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GRN 877

July 24, 2019

Rachel Morissette, Ph.D.
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
CPK-2 Building, Room 2092
5001 Campus Drive, HFS-225
College Park, MD 20740

Dear Dr. Morissette:

It is our opinion that the GRAS determination titled "Generally Recognized As Safe (GRAS) Notification for the Use of *Bifidobacterium longum* BB536 in Infant Formula" constitutes a new notification. *Bifidobacterium longum* BB536 produced by Morinaga Milk Industry Co., Ltd., subject of GRN 268, has been previously determined safe for use in conventional foods and beverages. This notification expands the intended use of *B. longum* BB536 to include non-exempt infant formula. The safety narrative was updated to include new published information since the filing of Morinaga's original *B. longum* BB536 GRN 268.

We thank you for taking the time to review this GRAS determination. Should you have additional questions, please let us know.

Sincerely,

Claire L, Kruger, Ph.D., D.A.B.T.
President

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Rockville, MD 20852
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000877	7/25/2019
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*
☒ New ☐ Amendment to GRN No._____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Claire Kruger	Authorized Representative
Organization *(if applicable)*	
Morinaga Milk Industry Co., Ltd.	
Mailing Address *(number and street)*	
331, Shiba 5-Chome, Minato-ku	

City	State or Province	Zip Code/Postal Code	Country
Tokyo		108-8384	Japan

Telephone Number	Fax Number	E-Mail Address
301-230-2181	301-230-2188	clairek@chromadex.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Claire Kruger	President
Organization *(if applicable)*	
ChromaDex Spherix Consulting	
Mailing Address *(number and street)*	
11821 Parklawn Drive, Suite 310	

City	State or Province	Zip Code/Postal Code	Country
Rockville	MD	20852	United States

Telephone Number	Fax Number	E-Mail Address
301-230-2181	301-230-2188	clairek@chromadex.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Bifidobacterium longum BB536

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Paper

☒ Electronic files on physical media

If applicable give number and type of physical media

1 CD

3. For paper submissions only:

Number of volumes 1

Total number of pages 43

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 268

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

In addition to the selected food uses outlined in GRN 268, Morinaga Milk Industry Co., Ltd. now intends to add *B. longum* BB536 to term infant formulas for healthy infants as BIFILON-50F. The intended use in infant formula is 1×10^8 CFU *B. longum* BB536 per gram of product.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Morinaga Milk Industry Co., Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Bifidobacterium longum BB536
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Morinaga Milk Industry Co., Ltd. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

331, Shiba 5-Chome, Minato-ku, Tokyo 108-8384 Japan
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
[redacted]	Claire L. Kruger, President, PhD, DABT	07/19/2019

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
1	Insert Clear Morinaga BB536 in Conventional Foods GRAS 7-19-19.pdf	Submission
2	Insert Clear Ahrne 1998.pdf	Submission
3	Insert Clear Akiyama 1994.pdf	Submission
4	Insert Clear Al-Sheraji 2012.pdf	Submission
5	Insert Clear ATCC Bifidobacterium longum.pdf	Submission
6	Insert Clear Bennet 1992.pdf	Submission
7	Insert Clear Benno 1984.pdf	Submission
8	Insert Clear Benno 1989.pdf	Submission
9	Insert Clear Borriello 2003.PDF	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
10	Insert Clear Carr 2002.pdf	Submission
11	Insert Clear Chouraqui 2008.pdf	Submission
12	Insert Clear Del Giudice 2017.pdf	Submission
13	Insert Clear Enomoto Allergology International 2014.pdf	Submission
14	Insert Clear Firmansyah 2011.pdf	Submission
15	Insert Clear FAO-WHO 2002.pdf	Submission
16	Insert Clear Grzeskowiak 2012.pdf	Submission
17	Insert Clear Hascoet 2011.pdf	Submission
18	Insert Clear Ishizeki 2013.pdf	Submission

