and nut products; plant protein products; processed fruits; processed vegetables and vegetable juices; snack foods; soft candy; soups and soup mixes; sugar; and sweet sauces, toppings, and syrups at a maximum level of approximately 2×10^9 colony forming units (CFU) and 2×10^9 inactivated CFU, respectively, per serving. On January 13, 2017, GRN 000660 received the Agency's response letter with no questions pertaining to Ganeden's conclusion that *B. coagulans* GBI-30, 6086 is GRAS for its intended use as an ingredient in

non-exempt powdered and liquid infant formulas for term infants at levels up to 2×10^8 CFU per 100 mL infant formula as consumed.

2.2 Other Ingredients

The other ingredients in the final product, Inactivated *B. coagulans* GBI-30, 6086, consist of maltodextrin, microcrystalline cellulose, organic inulin and/or sodium bicarbonate, which are used for bulking purposes, and milk powder and organic inulin, which can be used as diluents. Pursuant to 21 CFR 184.1444 (maltodextrin) and 184.1736 (sodium bicarbonate) these bulking agents are GRAS. The GRAS status of microcrystalline cellulose is included in the Select Committee on GRAS Substances (SCOGS) review of ethyl cellulose. Milk powder is GRAS due to common use pursuant to 21 CFR 182.1 and inulin is GRAS for use in foods pursuant to GRN Nos. 000477 and 000576. All corn-derived products used in the production of *B. coagulans* GBI-30, 6086 are manufactured to meet European Union quality standards (EC 1829, EC 1830), which do not allow for GMO products.

2.3 Manufacturing

The production process for Inactivated *B. coagulans* GBI-30, 6086 consists of fermentation followed by recovery, followed by a kill step, followed by spray-drying or freeze-drying. The purpose of the recovery process (centrifugation) is to retrieve and concentrate the Inactivated *B. coagulans* cells post-fermentation. The narrative below (broken into 14 steps) explains the manufacturing process.

2.3.1 Manufacturing Overview

Facility: Inactivated *B. coagulans* GBI-30, 6086 is manufactured using standard fermentation procedures. The production facilities are cGMP compliant for the production of food ingredients and designed for a clean and sanitary operation. SOP are followed and verification methods are in place. All personnel are trained in and follow hygienic practices in all areas of the facility from the delivery area for food grade raw materials all the way through to shipment of finished product.

Ingredients Receipt and Raw Material Selection from Approved Suppliers: All vendors supplying the food grade raw materials are approved by assuring products conform to specifications such as Kosher and Halal, come with a Certificate of Analysis (CoA), and the vendor has passed a third party audit. Only food grade ingredients and food contact packaging from approved vendors are used. Carriers are inspected for cleanliness of delivery vehicles as well as suitability of pallets. Expected expiration dates are also checked.

Storage: Storage areas use approved temperature and humidity conditions and are monitored for prevention of contamination, sanitary conditions, and proper physical stacking. Proper rotation of ingredient materials is used to insure a first-in-first-out use of ingredients.

Unpack and Weight: Media components for seed production and for fermentation are confirmed, weighed, and combined for each use.

Seed production: Media components for seed production are combined with filtered water in clean vessels and heat sterilized. The seed culture flasks are inoculated with material from low transfer Mother cultures held in Stock Culture into flasks and grown with shaking at the proper temperature before scale-up inoculation of second seed fermentation in small fermenters or carboys for growth.

Fermenter Tank Verification and Sterilization: Appropriately sized fermenter tanks are cleaned in place using standard procedures. Media components for final fermentation are combined with filtered water in clean vessels and heat sterilized in place.

Fermenter Inoculation: The sterilized medium in the fermenter is inoculated with an appropriate amount of culture that was grown in the small fermenter or carboy.

Growth of the Culture in the Final Fermenter: The culture conditions, which are developed for maximum growth of a standard product, include temperature, aeration, pH control, and agitation. Progress of the fermentation is followed by determining cell number increase by optical density measurements.

Inactivation Step: At the end of fermentation the biomass is concentrated (by centrifugation or cross flow filtration, etc.). The inactivation step combines changes in pH (with or without media components) along with a combination of temperature and/or pressure.

Centrifugation: The material in the fermenter is centrifuged to precipitate the mass. The pellet is washed to remove fermentation residues and re-concentrated.

Freeze Drying or Spray-Drying: The concentrated and washed material is then either freeze dried or spray dried. It is then blended with a GRAS, powdered diluent.

Quality Control: Each batch of the final product undergoes a rigorous QC testing procedure to confirm metal levels are below specification, to document no microbial contamination, and to ensure physical characteristics are within specified limits. Lots failing the QC steps are rejected and discarded by specified waste discharge procedures.

Certificate of Analysis: The QC results for each batch or lot are reported in a standard CoA.

Storage and Shipping: The final product is stored under proper conditions as outlined above and shipped in approved containers by approved shippers.

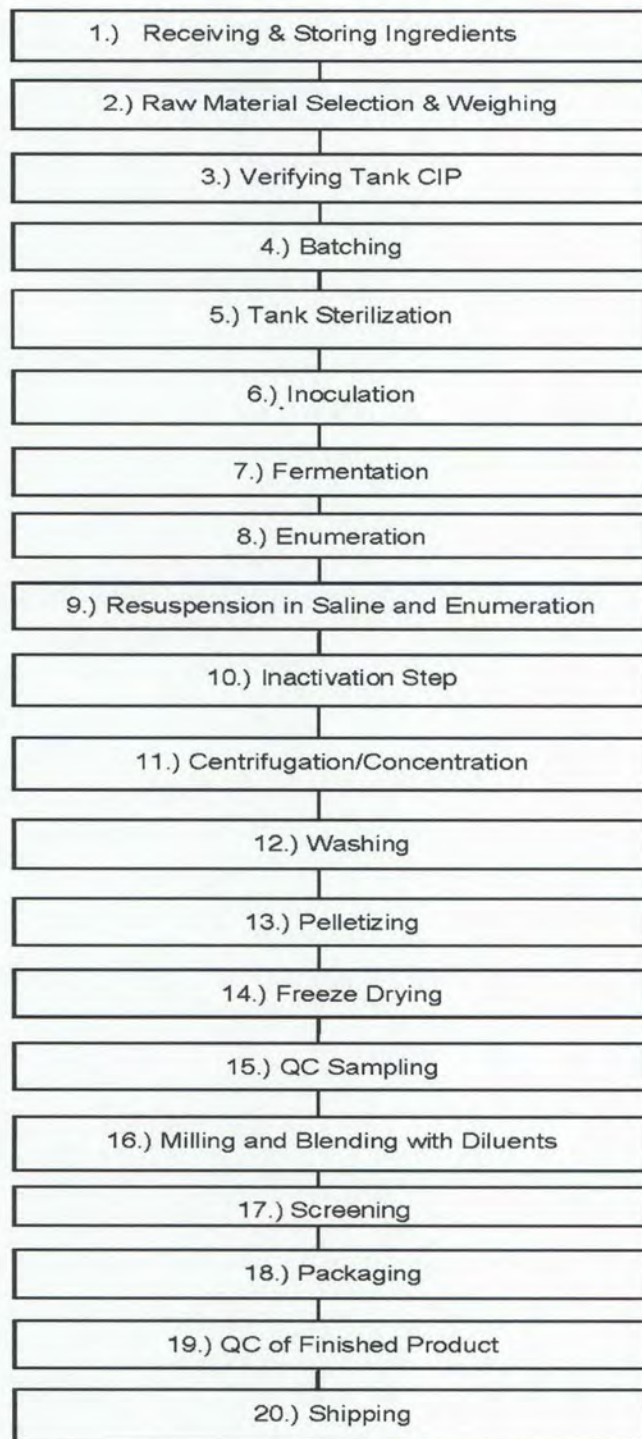


Figure 1. Manufacturing Flowchart

2.4 Specifications

The product specifications for Inactivated *B. coagulans* GBI-30, 6086, along with the specification methods, are listed in Table 1 below.

Table 1. Inactivated *B. coagulans* GBI-30, 6086 Specifications

Test Items	Specification	Method
<i>Bacillus coagulans</i> GBI-30, 6086		
Enumeration (before cell inactivation)	NLT 1.5×10^{10} (15 billion) colony forming units per gram	Internal Method
Physical Characteristics		
Appearance	Beige to White Powder	Visual inspection
Moisture	NMT 9%	AOAC 926.08
Sieve Test 1	100% through 40 mesh	Internal Method
Sieve Test 2	80% through 80 mesh	Internal Method
Heavy Metals		
Arsenic	NMT 1 ppm	EPA 3050/6020 USP 730
Cadmium	NMT 0.5 ppm	EPA 3050/6020 USP 730
Lead	NMT 3 ppm	EPA 3050/6020 USP 730
Mercury	NMT 0.1 ppm	EPA 3050/6020 USP 730
Microbiological Tests		
Standard Plate Count	NMT 5000 CFU/g	BAM CH.3
Yeast and Mold	NMT 100 CFU/g	BAM CH. 18
Total Coliforms	NMT 10 CFU/g	BAM CH. 4
<i>E. coli</i>	None Detected	BAM CH. 4
<i>Staphylococci</i> (Coag. Pos.)	None Detected	AOAC 975.55
<i>Salmonella</i>	None Detected	AOAC 999.08
<i>Listeria</i>	None Detected	AOAC 996.14
<i>P. aeruginosa</i>	None Detected	Internal Method
<i>Cronobacter sakazakii</i>	Absent/100 g	BAM CH. 29

Abbreviations: BAM, Bacteriological Analytical Manual; CFU, colony forming unit; Coag. Pos., coagulation positive; EPA, United States Environmental Protection Agency; LOD, limit of detection; ND, none detected; NLT, not less than; NMT, not more than; USP, United States Pharmacopeia.