PART VIII – LIST OF ATTACHMENTS *(continued)*

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
19	Insert Clear Lau 2018.pdf	Submission
20	Insert Clear Mah 2007.pdf	Submission
21	Insert Clear Picard 2005.PDF	Submission
22	Insert Clear Puccio 2007.pdf	Submission
23	Insert Clear Rouge 2009.pdf	Submission
24	Insert Clear Simakachorn 2011.pdf	Submission
25	Insert Clear Soh 2009.pdf	Submission
26	Insert Clear Tamaki 2016.pdf	Submission
27	Insert Clear Wu 2016.pdf	Submission

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

PART VIII – LIST OF ATTACHMENTS *(continued)*

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
28	Insert Clear Zhou 2000a.pdf	Submission
29	Insert Clear Zhou 2000b.pdf	Submission
30	Insert Clear Zhou 2001.PDF	Submission
31	Insert Clear IOM 2005.pdf	Submission
32	Insert Clear Martinez 2011.pdf	Submission
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	Insert Clear	
	Insert Clear	
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Generally Recognized As Safe (GRAS) Notification for the Use of *Bifidobacterium longum* BB536 in Infant Formula

Prepared for:

Morinaga Milk Industry Co., Ltd.
331, Shiba 5-Chome, Minato-ku
Tokyo 108-8384
Japan

Prepared by:

ChromaDex Spherix Consulting
A Business Unit of ChromaDex, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852

July 19, 2019

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I. SIGNED STATEMENT OF THE CONCLUSION OF GENERALLY RECOGNIZED AS SAFE (GRAS) AND CERTIFICATION OF CONFORMITY TO 21 CFR §170.205-170.260

A. SUBMISSION OF GRAS NOTICE

Morinaga Milk Industry Co., Ltd. is hereby submitting a GRAS notice in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

Morinaga Milk Industry Co., Ltd.
331, Shiba 5-Chome, Minato-ku
Tokyo 108-8384
Japan

C. COMMON OR USUAL NAME

The substance that is the subject of this Generally Recognized As Safe (GRAS) determination is *Bifidobacterium longum* BB536 (*B. longum* BB536) and was subject of GRAS Notification (GRN) 268. *B. longum* BB536 is a strain of the species *Bifidobacterium longum*. The bacterium has been deposited with the American Type Culture Collection (ATCC) and is designated BAA-999^{TM}. *B. longum* BB536 cultures are used by Morinaga Milk Industry Co, Ltd. to manufacture products sold under the trade name BIFILON-50F.

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

This notification does not contain any trade secret or confidential information.

E. INTENDED USE

In addition to the selected food uses outlined in GRN 268, Morinaga Milk Industry Co., Ltd. now intends to add *B. longum* BB536 to term infant formulas for healthy infants as BIFILON-50F. The intended use in infant formula is 1 x 10^8 CFU *B. longum* BB536 per gram of product.

F. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of *B. longum* BB536 as an ingredient in term infant formulas at a maximum level of 1 x 10^8 CFU *B. longum* BB536 per gram of product at the end of product shelf-life is based upon scientific procedures as described under 21 CFR §170.30(b). The

intake of *B. longum* BB536 from the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). To demonstrate that *B. longum* BB536 is safe, and GRAS, under the intended conditions of use, the safety of the intake of *B. longum* BB536 has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of *B. longum* BB536 as an ingredient in term infant formula has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1) Bifidobacteria are naturally occurring bacteria that contribute to the composition of the gut microflora of humans. Bifidobacteria represent up to 25% of the cultivatable fecal bacteria in adults and 80% in infants. *Bifidobacterium longum* has been detected in feces from infants and adults.

2) *Bifidobacterium longum* BB536, a strain of *B. longum,* is a Gram-positive anaerobic bacterium. *B. longum* BB536 was originally isolated from a healthy infant in 1969. The bacterium has been deposited with the American Type Culture Collection (ATCC) and is designated BAA-999™.

3) The maintenance of the original frozen culture has been tightly controlled to ensure purity and stability of the strain.

4) Finished products made with *B. longum* BB536 cultures reproducibly meet compositional standards and comply with limits on contaminants appropriate for food-grade ingredients. Product specifications are set to assure that *B. longum* BB536 is suitable for use in food.