2.4.1 Method for Enumeration

Enumeration is run at the end of fermentation. Based on R&D work, a known quantity of powder is produced once dried. The CFU per gram (pre-inactivation step) is calculated. Based on these results, it can be calculated how much diluent needs to be added to have a final product equivalent to 15 billion CFU cells inactivated.

2.4.2 Non-sequential Batch Analysis

Production conformity and consistency of Ganeden's Inactivated *B. coagulans* GBI-30, 6086 is tested in production lots. Results of five non-consecutive batch analyses, representing approximately 12 months of production are shown in the table below and were reasonably consistent and met the product specifications for enumeration, physical characteristics, heavy metals, and microbial analyses.

Table 2. Inactivated *B. coagulans* GBI-30, 6086 Batch Analyses

Test Items	Specification	Lot Number/Manufacture Date				
		160718	160809	160815	170118	170516
<i>B. coagulans</i> GBI-30, 6086		7/18/2016	8/19/2016	8/15/2016	1/18/2017	5/16/2017
Enumeration (before cell inactivation)	NLT 1.5×10^{10} (15 billion) colony forming units per gram	Conforms	Conforms	Conforms	Conforms	Conforms
Physical Characteristics						
Appearance	Beige to White Powder	Beige to white powder	Beige to white powder	Beige to white powder	White powder	White powder
Moisture	NMT 9%	4.76%	4.96%	4.66%	4.6%	4.1%
Sieve Test	100% through 40 mesh	100% through 40 mesh	100% through 40 mesh	100% through 40 mesh	100% through 40 mesh	100% through 40 mesh
Sieve Test	80% through 80 mesh	80% through 80 mesh	80% through 80 mesh	80% through 80 mesh	80% through 80 mesh	80% through 80 mesh
Heavy Metals						
Arsenic	NMT 1 ppm	0.02 ppm	0.03 ppm	0.02 ppm	0.02 ppm	0.01 ppm
Cadmium	NMT 0.5 ppm	0.002 ppm	0.002 ppm	<0.001 (LOD)	0.002 ppm	<0.001 (LOD)
Lead	NMT 3 ppm	<0.01 ppm (LOD)	0.04 ppm	<0.01 ppm (LOD)	<0.01 ppm (LOD)	<0.01 ppm (LOD)
Mercury	NMT 0.1 ppm	<0.005 ppm (LOD)	<0.005 (LOD)	<0.005 ppm (LOD)	<0.005 ppm (LOD)	<0.005 ppm (LOD)
Microbiological Tests						
Standard Plate Count	NMT 5000 CFU/g	1700 CFU/g	1530 CFU/g	500 CFU/g	<10 CFU/g	<10 CFU/g
Yeast and Mold	NMT 100 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Total Coliforms	NMT 10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
<i>E. coli</i>	None Detected	ND	ND	ND	ND	ND

<i>Staphylococci</i> (Coag. Pos.)	None Detected	ND	ND	ND	ND	ND
<i>Salmonella</i>	None Detected	ND	ND	ND	ND	ND
<i>Listeria</i>	None Detected	ND	ND	ND	ND	ND
<i>P. aeruginosa</i>	None Detected	ND	ND	ND	ND	ND
<i>Cronobacter sakazakii</i>	Absent/100 g	—	—	ND	ND	ND

Abbreviations: CFU, colony forming unit; Coag. Pos., coagulation positive; LOD, limit of detection; ND, none detected; NLT, not less than; NMT, not more than

2.4.3 Shelf–Life Stability

A three-year shelf–life from the time of manufacture has been recommended as an appropriate expiration period for the live *B. coagulans* GBI-30, 6086 spores. This recommendation is based upon accelerated and real-time shelf-life stability studies performed to assess the stability of *B. coagulans* GBI-30, 6086. As these products are Inactivated cells of the same strain, shelf life would be at least as long as the live cells.

2.5 Physical or Technical Effect

The physical or other technical effects that Inactivated *B. coagulans* GBI-30, 6086 is intended to produce do not relate to demonstrating the safety of Inactivated *B. coagulans* GBI-30, 6086.

Part 3: Dietary Exposure

Among healthy, full-term, formula-fed infants, highest energy consumption on a kcal/kg bw basis occurs in males 14–27 days old, who consume 121 and 143 kcal/kg bw/day at the 50th and 90th percentiles, respectively.⁹ In female infants, the highest energy consumption at the 50th percentile occurs in the same age group (14–27 days; 117 kcal/kg bw/day) while the highest consumption at the 90th percentile (143 kcal/kg bw/day) occurs in the 8–13 day old group (note, this is identical to 90th percentile consumption in 14–27 day old males and only slightly higher than 90th percentile consumption in 14–27 day old females at 136 kcal/kg bw/day). Although dated, Fomon's data is grossly consistent with more recent work^a based on the 2005–2012 National Health and Nutrition Examination Survey (NHANES) using the What We Eat In America (WWEIA) food category classification system¹⁰ and the Feeding Infants and Toddlers Study (FITS) 2008.¹¹ According to the NHANES analysis by Grimes et al., mean calorie intake by infants 0–5.9 months is 612.5 ± 6.4 kcal/day while infants 6–11.9 months consume 847.3 ± 13.3 kcal/day.¹⁰ Based on the FITS 2008 analysis by Butte et al., mean and 90th percentile calorie intake was 611 ± 6.9 and 779 kcal/day, respectively, in the 0–5 month age group and 854 ± 11.3 and 1183 kcal/day, respectively, in the 6–11 month age group.¹¹ Fomon's data provides finer graduations, reporting break down of calorie intake by gender on a mg/kg basis at the 10th, 50th, and 90th percentiles from 8 days to 111 days old (approximately 3.5 months) divided into six age intervals (8–13, 14–27, 28–41, 42–55, 56–83, and 84–111 days).⁹ That Fomon's data spans only approximately 3.5 months versus data spanning 12 months provided by Grimes et al. and Butte et al. allows for a more conservative estimate as the percentage of calorie intake from infant formula declines with increasing age.¹⁰

Most cow milk-based formulas for term infants (i.e., “standard” infant formulas) provide 67 kcal/100 mL (20 kcal/fl oz.) when ready for consumption, but formula concentrates may be mixed to yield higher calorie densities up to 101 kcal/100 mL (30 kcal/fl oz.).¹² Based on an infant formula comparison chart provided by Martinez and Ballew, 67 kcal/100 mL (20 kcal/fl oz.) is the recommended target for cow milk- soy protein-, and amino acid-based, as well as modified and

^a As an illustration of the gross consistency of Fomon's data with that of the more recent estimates, considering the high 90th percentile intake of 143 kcal/kg bw/day by 14–27 day old males and 8–13 day old females and using U.S. Centers for Disease Control Infant Growth Chart Data, male body weight at the 90th percentile ranges from approximately 4.8 kg on day 15 to 5.2 kg on day 30 while female body weight at the 90th percentile is approximately 4.5 kg on day 15, total daily calorie intake would range from approximately 686.4–743.6 kcal in 14–27 day old males and approximately 643.5 kcal per day in 8–13 day old females. This appears grossly accurate compared to the Butte et al. estimate of 779 kcals/day in males and females 0–5.9 months old at the 90th percentile.

extensively hydrolyzed, formulas for healthy term infants without special medical needs.¹² Using the most conservative estimates of 143 kcals/kg bw/day (the 90th percentile in girls 8–13 days and boys 14–27 days) and 67 kcal/100 mL formula as ready to consume and assuming formula accounts for 100% of energy consumption, approximately 213.4 mL/kg bw/day of infant formula would be consumed. At the maximum addition level of 2×10^8 CFU Inactivated *B. coagulans* GBI-30, 6086/100 mL of infant formula as ready for consumption a conservative high-end EDI is 4.27×10^8 CFU Inactivated *B. coagulans* GBI-30, 6086/kg bw. Considering the lowest no-observed-effect-level (NOEL), which was 1.34×10^{11} CFU/kg bw/day (based on a concentration of 33,300 mg of the test item per kg of feed corresponding to 1948 mg/kg bw/day with a pure *B. coagulans* GBI-30, 6086 cell mass concentration of 6.88×10^{10} CFU/g), obtained from the one-year chronic repeated-dose oral toxicity study summarized in subpart 6.1.6 of this GRAS notice, this would result in a conservative margin of safety (calculated as the NOEL/EDI) of 314-fold and supports a conclusion that the expanded intended use of Inactivated *B. coagulans* GBI-30, 6086 is reasonably certain to be safe. Because only very young, exclusively formula-fed infants would be expected to consume 100% of daily energy as infant formula and because the percent of daily energy intake for formula declines with increasing age, accounting for 65.4% and 47.1% of total daily energy intake in the 0–5.9 and 6–11.9 months groups, respectively,¹⁰ the true margin of safety is expected to be even greater.