5) Bifidobacteria are commonly consumed in fermented foods throughout the world. *B. longum* BB536 was first commercially available in Japan in 1977 and the availability of *B. longum* BB536 on the European market began in 1986.

6) *B. longum* BB536 has been tested for parameters outlined in the Food and Agriculture Organization of the United Nations/World Health Organization's (FAO/WHO) guidelines for the evaluation for microbes for probiotic use in foods. Results from these tests provide evidence that *B. longum* BB536 is safe for use in foods, namely:

- Available antibiotic resistance pattern suggests that *B. longum* BB536 does not present concerns for antibiotic resistance in humans.
- *B. longum* BB536 produces predominantly L-lactic acid, while production of D- lactic acid is negligible.
- *B. longum* BB536 has been reported to deconjugate bile salts. The production of deconjugated bile salts was concurrent with bacterial growth, and deconjugated bile salts were the only compound produced.
- Results from comparisons of amino acid sequences of known bacterial toxins with sequences of the predicted proteins from the genomic sequence of *B. longum* BB536 and genomic sequences of three known pathogens with sequences of the predicted proteins from the genomic sequence of *B. longum* BB536 indicate that there is no significant homology.
- *B. longum* BB536 was not observed to have hemolytic activity.

7) The LD_{50} of *B. longum* BB536 orally administered to mice was determined to be ~$5x10^{13}$ cfu/kg-bw. The LD_{50} of *B. longum* BB536 administered intraperitoneally to mice was determined to be ~$9x10^{11}$ cfu/kg-bw.

8) Results from repeat dose studies of *B. longum* BB536 administered to rats show no intake effects on body weight, body weight gain, or feed intake at doses up to $2x10^{12}$ cfu/kg bw/day. Findings for the studies provide support for the safe use of *B. longum* BB536 under the test conditions.

9) Seventeen clinical studies (reported in 14 papers) involving the administration of *B. longum* BB536 to healthy adults were identified and reviewed. The duration of *B. longum* BB536 consumption ranged from 6 days to 14 weeks. In three studies, intakes of *B. longum* BB536 were approximately 10^{11} cfu per day; participants consumed this dose for periods of 4, 13 or 14 weeks. In all other human studies reviewed, doses were in the range of approximately 10^9 to 10^{10} cfu *B. longum* BB536 per day. None of the studies reported any participant dropouts or adverse events due to the test articles. Findings from the studies provide support for the safe and well-tolerated use of *B. longum* BB536 under the test conditions.

10) Eleven clinical studies involving the administration of *B. longum* BB536 to unhealthy adults or children were identified and reviewed. Study durations ranged from 8 weeks to 1 year. Daily viable *B. longum* BB536 intakes for adults were in the range of 10^9 to10^{10} cfu in most studies, and approximately 10^{11} cfu *B. longum*

BB536 per day in one study. Daily doses of *B. longum* BB536 in populations of children were approximately 10^9 cfu. None of the studies reviewed reported adverse events or patient dropouts as a result of *B. longum* BB536 supplementation. Findings from the studies provide support for the safe and well-tolerated use of *B. longum* BB536 under the test conditions.

11) Fourteen studies involving the administration of *B. longum* BB536 to infants or toddlers were identified and reviewed. Study durations ranged from 5 days to 12 months. Infants and toddlers received formula supplemented with *B. longum* BB536 ranging from 10^7-10^9 cfu. None of the studies reported adverse events due to *B. longum* BB536 supplementation. Findings from the studies provide support for the safe and well-tolerated use of *B. longum* BB536 in infants and toddlers under the test conditions.

12) Research on bifidobacteria has been conducted on several strains of *Bifidobacterium* longum; results from studies of other strains of *B. longum* provide corroborative evidence for the safety of human consumption of *B. longum* BB536.

13) While other bifidobacterial strains are not identical to *B. longum* BB536, they share many common characteristics. Therefore, studies on other bifidobacteria species commonly used as probiotics or in food production, such as *B. breve* and *B. infantis*, can be used to provide corroborative evidence for the safety of *B. longum* BB536. Results from acute studies in rats demonstrate that under conditions of the tests, neither *B. breve* nor *B. infantis* presented toxicological concerns at the highest doses tested. Results from subchronic toxicity studies of *B. infantis* and *B. breve* in rats demonstrate that under conditions of the tests, No Observed Adverse Effect Levels (NOAELs) were determined to be the doses tested. The doses tested were $2.3x10^{11}$ cfu *B. breve*/kg-bw/day and $7.6x10^{10}$ cfu *B. infantis*/kg-bw/day. Results from these studies of *B. breve* and *B. infantis* provide corroborative data to support the available evidence that *B. longum* BB536 is safe for human consumption.

14) Assuming addition of $1x10^8$ cfu of *B. longum* BB536 per gram of infant formula, the formula is the sole source of nutrition, and the caloric requirements of a one month-old infant and six month-old infant are 472 kcal/day and 645 kcal/day, the estimated intakes of *B. longum* BB536 from infant formula are $9.9x10^9$ and $1.35x10^{10}$ cfu, respectively.

Determination of the GRAS status of *B. longum* BB536 under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, FACN, FASN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, and Thomas E. Sox, PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of *B. longum* BB536 and the potential human exposure to *B. longum* BB536 resulting from its intended use as an ingredient in term infant formula and have concluded:

> *There is no evidence in the available information on B. longum BB536 that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when B. longum BB536 is used at levels that might reasonably be expected from the proposed applications. B. longum BB536 is GRAS for use in infant formula as proposed by Morinaga Milk Industry Co, Ltd.*

Therefore, *B. longum* BB536 is safe and GRAS at the proposed levels of addition to term infant formula. *B. longum* BB536 is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

G. PREMARKET APPROVAL

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the substance is GRAS under the conditions of intended use.

H. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, PhD, DABT, President, ChromaDex Spherix Consulting, A Business Unit of ChromaDex, Inc., at 11821 Parklawn Drive, Suite 310, Rockville, MD 20852. Telephone: 301-230-2180; Email: clairek@chromadex.com, or be sent to FDA upon request.

I. FREEDOM OF INFORMATION ACT (FOIA)

Parts 2 through 7 of this notification do not contain data or information that is exempt from disclosure under the FOIA.

J. INFORMATION INCLUDED IN THE GRAS NOTIFICATION

To the best of our knowledge, the information contained in this GRAS notification is complete, representative and balanced. It contains both favorable and unfavorable information, known to Morinaga Milk Industry Co., Ltd. and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

July 19, 2019

Claire L. Kruger, PhD, DABT
President, ChromaDex Spherix Consulting
Authorized Representative of Morinaga Milk Industry Co., Ltd.

Date

II. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT OF THE NOTIFIED SUBSTANCE

A. COMMON OR USUAL NAME

Bifidobacterium longum BB536.

B. TRADE NAME

BIFILON-50F

C. DESCRIPTION OF *BIFIDOBACTERIUM LONGUM* BB536

Incorporated by reference from GRN 268, pages 7-9. *B. longum* BB536 has also been known as BL999 or BAA-999. *B. longum* BB536, also known as BL999 or BAA-999, is a Gram-positive, anaerobic rod or Y-shaped bacterium . It has been shown to be genetically similar to the reference *B. longum* strain, *B. longum* E194b, and genetically dissimilar to *B. animalis* R 101-8. Its carbohydrate fermentation pattern was also determined, in which it can ferment sugars such as arabinose, xylose, galactose, and lactose, but not ribose, starch, inulin, or sorbitol.

1. Genotypic Identification

In order to genotypically verify that their *B. longum* BB536 stock cultures are homogenous and not contaminated with another species, Morinaga compared the 16S rDNA sequence of their original culture to that of *B. longum* JCM1217 (Type strain). The 16S rDNA gene sequence of *B. longum* BB536 was obtained from its whole genome sequence. The whole genome was attained by single-molecule real-time DNA sequencing (PacBio). The 16S rDNA sequence of *B. longum* JCM1217 (Accession number AP010888,locus_tag=BLLJ_16SrRNA01) was obtained from the National Center for Biotechnology Information (NCBI) website. Sequences were aligned using the BLASTN software (Version 2.9.0+). Alignment results indicate 99% sequence homology and verify the species of Morinaga's working stocks (Table 1).