Because both the live and inactivated *B. coagulans* GBI-30, 6086 are GRAS for use in a variety of foods as described in GRN Nos. 000399 and 000670, it is possible that when solid food introduction to the infant is begun, exposure could occur from both infant formula as well as conventional food. Because energy intake from formula declines with increasing age and consumption of solid food and serving amounts of conventional foods are expected to be much smaller than typical adult serving sizes and our EDI estimates are conservative, any potential exposure from conventional foods is expected to be largely substitutive to exposure from infant formula and remain well within an acceptable margin of safety. Additionally, *B. coagulans* GBI-30, 6086 is GRAS for use in non-exempt term infant formula as described in GRN 000660, and this use is also expected to be entirely substitutive.

Part 4: Self-limiting Levels of Use

There are no known inherent self-limiting levels of use.

Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for Inactivated *B. coagulans* GBI-30, 6086 is based on scientific procedures, and as such, experience based on common use in food prior to 1958 is not considered pivotal information. Inactivated *B. coagulans* GBI-30, 6086, and to the best of our knowledge, any other *B. coagulans* preparations, were not commonly used in foods prior to 1958.

Part 6: Narrative

6.1 Toxicology Studies

Ganeden sponsored a series of independent toxicological studies to investigate the potential mutagenic activity, acute oral toxicity, subchronic and chronic repeated-dose oral toxicity, and one-generation reproduction toxicity as well as acute eye and skin irritation potential of pure *B. coagulans* GBI-30, 6086.^{13, 14} The test item used in the studies was the pure cell mass consisting solely of *B. coagulans* GBI-30, 6086. The concentration varied slightly from batch to batch and, therefore, was reported for each study. All studies were conducted in accordance with Good Laboratory Practice (GLP), and the animal studies were conducted in compliance with regulations governing animal welfare and protection and under the permission of the Institutional Animal Care and Use Committee of the performing laboratory according to SOP for animal protection (except that the micronucleus test in mice reported only that animal care was according to SOP and the animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility). The study summaries below were reproduced here, with minor revisions, from GRN Nos. 000399 and 000660.

6.1.1 Bacterial Reverse Mutation Assay

The bacterial reverse mutation assay was performed to evaluate whether *B. coagulans* GBI-30, 6086 cell mass has mutagenic properties.¹³ The study was conducted according to Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals: Bacterial Reverse Mutation Test (Guideline 471, as adopted July 21, 1997). The plate incorporation method was employed for this study with five doses in triplicate on four *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and one *E. coli* (WP2 [uvrA]) tester strains with and without an S9 activation system. Doses used in the definitive study were: 10, 50, 100, 500, and 5,000 µg per plate. The plates were read after 72 hours of incubation. Distilled water was used as a vehicle control and the appropriate positive control was used for each of the tester strains with and without S9 metabolic activation.

There were no revertants exceeding three times the background average either with or without the S9 metabolic activation system. In addition, no dose-dependent increase in revertants was observed. In conclusion, the results of this study showed that the *B. coagulans* cell mass (*B. coagulans* GBI-30, 6086) had no mutagenic effect on any strain used in this test. Furthermore, the results of the repeat assay confirmed the results of the definitive assay.

6.1.2 In Vitro Chromosomal Aberration Assay

The chromosomal aberration assay in cultured mammalian cells was conducted to investigate for any potential the test article may have for causing structural damage to either chromosomes or chromatids.¹³ Chinese hamster ovary cells (CHO-K1), and test item concentrations of 312.5, 625, 1250, 2500 and 5000 µg/mL were utilized in the study. The test item was originally obtained at a concentration of 1.93×10^{11} CFUs/g. Three treatment schemes were utilized, including incubation of the test article with cells for 3 h both with and without an S9 metabolic activation system (Schemes I and II, respectively), and incubation for 20 h without S9 (Scheme III).

Cells in the negative control group had 20 ± 2 chromosomes upon karyotypic analysis. The percentage of chromosomal aberrations measured in the negative control groups was zero. None of the dose levels of *B. coagulans* GBI-30, 6086 tested produced any statistically significant increase in aberrant cells, while the positive control groups (one micromolar mitomycin C) did induce a significant increase when compared with the negative controls as expected. Therefore, under the conditions of the assay, *B. coagulans* GBI-30, 6086 produced a negative response for induction of structural chromosomal aberrations both with and without the metabolic activation system in Chinese hamster ovary cells.

6.1.3 In Vivo Peripheral Blood Micronucleus Assay

The micronucleus test was conducted to investigate for the formation of micronuclei containing chromosomal fragments or whole chromosomes, which are indicative of cytogenetic damage.¹³ Male mice of strain BALB/dByJNarl, aged 7–8 weeks were assigned randomly to five groups of five, and were housed five animals per cage. The test item was obtained at a concentration of 1.93×10^{11} CFUs/g. Mitomycin C served as the positive control. Doses of test item included 500, 1000 and 2000 mg/kg bw/day given orally for three days.

There were no differences in body weight between the treatment groups compared to the control group and no signs of toxicity were noted in clinical observations following administration of the test item at doses of 500, 1000 and 2000 mg/kg bw/day. Animals in the positive control group showed a significant increase in the frequency of micronuclei compared to the negative controls. None of the treatment groups were positive for statistically significant induction of micronuclei in reticulocytes, and the ratio of reticulocytes to total erythrocytes in these groups showed no significant decrease compared to the negative control group. The average reticulocyte to total erythrocytes ratio in the negative control group was 3.87%. The treatment groups were 3.69%, 3.65% and 3.69% in the 500, 1000 and 2000 mg/kg bw/day groups, respectively. The positive control caused a 32.8% decrease in the ratio.

The incidence of micronucleated reticulocytes in the peripheral blood per 1000 reticulocytes was 1.8 ± 0.8 in the negative control group, which was within the historical reference range. The positive control group had a mean frequency of 31.2 ± 5.5 , which was a statistically significant increase compared with the negative control group. The test article dose groups had 1.3 ± 1.2 , 2.2 ± 1.0 and 0.9 ± 0.4 micronucleated reticulocytes per 1000 reticulocytes, at the test dose levels of 500, 1000, and 2000 mg/kg bw/day, respectively. These values were not statistically significant and, thus, did not demonstrate any signs of toxicity with administration of *B. coagulans* GBI-30, 6086 in the mouse peripheral blood micronucleus assay.

6.1.4 Acute Oral Toxicity Study in Rats

An acute oral toxicity study of a *B. coagulans* cell mass with a 14-day post-treatment observation period in rats (limit test) was performed using *B. coagulans* GBI-30, 6086 cell mass from lot number BAC-PF071, which had a concentration of 1.04×10^{11} CFUs/g per the certificate of analysis.¹³ A single oral dose of 5,000 mg/kg body weight of *B. coagulans* GBI-30, 6086 was administered by gavage to the treatment group consisting of five male and five female CRL:(WI) BR Wistar rats. Methylcellulose solution of 1% was administered to the control group (same number of animals) by oral gavage. The animals were weighed, observed for clinical signs, and checked for lethality for each of 14 days. A gross and histopathological examination was performed on day 15. The study was performed using guidelines from FDA Redbook II Draft Guidance, Acute Oral Toxicity Tests (1993) and OECD Guidelines for the Testing of Chemicals No. 423 (adopted December 17, 2001).

No mortality, adverse clinical signs, or weight-loss was observed. All animals survived until the time of the necropsy. All of the organs of the male and female rats were free from gross pathological changes related to the single oral administration of 5,000 mg/kg *B. coagulans* GBI-30, 6086.

It should be noted that when manufacturing the final product, *B. coagulans* GBI-30, 6086 is mixed with a diluent to establish a consistent concentration of 15×10^9 CFUs/g. However, for the purpose of this study, uncut *B. coagulans* GBI-30, 6086 cell mass was used. The concentration of this uncut test article (lot # BAC-PF071) was 1.04×10^{11} CFUs/g (hence 5.2×10^{11} CFUs/kg in this study), which is 6.933 times greater concentration than *B. coagulans* GBI-30, 6086—the finished product. So, while the LD-50 is greater than 5,000 mg/kg in this study, for the undiluted *B. coagulans* cell mass, it is equivalent to 34,655 mg/kg of *B. coagulans* GBI-30, 6086, the product under consideration in this GRAS notice.