Table 1. *B. longum* BB536 and *B. longum* JCM1217 16S rDNA Sequence Alignment

Sequence Alignment	Identities	Gaps
B. longum BB536 and *B. longum* JCM1217 16S rDNA (Accession number AP010888)	99%	0%

D. PRODUCTION PROCESS

The production process has not changed since the original GRAS Notification (GRN 268), therefore we are incorporating by reference from GRN 268, pages 9-11. The culturing process is a series of expansions of a working culture, which are derived from the original stocks of *B. longum* BB536, yielding a manufacturing culture. The manufacturing culture provides the material for the non-culturing process. This process concentrates, resuspends, and freeze dries the material into a powder where it is mixed with cornstarch as a carrier into the final product, BIFILON-50F. For reference, a schematic of the production process has been included (Figure 1).

1. Strain Maintenance

The original isolate culture is stored at -80°C in a deep freezer at Morinaga Milk Industry as the original frozen culture of *B. longum* BB536 and is deposited in the American Type Culture (ATCC), designated BAA-999™, and the National Institute of Technology and Evaluation (NITE), designated BP-02621. The maintenance of the original frozen culture has been tightly controlled at the Morinaga Milk Industry.

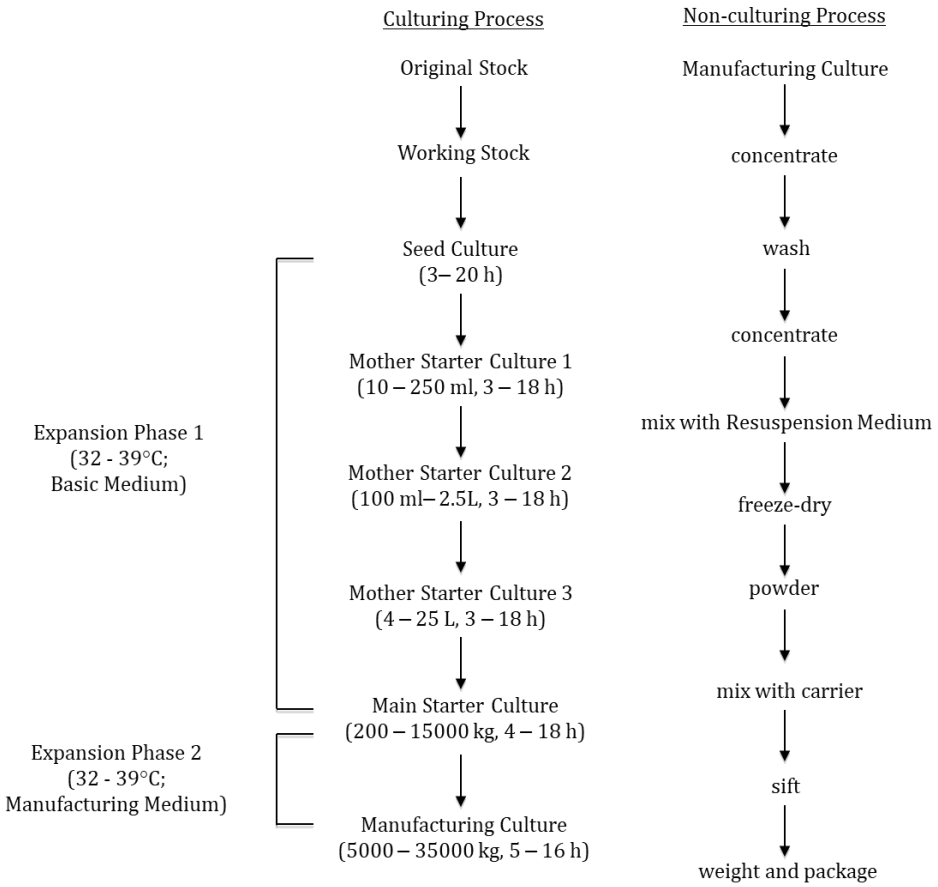

Figure 1. *B. longum* BB536 Production Process Schematic

Original and working stocks are maintained at Morinaga Milk Industry.

2. Culture and Suspension Media

The fermentation uses a defined growth medium and controlled fermentation growth conditions; components of the fermentation media are food grade.

3. Compliance

All Morinaga manufacturing facilities have been certified as meeting the Food Safety System Certification (FSSC) 22000 and International Organization for Standardization (ISO) 22000:2005 or Hazard Analysis and Critical Control Points (HACCP). All raw materials, processing aids, and food contact substances comply with the conditions of use specified in the United States Code of Federal Regulations and Food Chemical Codex monographs.

Procedures are in place describing required actions when parameters are out of specification. The production system has been certified to meet the requirements of Hazard Analysis and Critical Control Points (HACCP) Codex Alimentarius at all facilities.

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

The finished product made with *B. longum* BB536 cultures to be included in non-exempt infant formula is BIFILON-50F. BIFILON-50F is manufactured without milk-based ingredients and uses cornstarch as a carrier.

1. Verification of *Bifidobacterium longum* BB536 Cells in Finished Products

Morinaga undertook a study to demonstrate that the *B. longum* BB536 cells in Morinaga products are identical to the original *B. longum* BB536 cells isolated in 1969. The electrophoretic patterns of RAPD-PCR products of BB536 cells in finished product were identical to the original *Bifidobacterium longum* BB536 culture (Appendix, Study Nos 2018-B0134E, 2018-B0505E, 2018-B0862E). These results demonstrate that the *B. longum* BB536 cells in finished products are identical to the original *B. longum* BB536 culture. Because the cells are the same, safety studies conducted with the original *B. longum* BB536 culture are relevant to the safety of the *B. longum* BB536 cells found in the products that are the subjects of this GRAS Notification.

2. Specifications and Lot Data for Finished Products of *Bifidobacterium longum* BB536

To ensure a consistent food-grade product, each batch of BIFILON-50F made from *Bifidobacterium longum* BB536 is evaluated against an established set of product specifications (Table 2). Importantly, the specifications for BIFILON-50F remain unchanged from the original GRN 268. Due to the inclusion in infant formula, a negative specification for *Cronobacter*

sakazakii has been included in this notification. Data from six non-consecutive batches of BIFILON-50F (three from each plant) show that the manufacturing process produces a finished product that reproducibly complies with the product specifications, regardless of manufacturing location. The analytical methods used for *B. longum* BB536 identification (RAPD) and enumeration are described in GRN 268, pages 22-23 and are incorporated by reference.

F. STABILITY OF *BIFIDOBACTERIUM LONGUM* BB536

The stability of *B. longum* BB536 in the BIFILON-50F as specified in GNR 268, page 23 are incorporated by reference. Morinaga evaluated the survival of *B. longum* BB536 in the BIFILON-50F over 18 to 36-month periods. Stability studies demonstrate acceptable stability. Importantly, determining the stability of BIFILON-50F is an ongoing process and will continue to be monitored in order to support the intended shelf-life of the finished product.

Table 2. BIFILON-50F Specifications and Lot Data

Parameter	Method	Specification±	Lot No. and Manufacturing Location					
			Tone Plant	Tone Plant	Tone Plant	Fukui Plant	Fukui Plant	Fukui Plant
Physical Characteristics								
Bifidobacterium longum BB536 Identity	RAPD PCR	Identified	Identified	Identified	Identified	Identified	Identified	Identified
Appearance	Visual inspection	White to slightly brown powder	White to slightly brown powder	White to slightly brown powder	White to slightly brown powder	White to slightly brown powder	White to slightly brown powder	White to slightly brown powder
Foreign Body	Visual inspection	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Odor and Taste	Sensory Evaluation	No abnormal odor and taste	No abnormal odor and taste	No abnormal odor and taste	No abnormal odor and taste	No abnormal odor and taste	No abnormal odor and taste	No abnormal odor and taste
Moisture	Gravimetric method at 105°C, 4h	< 6g/100g	2.2	2.1	1.7	1.7	1.7	1.6
Casein	ELISA	< 10ppm	< 10	< 10	< 10	< 10	< 10	< 10
β-Lactoglobulin	ELISA	< 10ppm	< 10	< 10	< 10	< 10	< 10	< 10
Microbiological Characteristics								
Anaerobic cfu (including *Bifidobacterium longum* BB536)	BL Agar	> $8.0x10^{10}$	$1.4x10^{11}$	$1.4x10^{11}$	$1.3x10^{11}$	$1.3x10^{11}$	$1.2x10^{11}$	$1.1x10^{11}$
Total aerobic bacteria	Standard Plate Count Agar or ISO 4833-1	< 300 cfu/g	< 300	< 300	< 300	< 300	< 300	< 300
Mold	Potato Dextrose Agar or ISO 21527-2	< 30 cfu/g	< 30	< 30	< 30	< 30	< 30	< 30
Yeast	Potato Dextrose	< 30 cfu/g	< 30	< 30	< 30	< 30	< 30	< 30