6.1.5 90-day Repeated-Dose Oral Toxicity Study in Rats

The study was conducted to evaluate for repeated oral toxicity of *B. coagulans* GBI-30, 6086 cell mass in rats over a 13-week period and to characterize the potential adverse effects of the substance and indicate target organs.¹³ The study was performed using guidelines from FDA Redbook II Draft Guidance, Short-term Toxicity Studies with Rodents (2003) and OECD Guidelines for the Testing of Chemicals No. 408 (adopted September 21, 1998). The test item was administered orally by gavage to CRL:(WI) BR Wistar rats for 90 consecutive days. Dose levels administered were as follows: 0 mg/kg body weight per day (vehicle control), 100, 300, and 1000 mg/kg body weight per day in both male and female animals. The test item was suspended in 1% aqueous methylcellulose solution. The treatment volume was 10 mL/kg body weight in each group.

General clinical observations were made once daily. Detailed clinical observations were made prior to the first exposure and once per week thereafter. Behavioral (spontaneous activity, motor affective responses, sensori-motor responses), neurological (posture, muscle tone, equilibrium and gait, central nervous system excitation) and autonomic (eye, secretion, excretion) functions were observed. Functional Observation Battery was performed on the 13th week of the treatment. Body weight and food consumption were measured weekly. Blood sampling for clinical pathology and gross pathology were conducted at the end of the treatment period. Selected organs were weighed. Gross and histopathological examinations were performed on fixed organs and tissues from the control and highest dose group (and middle dose groups as appropriate).

No mortalities were observed throughout the study. The daily general observations and the weekly detailed observations did not result in test item-related clinical signs. In evaluation of the Functional Observation Battery results, only one individual variation was found without a trend.

The mean body weight of the males in the 100 mg/kg group was below that of the controls on day 50, and from days 71 to 90 in the study. The difference was 6–7% lower than the control groups, but was not considered related to the test item because of a lack of a dose response. The mean body weight of the males in the 1000 mg/kg group was lower than the control groups, in this case, from days 22–90. No statistically significant differences in body weight were noted in the male or female rats in the 300 mg/kg group, when compared to the control. Similar effects were not observed in the female rats in any of the treatment groups.

Average daily food consumption was similar in all groups except for a slightly lower mean value ($p < 0.05$) with the males in the highest dose group during week 8, and a slightly higher mean value ($p < 0.01$) with the females in the highest dose group during week 6. Water consumption for all of the male dose groups was similar when compared with the controls. Statistically significant lower water consumption ($p < 0.05$) was noted in the females in the 100 mg/kg group during

days 57–58 and 88–89, and in the females in the 300 mg/kg group ($p < 0.05$) on days 57–58, 85–86 and 88–89. Females in the 1000 mg/kg dose group also had decreased water consumption, but only during days 57–58 ($p < 0.01$) and 88–89 ($p < 0.05$).

Some statistically significant differences were observed from the results of the hematology and clinical chemistry parameters tested. However, since they fell into the historically normal range for the laboratory, it was concluded that no clinically relevant test item-related hematological or clinical chemistry changes were observed in either the male or female rats receiving *B. coagulans* GBI-30, 6086 at 100, 300, or 1000 mg/kg/day.

Gross pathological evaluation at the end of the study revealed several cases of pinprick-sized hemorrhages and pale, pillow-like raised areas in the lungs in all groups, which were likely caused by exsanguination of the animals. No treatment-related histopathological findings were noted upon examination of the animals at the end of the study.

Absolute organ weights differed only in the brains of the males in the 1000 mg/kg group (lower), the liver in the 100 and 1000 mg/kg groups (lower), and the testes in the 100 mg/kg group (lower). However, the differences were considered to be the consequence of the lower mean body weights of these groups. Importantly, the relative organ weights compared to body weights did not differ for these organs.

The relative kidney weight was lower than the control in the males in the 300 mg/kg group, and higher in the males in the 1000 mg/kg group. The changes were not considered to be of biological significance or related to the test item, most importantly because they were not corroborated with any histological findings. The relative weight of the adrenal glands was lower than the control group for the females in the 300 mg/kg group, but was due to individual variation and not considered related to administration of the test item because of a lack of a dose response.

No toxicologically significant differences between the treated groups (100, 300 and 1000 mg/kg bw/day) and the controls were observed with respect to food consumption, water consumption, sensory reactivity, general and behavioral conditions, hematological and clinical chemistry evaluations. *B. coagulans* GBI-30, 6086 caused neither treatment-related macroscopic or microscopic signs nor changes in the organ weights of the male and female rats at 100, 300 and 1000 mg/kg/day after the 13-week treatment period. The test item was well tolerated.

Since there were no signs of toxicity noted with respect to gross or histopathological examinations, nor with hematology, clinical chemistry, or organ weights for the 1000 mg/kg dose group, the differences in the mean body weight of the males described above is not considered related to the test item, but rather a result of biological variation. Hence, the NOAEL for both males and females is

considered to be 1000 mg/kg body weight per day, which was the highest dose tested.

As described above, it should be noted that in manufacturing of the final product, *B. coagulans* GBI-30, 6086 is diluted with maltodextrin, microcrystalline cellulose and/or sodium bicarbonate to a concentration of 15×10^9 CFUs/g. For the purpose of this study, undiluted *B. coagulans* cell mass was used. The concentration of this undiluted test item (lot # BAC-DVSA-BCO- 001-PG043) was 1.36×10^{11} CFUs/g, which is 9.07 times greater concentration than *B. coagulans* GBI-30, 6086. So, the NOAEL of 1,000 mg/kg for the undiluted *B. coagulans* cell mass used in this study is equivalent to 9,070 mg/kg of *B. coagulans* GBI-30, 6086.

6.1.6 One-year Chronic Repeated-Dose Oral Toxicity Study Combined with a One-generation Reproduction Toxicity Study

Introduction

This study was conducted to evaluate the potential toxicity of chronic oral consumption of *B. coagulans* cell mass as *B. coagulans* GBI-30, 6086, and to assess for any reproductive toxicity, as well as to confirm the findings of the 90-day repeated-dose oral toxicity study in the rat described above.¹⁴ The study was designed and conducted according to the guidelines suggested by the Toxicological Principles for the Safety Assessment of Food Ingredients (USFDA Office of Food Additive Safety Redbook 2000) IV.C.5a. Chronic Toxicity Studies with Rodents (July, 2007) and IV.C.9a Guidelines for Reproduction Studies (July, 2000) and OECD Guidelines for the Testing of Chemicals, No. 452 (adopted May 12, 1981).

Table 3. Test item dose levels for the main group (20 animals/sex/group)

Group	Test article concentration in diet (ppm)		Target dose level mg/kg bw/day	Actual dose level (mg/kg bw/day)	
	Nominal	Real		Male	Female
1	0	0	0	0	0
2	10 000	10 000	600	585	761
3	20 000	20 000	1200	1146	1467
4	33 300	33 300	2000	1948	2526

Table 4. Test item dose levels in the satellite group (10 males and 20 females per group)

Group	Test item concentration in diet (ppm)		Target dose level mg/kg bw/day	Actual dose level (mg/kg bw/day)	
	Nominal	Real		Male	Female
1	0	0	0	0	0

2	10 000	10 000	600	715	1079
3	20 000	20 000	1200	1348	2082
4	33 300	33 300	2000	2372	3558

The concentration, homogeneity, and viability of the test item were analyzed for each of the doses for each batch of feed produced throughout the study. Each lot of feed produced was found to be viable, met or exceeded the target dose level, and were homogeneous throughout the lots produced.

Hsd.Brl.Han Wistar rats between 7–9 weeks of age were used for the study. The animals were housed individually in type II polypropylene/polycarbonate cages. The rats were acclimatized for 14 days prior to the start of the study. Only healthy animals, free from any clinical signs were used.

For the main study, 20 animals per sex per group were randomized. For the reproduction study, satellite groups consisting 10 males and 20 females were randomized. For mating, a ratio of one male to one female was used with a three hour per day mating time each morning for three weeks. The presence of a vaginal plug or sperm indicated the day of mating, which was considered day 0 of the pregnancy.

General clinical observations were made once daily at roughly the same time each day. Detailed clinical observations for the main group were made prior to the start of the study and then once weekly in an arena outside of the home cage through to the end of the study. Observations included: skin, fur, eyes, mucous membranes, lachrymation, piloerection, pupil size, respiration, circulation, central nervous system, somato-motor system activity, behavior, gait, posture, response to handling, as well as particular attention for the observation of any tremors, convulsions, excessive salivation, diarrhea, lethargy, sleep, and coma.

All animals were weighed at the time of randomization and day 0 of the study. Body mass was recorded weekly for the first 13 weeks and then monthly for the duration of the study.

In the satellite groups, male rats were weighed weekly until necropsy. The females in the same group were weighed weekly through mating and then on gestation days: 0, 7, 14, and 21 as well as on lactation days: 0, 7, 14, and 21. The live F1 generation pups were counted and weighed on the morning after birth and on days 4 and 7, and then weekly thereafter up to weaning (postnatal day 21). All pups found dead were subject to necropsy.

Food was weighed weekly and the daily average food consumption per rat was calculated.

In the reproduction study, the females were allowed to litter and rear their offspring. The development of the pups was assessed by the surface righting reflex, pinna detachment and eye opening.