Table 2. BIFILON-50F Specifications and Lot Data

Parameter	Method	Specification±	Lot No. and Manufacturing Location					
			Tone Plant	Tone Plant	Tone Plant	Fukui Plant	Fukui Plant	Fukui Plant
	Agar or ISO 21527-2							
Enterobacteriaceae	BPW/VRBD or ISO 21528-1	Not Detected/10g	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Staphylococcus aureus	Mannitol Salt Agar with egg yolk or ISO 6888-1	Not Detected/0.01g	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Salmonella	BPW/Selenite Broth/DHL agar or ISO 6579	Not Detected/25g	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Cronobacter sakazakii	BPW/VRBD or ISO 22964	Not Detected/25g	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Heavy Metals								
Lead	ICP-MS	< 0.5ppm	< 0.5ppm	< 0.5ppm	< 0.5ppm	< 0.5ppm	< 0.5ppm	< 0.5ppm
Arsenic	X-ray Fluorescence	< 1ppm	< 1ppm	< 1ppm	< 1ppm	< 1ppm	< 1ppm	< 1ppm
Cadmium	ICP-MS	< 0.5ppm	< 0.5ppm	< 0.5ppm	< 0.5ppm	< 0.5ppm	< 0.5ppm	< 0.5ppm
Mercury	ICP-MS	< 0.1ppm	< 0.1ppm	< 0.1ppm	< 0.1ppm	< 0.1ppm	< 0.1ppm	< 0.1ppm

± Specifications are unchanged from GRN268

BL – Blood Liver; BPW – Buffered Peptone Water; cfu – colony forming units; DHL – Deoxycholate Hydrogen sulfide Lactose; ELISA – enzyme-linked immunosorbent assay; ICP-MS – Inductively coupled plasma mass spectrometry; ppm – parts per million; RAPD – Random Amplification of Polymorphic DNA; VRBD – Violet Red Bile Dextrose

Limit of Quantification (LOQ): Lead = < 0.04 ppm; Arsenic = < 1 ppm; Mercury = < 0.04 ppm; Cadmium = < 0.04 ppm

III. DIETARY EXPOSURE

A. INTENDED EFFECT

As described in GRN 268, page 36, the subject of the GRAS determination, *Bifidobacterium longum* BB536, is a bacterium naturally found in the colon that contributes to the composition of the gut microflora. The intended effect is to provide a dietary source of *B. longum* BB536 to term infants.

B. HISTORY OF USE

1. Naturally Occurring in Humans

Bifidobacteria are a natural component of the normal human gut microflora. Bifidobacteria comprise up to 25% of the cultivatable fecal bacteria in adults and 80% in infants (Picard et al. 2005), and *Bifidobacterium longum* has been detected in feces from infants and adults (Benno et al. 1984, Benno et al. 1989). *Bifidobacterium longum* BB536 was isolated from a healthy infant.