Clinical testing consisted of: ophthalmoscopy, pathology, hematology, coagulation studies, clinical chemistry, and urinalysis.

Pathological examination began with necropsies on week 53. The animals were observed for external appearance of the cranial, thoracic, and abdominal cavities. The adrenal glands, aorta, femur bone marrow, brain, eyes, mammary gland (females), gonads (both sexes), gross lesions, heart, kidneys, large intestine, small intestine, liver, lungs, lymph nodes (submandibular and mesenteric), quadriceps muscle, esophagus, pancreas, pituitary gland, prostate, submandibular salivary gland, sciatic nerve, seminal vesicle, skin, spinal cord (cervical, mid-thoracic, lumbar), spleen, sternum, stomach, thymus gland, thyroid gland, parathyroid gland, trachea, and the urinary bladder were preserved for histopathological examination.

The liver, brain, heart, thymus, spleen, kidneys, testes, epididymides, uterus, thyroid, parathyroid, adrenal glands, and ovaries were weighed. Paired organs were weighed together.

Complete histopathological examination was performed in the control and the highest dose group tested in the main study (groups 1 and 4 respectively). The following organs of all male and females parent animals in the satellite group were examined histopathologically: ovaries, uterus, cervix, vagina, testes, epididymides, seminal vesicles, prostate, coagulating gland, and pituitary gland.

Main Study

There was no test item-related mortality. One female animal of 1,200 mg/kg bw/day was found dead on day 137 as a consequence of an individual disorder, which was concluded on the basis of clinical observations. Gross pathology and histopathological evaluation was not possible because autolysis of the organ systems had occurred prior to discovering the dead animal. Two female animals (one in the control group and one in the 2,000 mg/kg bw/day group) were euthanized during the treatment period because of their moribund condition. Gross pathological and histopathological examinations revealed individual disease in both animals such as diffuse subacute dermatitis resulting in cachexia, and generalized fibrosarcoma, respectively. This was not considered due to the test item.

General daily and detailed clinical observations did not reveal any toxic signs related to the test item. Any clinical signs observed occurred mainly in female animals (irritability, decreased body tone and grip tone, decreased righting reflex, alopecia and scars on the skin, sanguineous eye with swelling) were with low incidence. These observations are seen occasionally in experimental rats and were not related to the doses in this study.

No test item-related body weight, or body weight gain changes were observed during the study. The mean daily food consumption was similar in the control and test item treated groups.

There were no eye alterations observed in any of the groups in the study. Laboratory examinations did not reveal any test item-related pathological changes in the evaluated hematological, clinical chemistry or urine parameters at the end of the 3rd week, as well as the 3rd, 6th or 12th months. Changes observable in all groups of treated male and female rats were not toxicologically relevant as alterations noted were either not related to administered dose, lying well within the historical background range or were not correlated with other hematological or histopathological alterations.

Macroscopic alterations observed during terminal necropsy were comparable in both the treatment groups and the control animals, and could not be attributed to administration of the test item.

There were no test item-related changes in the examined organ weights. Histopathological evaluation of the organs of the experimental animals did not reveal lesions attributable to the effect of test item.

In conclusion, *B. coagulans* GBI-30, 6086 caused no signs of toxicity in male or female Hsd.Brl.Han: Wistar rats after one year of diet-mixed administration.

Based on the observations made in this dietary toxicity study, the dietary No Observed Effect Level (NOEL) was determined to be 33,300 ppm for male and female Hsd.Brl.Han: Wistar rats which corresponds to a mean daily intake of:

- NOEL for male rats: 1948 mg/kg bw/day (mean value)
- NOEL for female rats: 2525 mg/kg bw/day (mean value)

Reproduction study

No animals of parental generation died during the observation period. There were no test item-related effects on the general state and behavior of parental animals during the pre-mating, mating, gestation and lactation periods.

The body weight and body weight gain of male and female parental animals was unaffected at the examined dose levels during the pre-mating period. There was no effect on body weight or body weight gain of the dams during the gestation and lactation period at the examined dose levels.

No differences were seen in food consumption in males of all three treatment groups during the pre-mating and post-mating periods, or in females of all treatment groups during the pre-mating, gestation and lactation periods, when compared to controls.

There was no test item influence on the estrous cycle, mating, fertility, or gestation period or for the delivery of dams when compared with the control animals. Reproductive performance of males and females were unaffected by treatment with test item.

Macroscopic observations of organs and tissues did not reveal alterations due to the effect of the test item in P generation. No test item-related organ weight changes were found in parental male or female animals.

No histopathological alterations related to the test item effect were found. In the male animals, the investigated organs of the reproductive system (testes, epididymides, seminal vesicles, prostate, coagulating gland) were histologically normal. In dams, the ovaries had a normal structure characteristic of the species, age and phase of the active sexual cycle. The uterus, cervix, and vagina had a normal structure in accordance with the phase of sexual cycle in the investigated animals.

No test item-related alterations were found in the F1 generation. Viability and lactation indices and sex ratios were similar among the control and treated groups. A statistically significant difference in mortality compared to controls appeared in the low-dose pups between postnatal days 7 and 14 and was not considered toxicologically relevant or related to administration of the test item due to the lack of a dose-response and the low magnitude of the difference. Clinically and biologically irrelevant but statistically significant minor increases in body weight and body weight gain in treated pup groups compared to controls were observed and were not related to dose (postnatal days 7-14-21). Therefore, these slight variations were not considered to be adverse or biologically significant outcomes. There were no statistically significant and/or clinically significant differences in clinical observations and postnatal development of the treated F1 pups compared to controls.

No test item-related alterations were observed on gross pathological examination of stillborns and pups found dead during the observation period. Necropsy observations were of normal physiological phenomena in fetuses/pups that occurred as spontaneous findings and with similar frequency among treated and control group pups and were recorded to determine whether dead newborns were live-born or stillborn. Pups that were cannibalized prior to discovery could not undergo necropsy; cannibalization occurred with similar frequency among treated and control groups and was not dose-related. Fetuses/pups were stillborn, found dead, and cannibalized, respectively, as follows: 2, 0, and 3 of 179 (controls); 4, 1, and 11 of 164 (low-dose); 2, 2, and 6 of 184 (mid-dose); and 0, 2, and 4 of 206 (high-dose).

In conclusion, *B. coagulans* GBI-30, 6086 caused no signs of toxicity in the parental generation (male or female) of Hsd.Brl.Han:Wistar rats during the course of this one generation reproductive toxicity study. Reproductive performance of

males and females were unaffected by the treatment and there was no effect on mortality or postnatal development of pups.

Based on the observations, the dietary No Observed Effect Level (NOEL) for the reproductive study was determined to be 33,300 ppm for male and female Hsd.Brl.Han:Wistar, which corresponds to a mean daily intake of the following:

- NOEL for parental male rats: 2372 mg/kg bw/day (mean value)
- NOEL for parental female rats: 3558 mg/kg bw/day (mean value)
- NOEL for reproductive performance of male rats: 2372 mg/kg bw/day (mean value)
- NOEL for reproductive performance of female rats: 3558 mg/kg bw/day (mean value)
- NOEL for F1 Offspring: 3558 mg/kg bw/day (mean value)

The NOEL for F1 offspring was considered as identical to that determined for the dams—3558 mg/kg bw/day (mean value) (equivalent to 2.45×10^{11} CFU/kg bw/day). However, due to the very low body weight of pups with respect to the dams, a very high, albeit transient, exposure to the test item can be presumed from the pups' sharing of the maternal feed when the pups started self-feeding (postnatal day 14).¹⁵ Beyond the data provided by the genotoxicity, general oral toxicity, and reproductive performance evaluations, the postnatal observations in the F1 generation of the reproduction toxicity study is of some additional importance to the consideration of use of *B. coagulans* GBI-30, 6086 in infant formula.

6.1.7 Eye Irritation Study in Rabbits

An acute eye irritation study was performed in New Zealand White rabbits using undiluted *B. coagulans* GBI-30, 6086 cell mass from lot number BAC-DVSA-BCO-001-PG044, which had a concentration of 1.93×10^{11} CFUs/g per the certificate of analysis.¹³ The study concluded that *B. coagulans* GBI-30, 6086 applied to the mucosa of the rabbit's eyes resulted in a slight to moderate conjunctival irritant effect that was fully reversible within 72 hours. According to EC criteria for classification and labeling requirements for dangerous substances and preparations, obligatory labeling of the test item is not required with regard to eye irritation.

6.1.8 Skin Irritation Study in Rabbits

An acute skin irritation study was performed in New Zealand White rabbits using the undiluted *B. coagulans* cell mass (*B. coagulans* GBI-30, 6086) from lot

number BAC-DVSA-BCO-001-PG044, which had a concentration of 1.93×10^{11} CFUs/g per the certificate of analysis.¹³ The irritation effect of the test item was evaluated according to the Draize method (OECD 404, 2002). According to EEC directive 2001/59/EEC the test article is not classified as irritating to the skin. The observed clinical sign of very slight erythema on the treated skin surface was evaluated as fully reversible.

6.2 Neonatal Exposure to Bacteria

During the first months of life, barriers to bacterial colonization of the gut and translocation through the intestinal wall are immature compared to those found in childhood and older individuals. In addition the intestine is sterile at birth and is colonized rapidly as infants are exposed to microorganisms present in their environment (e.g., birth canal and maternal feces during non-cesarean birth, skin contact, breast milk, surrounding environment).^{16, 17} For example, human milk may contain up to 13 million CFU/mL of a variety of bacterial species.¹⁸ Thus, from an evolutionary perspective, infants are evolved to respond to oral exposure to a wide variety of pathogenic bacteria and other microorganisms without adverse outcomes, and in fact, this early exposure supports the development of a healthy gut barrier and host immune response.¹⁹ Furthermore, available data suggest that a stable intestinal microflora develops quickly in breastfed infants and that the introduction of solid food and/or infant formula (containing no added bacteria or prebiotics) and weaning lead to a qualitative and quantitative change in this microflora (although the bifidobacterial component remains stable while populations of other species increase).²⁰⁻²²

Additionally, a number of other bacterial species have been determined GRAS for use in infant formula. In our searches of FDA's GRAS Notice Inventory Database, nine GRAS notices pertaining to probiotic use in infant formula were located. These are summarized in the table below:

Table 5. FDA GRAS Notices for Probiotic use in Infant Formula

GRN No.	Organism	Intended Use	Addition Level*	EDI	Findings	Outcome / Date
49 ²³	<i>Bifidobacterium lactis</i> Bb12	Ingredient in milk-based infant formula by infants ≥ 4 mos old	10^6 – 10^8 CFU/g	Not calculated	No adverse events were reported	FDA has no comments 3/19/2002
49 ²³	<i>Streptococcus thermophilus</i> Th4	Ingredient in milk-based infant formula by infants ≥ 4 mos old	10^6 – 10^8 CFU/g	Not calculated	No adverse events were reported	FDA has no comments 3/19/2002

GRN No.	Organism	Intended Use	Addition Level*	EDI	Findings	Outcome / Date
231 ²⁴	<i>Lactobacillus casei</i> subsp. <i>rhamnosus</i> GG	Ingredient in term infant formulas	10 ⁸ CFU/g	10 ⁸ –10 ¹⁰ CFU/day	No adverse events were reported	FDA has no comments 5/29/2008
268 ²⁵	<i>Bifidobacterium longum</i> BB536	Ingredient in milk-based powdered follow-on infant formulas for term infants ≥ 9 mos old	10 ¹⁰ CFU/g	12 x 10 ¹⁰ CFU/day for 9-12 month olds; 9.2 x 10 ¹⁰ CFU/day for 12–23 month olds	No evidence for a potential health hazard	FDA has no comments 7/8/2009
281 ²⁶	<i>Lactobacillus rhamnosus</i> HN001	Ingredient in milk-based powdered term infant formula and follow-on formula	10 ⁸ CFU/g (1.35 x 10 ⁹ CFU/100 mL ready to consume)	10 ⁹ –10 ¹⁰ CFU/day	No adverse events in studies relevant to human consumption were reported	FDA has no comments 8/31/2009
410 ²⁷	<i>Lactobacillus reuteri</i> DSM 17938	Ingredient in powdered whey-based term infant formula	10 ⁶ –10 ⁸ CFU/g	a	No adverse events were reported	FDA has no comments 3/26/2012
454 ²⁸	<i>Bifidobacterium breve</i> M-16V	Ingredient in non-exempt powdered term infant formula containing partially hydrolyzed milk or soy proteins	10 ⁸ CFU/g	9.9 x 10 ⁹ CFU/day for one month old; 1.35 x 10 ¹⁰ CFU/day for 6 month old	No adverse events were reported	FDA has no comments 9/27/2013
455 ²⁹	<i>Bifidobacterium breve</i> M-16V	Ingredient in exempt term powdered amino acid-based infant formulas	10 ⁸ CFU/g	9.9 x 10 ⁹ CFU/day for one month old; 1.35 x 10 ¹⁰ CFU/day for 6 month old	No adverse events were reported	FDA has no comments 9/30/2013

GRN No.	Organism	Intended Use	Addition Level*	EDI	Findings	Outcome / Date
531 ³⁰	<i>Lactobacillus fermentum</i> CECT5716	Ingredient in powdered milk-based infant formulas	10 ⁷ CFU/g	2 x 10 ⁸ CFU/day	No adverse events were reported in animal and human studies; a single case report was noted for an adverse event in an immunocompromised individual	FDA has no comments 3/20/2013
660 ⁷	<i>Bacillus coagulans</i> GBI-30, 6086	Ingredient in non-exempt powdered and liquid infant formulas for term infants	2 x 10 ⁸ CFUs per 100 mL infant formula as ready for consumption	4.27 x 10 ⁸ CFU <i>B. coagulans</i> GBI-30, 6086/kg bw/day	No adverse events were reported in animal and human studies	FDA has no comments 3/20/2013

*According to the reviewed GRNs, approximately 14 grams powdered formula is used to reconstitute 100 mL of ready to consume formula (FDA notes this amount varies slightly).

6.3 Studies on *Bacillus coagulans* in Infants

In addition to the lack of adverse effects of *B. coagulans* in human clinical trials in adults reported in GRN 000399 on page 19 under the subheading Additional Support of Safety and incorporated herein by reference, new literature searches were conducted as part of GRN 000660 in order to investigate results of studies conducted specifically in infants and their summaries are reproduced here with minor revisions. An additional literature search conducted on July 18, 2017 did not find any additional relevant studies published since the submission of GRN 000660. A total of four randomized blinded placebo-controlled clinical trials in which infants were administered *B. coagulans* were located. None of the trials reported any treatment-related adverse effects. These trials are summarized with respect to safety aspects below (note that four of the five trials summarized below

refer to *B. coagulans* as *Lactobacillus sporogenes*; this miscalling of *B. coagulans* is common within the probiotic industry^{b,31}).

The use of *B. coagulans* (miscalled by the authors of this study as *Lactobacillus sporogenes*^b) for prevention of necrotizing enterocolitis (NEC) in very low birth weight infants (preterm infants <33 weeks gestational age or birth weight <1500 g who survived to feed enterally) was investigated.³² Infants of the treatment group were given a daily bolus of 3.5×10^8 CFU *B. coagulans* mixed in breast milk or mixed feeding (breast milk and formula) while the control group received breast milk or formula only (as the addition of treatment powder did not change the appearance of the test item, which was provided to subjects' caregivers under a blinded protocol). Treatment was initiated on the first feed and continued until discharge from the neonatal intensive care unit. Two hundred twenty-one infants completed the study and adverse outcomes (with the exception of feeding intolerance, which was significantly lower in the active treatment group) occurred with similar incidence in both groups. No adverse events attributable to the test item occurred during the study. While sepsis occurred with similar incidence in the active treatment and control groups (23.4 vs 26.4%, respectively, $p=0.613$), the pathogens were mostly catheter-related and *B. coagulans* was not found in any of the cultures.

One hundred twelve healthy term newborns were randomized to receive either 1×10^8 CFU *B. coagulans* (miscalled by the authors of this study as *L. sporogenes*^b) or placebo daily for 12 months in a clinical trial to investigate the potential preventive effect of the probiotic on acute diarrheal disease, a common cause of infant mortality in India.³³ No adverse effects, withdrawals, or loss to follow up were reported, and while there was a trend for body weight at one year to be higher in the treated group, this was not statistically significant.

Another trial investigated the use of *B. coagulans* spores^c (miscalled by the authors of this study as *L. sporogenes*^b but also acknowledged in the paper's

^b As mentioned above, *Bacillus coagulans* was first described by Hammer in 1915. In 1933 Horowitz-Wlassowa and Nowotelnow isolated and described *Lactobacillus sporogenes*. Later, *L. sporogenes* was reclassified as *B. coagulans* based on shared characteristics, and this reclassification is well-known. In Bergey's Manual of Determinative Bacteriology, 8th edition, it is noted that spore-bearing rods producing lactic acid, facultative or aerobic and catalase positive are to be classified within the genus *Bacillus*. Unfortunately, the use of *L. sporogenes* still widely persists in the dietary supplement marketplace and also in the scientific literature.

^c Of the four clinical trials summarized here, this is the only one to specifically state spores were used; however, we are unaware of any application ever administering, or the availability of, live *B. coagulans* in other but the spore form. And as it would be highly unusual for live *B. coagulans* to be administered in the germinating form, we would

Introduction as *B. coagulans*) versus placebo for acute watery diarrhea in 148 male subjects 6–24 months old.³⁴ Subjects received 1.2×10^8 CFU or placebo twice daily (2.4×10^8 daily) for up to five consecutive days or recovery, whichever occurred first. No adverse events or complications were observed during the treatment period or the 15-day no-treatment follow-up period.

No adverse events were reported in a published abstract summarizing a trial in which 19 infants (< 1 year of age; mean 5.5 months) with gastroesophageal reflux were given *B. coagulans* (dose not reported) for seven consecutive days.³⁵

6.4 Virulence and Toxicity

As shown in GRN 000399 under the subheading Additional Support of Safety on pages 18–19 and incorporated herein by reference, US FDA, Health Canada, and EFSA recognize *B. coagulans* as a non-pathogenic, non-toxicogenic organism. Furthermore, EFSA's granting of qualified presumption of safety status to *B. coagulans* beginning in 2007 and maintained through the current 2016 list, in addition to affirming non-pathogenicity, provides that the agency has no current safety concerns for the organism's intended uses in foods so long as the strain satisfies the qualifications of absence of toxigenic potential (e.g., toxins that can be produced in food and enterotoxins) and acquired antibiotic resistance genes.³⁶⁻⁴⁵

6.4.1 *Bacillus coagulans* GBI-30, 6086

An unpublished PCR assay, as reported in GRN 000399 on page 19 under the subheading Additional Support of Safety and incorporated herein by reference, showed no evidence that *B. coagulans* GBI-30, 6086 contains genes that encode for enterotoxins or hemolysins.

Since the submission of GRN 000399, additional work, based on the complete genomic sequence of *B. coagulans* GBI-30, 6086 has been carried out confirming and adding to this evidence.⁴ In this study, putative virulence factors (VF) were identified according the method of Chen et al.⁴⁶ using a local Protein-protein Basic Local Search Tool (BLASTP) with protein sequences derived from the *B. coagulans* GBI-30, 6086 annotated genes to query the Virulence Factor Database (VFDB). Basic Local Alignment Search Tool (BLAST) results were considered in the study if they demonstrated greater than 30% identity and 70% coverage.

Genes encoding for the hemolysin BL (HBL complex; hblC, hblD, hblA and hblB: AJ007794), the non-hemolytic enterotoxin NHE (NHE complex; nheA, nheB and

expect that so doing would be cause for specific discussion in the study. Therefore, administration of spores is presumed in all studies reported.

nheC: Y19005), the enterotoxin T (bceT; D17312), the cytotoxin K (cytK; AJ277962), and the cereulide (cesA, cesH, cesP, cesT, cesB, cesC, cesD; DQ360825) were searched for on the genome of *B. coagulans* GBI-30, 6086 using BLASTX in order to further evaluate the potential for presence of enterotoxin genes. Additionally, searches were conducted to evaluate the presence of genes involved in the synthesis of lipopeptides as fengycin (fenA, AF023464; fenB, Appl Microbiol Biotechnol BACFENB; fenD, CAA09819; fenE, AF023465), surfactins (srfAA, D13262; srfAB, AF233756; srfAC, CAB12145), and lychenisin (lchAA, lchAB, lchAC; AJ005061).

B. coagulans GBI-30, 6086 was grown in the presence of arginine, histidine, lysine, ornithine, putrescine and tyrosine, and supernatants were obtained for HPLC determination and quantification of biogenic amine production according to the methods of Martuscelli et al., Tabanelli et al., and Tabanelli et al.,⁴⁷⁻⁴⁹ respectively. BLASTX was also searched against the genome of *B. coagulans* GBI-30, 6086 for the presence of genes related to biogenic amines.

The majority of the 200 putative VFs identified in the genome of *B. coagulans* GBI-30, 6086 using VFDB were defensive (multidrug transports and resistance proteins, a peroxidase, and an alkyl hydroperoxide reductase) or non-classical VFs related to normal cellular activities (Clusters of Orthologous Groups (COG) database (<http://www.ncbi.nlm.nih.gov/COG/>)). The majority of putative VFs were related to extracellular structures and may represent beneficial traits for adhesion to host cells or the sporulation mechanism (VFDB). Furthermore, possible VFs with relatively low similarity to known VFs can be detected by BLAST similarity. Therefore, the putative VFs identified in the genome of *B. coagulans* GBI-30, 6086 were not considered to be harmful.

B. coagulans GBI-30, 6086 does not carry any known enterotoxin or hemolysin genes as determined by the BLASTX analysis. Additionally, the genome of *B. coagulans* GBI-30, 6086 was determined not to contain genes encoding for the harmful cyclic lipopeptides—surfactins—or other lipopeptides with toxin activity, such as fengycin and lychenisin.

The HPLC analysis for biogenic amines demonstrated that *B. coagulans* GBI-30, 6086 did not produce tyramine, histamine, putrescine, cadaverine and phenylethylamine, and the polyamines, spermine and spermidine under the conditions of the assay. In the genomic analysis, with the exception of genes encoding for the metabolic pathways from arginine to putrescine and putrescine to spermidine, genes related to the production of biogenic amines were absent. Because the corresponding biogenic amines related to the identified pathways were not produced, it is presumed the genes were either not functional or not expressed at a sufficient level for production of detectable amounts.

6.4.2 *Bacillus coagulans* in general

The D(-)-lactate isomer of lactic acid is not produced in human metabolism, but human exposure can occur from bacterial production.¹⁷ Because human cells do not efficiently metabolize and excrete D(-)-lactate and infants have immature renal systems and decreased intestinal barrier function, newborns and neonates are at elevated risk of developing D-lactic acidosis if exposure occurs. *B. coagulans* does not produce D(-)-lactate.^{50, 51}

With the exception of the members of the *Bacillus cereus* group (e.g., *B. cereus*, *B. anthracis*, *B. thuringiensis*), the virulence of members of the *Bacillus* genus may be considered very low, and identified risk factors for *Bacillus* bacteremia include drug addiction, hemodialysis, and leukemia (all of which may contribute to immunosuppression).⁵² Based on two retrospective studies investigating *Bacillus* bacteremia, the presence of central venous catheters may increase risk of *Bacillus* bacteremia in immunocompromised patients.^{52, 53}

Of 1038 bacteremias occurring in patients of the University of Maryland Cancer Center, Baltimore between January 1978 and June 1986, 24 episodes of *Bacillus* bacteremia were documented, yet only a single case was documented as *B. coagulans* bacteremia.⁵³ The classification of the *B. coagulans* bacteremia as a definite clinical infection or possible infection was not reported nor were the specific patient details (e.g., source of infection, signs, symptoms, outcomes). Based on the reported results and previous reports cited and discussed by the authors, no other cases of infection or possible infection from *B. coagulans* have been documented in the English literature dating to the 1950s, and the majority of cases of *Bacillus* bacteremias were due to *Bacillus cereus*. Thus, *B. coagulans* may be considered non-virulent.

6.5 Antibiotic Resistance

Phenotypic and genomic-sequencing analyses for antibiotic susceptibility/resistance of *B. coagulans* GBI-30, 6086 have been conducted with attention to transferable antibiotic resistance.⁴ Minimum inhibitory concentrations (MIC) of 15 antibiotics (ampicillin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, kanamycin, linezolid, neomycin, rifampicin, streptomycin, tetracycline, trimethoprim, vancomycin and virginiamycin) were determined according to standard protocol ISO 10932:2010 (IDF 223:2010). Putative antibiotic resistant genes were identified according to the method of McArthur et al.⁵⁴ using BLASTP with protein sequences derived from the *B. coagulans* GBI-30, 6086 annotated genes to query the Comprehensive Antibiotic Resistance Database (CARD) (<http://arpcard.mcmaster.ca>). ProphageFinder (<http://bioinformatics.uwp.edu/~phage/DOEResults.php>) was used to identify putative prophage sequences, and CRISPRFinder⁵⁵ was used to identify CRISPRs.

The results of the phenotypic analysis were compared to MIC cut-off values for *Bacillus* species as defined by EFSA,⁵⁶ and *B. coagulans* GBI-30, 6086 was found susceptible to 13 of the 15 antibiotics tested as follows: ampicillin (0.125 mg/L), chloramphenicol (0.25 mg/L), ciprofloxacin (0.03 mg/L), clindamycin (0.125 mg/L), erythromycin (0.125 mg/L), gentamycin (0.031 mg/L), linezolid (0.06 mg/L), neomycin (2 mg/L), rifampicin (0.016 mg/L), tetracycline (0.25 mg/L), trimethoprim (0.063 mg/L), vancomycin (0.063 mg/L) and virginiamycin (0.016 mg/L). *B. coagulans* GBI-30, 6086 was resistant to the two of the 15 antibiotics, kanamycin and streptomycin, with MICs greater than 1500 mg/L.

The majority, 57, of the 109 putative antibiotic resistance genes identified in the genomic analysis were transporters. The remaining are broken down as follows: genes modulating the antibiotic efflux (9); genes associated with resistance to daptomycin (6), polymyxin (1), streptothricin (1), penicillin (5), vancomycin (13), elfamycin (1), rifampin (2), sulphonamide (1), macrolides (as erythromycin, streptogramin and chloramphenicol) (2), fluoroquinolone (2), aminocoumarin (2), and trimethoprim (1); other genes related to a non-specified antibiotic resistance (4); and aminoglycosides (2).

Because the resistance exhibited in the phenotypic analysis was confined to aminoglycoside antibiotics, these two genes were further evaluated while it was assumed that the additional genes retrieved in silico were not functional, not expressed at sufficient levels, or only partially similar to known antibiotic resistance genes, and these genes were, therefore, not considered to represent harmful traits of *B. coagulans* GBI-30, 6086. The two identified aminoglycoside resistance genes were IE89_07115 and IE89_03650.

IE89_07115 encodes for the ribosomal protein S12 of subunit 30S, and the authors reported that the mechanism of aminoglycoside resistance “can be mediated by 16S rRNA methylases and methyltransferases or intrinsic mechanisms as chromosomal mutations.” Because no active rRNA methylases and only a single active 16S rRNA methyltransferase, lacking similarity to genes involved in aminoglycoside resistance, was retrieved by CARD in the *B. coagulans* GBI-30, 6086 genome, intrinsic resistance due to mutation was assumed. The genomic regions surrounding IE89_07115 lack mobile elements and the gene is co-localized with other genes that encode for essential chromosomal genetic information. Based on the above factors, the region was considered highly stable and the risk of gene transfer was considered low.

IE89_03650 encodes for an aminoglycoside 3-*N*-acetyltransferase as determined by the CARD query. Flanking regions lack mobile elements and show that IE89_03650 is co-localized with a gene encoding for a multidrug transporter MatE (an organization detectable in all available *B. coagulans* genomes cataloged in NCBI). Again, these factors indicate a very low risk of transferability of this gene.

Nine complete transposase-encoding genes were identified in the genome of *B. coagulans* GBI-30, 6086 using the NCBI Prokaryotic Genomes Annotation Pipeline; however, their flanking regions were not associated with antibiotic resistance or other putatively adverse genes. Two prophage-like elements were identified using ProphageFinder; however, these were considered defective and non-functional phages as the essential tail tape measure protein gene was not present, and *attL* and *attR* sites in both prophage regions were not identified. Three CRISPR arrays were identified. Two were located in adjacent contigs, suggesting the possibility that they are part of the same array, and five CRISPR-associated (cas) proteins near the CRISPR *locus* on the first contig were also identified. The third CRISPR array was located in a contig with no proteins annotated. The presence of a CRISPR system represents a barrier to entry of foreign DNA, and may promote genome stability, including limiting of the spread of antibiotic resistance by counteracting multiple routes of horizontal gene transfer.

Based on the above analysis, it is concluded that *B. coagulans* GBI-30, 6086 is susceptible to the majority of major classes of antibiotics representing a wide range of determinants of resistance and currently does not harbor transferable antibiotic resistance genes.

6.6 Allergenicity

No reports of allergic reactions to *B. coagulans* were found in our searches of the scientific literature and government databases, including FDA's Safety Information and Adverse Event Reporting Program, MedWatch, and FDA's Recalls, Market Withdrawals, & Safety Alerts search engine (accessed July 18, 2017).

6.7 Adverse Events

No FDA letters regarding concern for safety to companies that market products containing *B. coagulans* were located. A search of MedWatch, FDA's adverse event reporting program, and FDA's Recalls, Market Withdrawals, & Safety Alerts search engine did not uncover any mention of *B. coagulans* products. (accessed July 18, 2017).

6.8 Basis for the GRAS Conclusion

The scientific procedures establishing the safety of Inactivated *B. coagulans* GBI-30, 6086 comprise the technical element of the GRAS standard. The common knowledge element is comprised of the general availability and general acceptance, throughout the scientific community of qualified experts, of the

technical element. Together, the technical element and the common knowledge element form the basis for Ganeden's conclusion of GRAS status of Inactivated *B. coagulans* GBI-30, 6086 for its intended use.

6.8.1 Technical Element

Inactivated *B. coagulans* GBI-30, 6086 has been the subject of a thorough safety assessment as described above. While the scientific studies described above were conducted on live spore preparations, there is no scientific basis to conclude a difference between inactivated vegetative cells or spores. The technical reason for inducing sporulation in the live product is to mitigate die off in various 'in food' environments, during shelf-life, and under the harsh acidic conditions of the human stomach. In the environment of the human colon, the live product spores are expected to return to the live vegetative state. As the inactivated cells are dead, there is no need to induce sporulation prior to killing for the purpose of preventing die off. The totality of evidence supporting the safety of *B. coagulans* GBI-30, 6086, whether vegetative or inactivated, is comprised of data and information that establish the safety of Inactivated *B. coagulans* GBI-30, 6086 under the conditions of its intended use (the technical element) and data and information that is corroborative of safety. The scientific data, information, and methods forming the technical element of this conclusion are:

- The establishment of identity, demonstrating that the organism is a pure strain of *B. coagulans* Hammer, does not produce D(-)-lactate, and is recognized as a non-pathogenic and non-toxicogenic, and also demonstrating that the fully sequenced and characterized genome of *B. coagulans* GBI-30, 6086 is not a source of putative virulence factors, genes for known enterotoxins, hemolysins, or other toxins, or biogenic amines and does not harbor transferable antibiotic resistance genes;
- The method of manufacture and specifications, demonstrating the safe production and the high quality control standards of Inactivated *B. coagulans* GBI-30, 6086;
- The bacterial reverse mutation test, in vitro mammalian chromosomal aberration test, and in vivo mammalian micronucleus test, establishing the lack of genotoxic potential of *B. coagulans* GBI-30, 6086;
- The ninety-day and one-year repeated-dose oral toxicity studies in rats and the one-generation reproduction toxicity study (conducted jointly with the one-year study), establishing the lack of adverse health effects and or target organs, including those systems of particular susceptibility in infants (neurological, immune, skeletal, reproductive, and endocrine),¹⁵ and lack of reproductive and developmental toxicity of repeated exposure to *B. coagulans* GBI-30, 6086 in rats; and

- The dietary exposure estimate establishing an adequate margin of safety (MOS) for the intended conditions of use Inactivated *B. coagulans* GBI-30, 6086 as food for human infants.

In the one-year chronic repeated-dose oral toxicity study summarized in subpart 6.1.6 of this GRAS notice, the lowest NOEL was 1.34×10^{11} CFU/kg bw/day *B. coagulans* GBI-30, 6086 in male Hsd.Brl.Han Wistar rats). Based on the intended use of the ingredient in non-exempt powdered and liquid infant formulas for term infants at levels up to 2×10^8 CFU per 100 mL infant formula as consumed and conservative estimates of consumption of approximately 213.4 mL/kg bw/day of infant formula at the 90th percentile of highest users (girls 8–13 days and boys 14–27 days), resulting in an EDI of 4.27×10^8 CFU Inactivated *B. coagulans* GBI-30, 6086/kg bw, the NOEL allows for an adequate and conservative MOS (NOEL/EDI) of 314-fold when compared to the estimated human exposure level, which supports a conclusion that the intended use of Inactivated *B. coagulans* GBI-30, 6086 is reasonably certain to be safe.

The safety of Inactivated *B. coagulans* GBI-30, 6086 is corroborated by an acute oral toxicity study in rats, establishing that the LD-50 is greater than 34,655 mg/kg of *B. coagulans* GBI-30, 6086; eye and skin irritation studies in rabbits; the GRAS status of *B. coagulans* GBI-30, 6086 for use in various foods and infant formulas and the GRAS status of Inactivated *B. coagulans* GBI-30, 6086 for use in various foods; the lack of serious adverse events reported in clinical trials using various preparations of *B. coagulans* in infants, as reported herein, and various preparations of *B. coagulans*, including *B. coagulans* GBI-30, 6086, in children and adults, as reported in GRN 000399 and incorporated by reference in this notice; the non-virulent nature of *B. coagulans* in general; and the body of scientific literature, and eight GRAS notices to FDA that received letters with no questions, demonstrating the general safety of probiotic organisms when ingested by infants.

6.8.2 Common Knowledge Element

The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part 7 of this GRAS notice contains the citations for the published studies. This publically available data and information fulfills the requirement for general availability of the scientific data, information, and methods relied on to establish the technical element of the GRAS standard. The peer-review of the published studies and lack of Letters to the Editor or other dissenting opinions, as well as FDA's lack of questions regarding the conclusions of GRAS status of the intended uses of *B. coagulans* GBI-30, 6086 in GRN Nos. 000399 and 000660 and the intended uses of Inactivated *B. coagulans* GBI-30, 6086 in GRN 000670 provides ample evidence of consensus among qualified

experts that there is reasonable certainty that consumption of Inactivated *B. coagulans* GBI-30, 6086 for its intended use in non-exempt powdered and liquid infant formulas for term infants at levels up to 2×10^8 CFU per 100 mL infant formula as consumed is not harmful. The general availability and acceptance of this scientific data, information, and methods satisfies the common knowledge element of this GRAS conclusion.

6.9 Data and Information that is Inconsistent with the GRAS Conclusion

We have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

6.10 Information that is Exempt from Disclosure under FOIA

There is no data or information in this GRAS notice that is considered exempt from disclosure under FOIA as trade secret or commercial or financial information that is privileged or confidential.

Part 7: Supporting Data and Information

7.1 Data and Information that are *not* Generally Available

All of the information described in Part 6 of this GRAS notice is generally available.

7.2 References that *are* Generally Available

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