2. Bifidobacteria Added to Foods

Bifidobacteria have been consumed in fermented foods for decades and currently used commercial strains include *Bifidobacterium animalis* ssp. lactis strain Bf-6, *Bifidobacterium lactis* Bb-12, *Bifidobacterium lactis* DR10 (HN019), *Bifidobacterium longum* BB536, *Bifidobacterium breve* Yakult, *Bifidobacterium breve* SBT-2928, and *Bifidobacterium breve* C50. In the United States *B. animalis* ssp. lactis Bf-6 has been approved for use in conventional foods (GRN 377), *B. lactis* Bb-12 has been determined to be GRAS for use in formulas for infants four months of age and older (GRN 49), *B. longum* BB536 has been approved for use in selected foods (GRN 268), *B. breve* M-16V has been approved for use in selected foods and infant formulas (GRN 453, 454, 455), and *Bifidobacterium longum* subsp. *infantis* R0033 has been approved for use in infant formulas (GRN 758). Other probiotics, such as *Lactobacillus reuteri* DSM 17938, *Lactobacillus fermentum* CECT5716, and *Bacillus coagulans* GBI-30, 6086, have been approved for use in term infant formulas (GRN 410, 531, and 660). Furthermore, there is no evidence to date showing that the consumption of non-viable bifidobacteria in fermented foods is unsafe.

Bifidobacterium longum BB536 was first commercially available in Japan in 1977 with the launch of Morinaga Bifidus Milk. At present, several products containing *B. longum* BB536 are available on the Japanese market. The availability of *B. longum* BB536 in Europe began in 1986 with the production of Morinaga Bifidus Yogurt in France, followed by Sweden in 1989. In 1994, *B. longum* BB536 was sold as a frozen starter to dairy companies in Germany, Russia, Poland and other EU countries.

C. INTENDED USE

In addition to the selected food uses outlined in GRN 268, Morinaga Milk Industry Co., Ltd. now intends to add *B. longum* BB536 to term non-exempt infant formulas for healthy infants as BIFILON-50F. The intended use in infant formula is 1 x 10^8 CFU *B. longum* BB536 per gram of infant formula. Morinaga Milk Industry will ensure the stability and viability of *B. longum* BB536 so that the products deliver the declared levels in a serving of stated size throughout the product shelf-life.

D. ESTIMATED DAILY INTAKE

Powdered term infant formulas will contain 10^8 cfu *B. longum* BB536/g to produce an intended target intake level of 10^9 - 10^{10} cfu *B. longum* BB536/day. Infant formulas in the US market typically provide 0.67 kcal/ml (20 kcal/fl oz) (Martinez and Ballew, 2011). Assuming that these formulas are the sole source of nutrition, reconstituted at 14.1 g/100 ml with a caloric density of 0.67 kcal/ml, and the caloric requirements of one month-old and a six month-old infant are 472 kcal/day and 645 kcal/day (Institute of Medicine (US) Panel on Macronutrients and Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005), the addition of 10^8 cfu *B. longum* BB536/g infant formula will result in intakes of 9.9 x 10^9 and 1.35 x 10^{10} cfu *B. longum* BB536/day. These levels are consistent with intake levels reported in other GRAS notifications where *B. lactis* Bb12, *Streptococcus thermophilus* Th4, *L. reuteri* DSM 17938, *B. breve* M-16V are used in infant formulas (GRN 49; GRN 410; GRN 454, 455).

IV. SELF-LIMITING LEVELS OF USE

This part does not apply.

V. COMMON USE IN FOOD BEFORE 1958

This part does not apply.

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

A. REVIEW OF BIFIDOBACTERIA

The safe use of bifidobacteria in food products is supported by the long historical consumption of fermented milks and the growing knowledge about bifidobacteria taxonomy and physiology. Lactic acid-producing bacteria in foods are thought to have little or no pathogenic potential (Picard et al. 2005). Bifidobacteria are naturally present as the dominant colonic microbiota and represent up to 25% of the cultivable fecal bacteria in adults and 80% in infants (Picard et al. 2005).

Bifidobacterium genera are generally held to be non-pathogenic to humans (Carr et al. 2002). There is also evidence from the literature that *Bifidobacterium* spp. lack invasive properties, i.e., the bacteria will not pass the epithelial boundary of the intestine and reach deep tissue, and that they are not mucinolytic (Zhou et al. 2000a, 2000b; 2001). These genera have been used in a variety of food products for centuries and are regularly consumed by humans on a daily basis. In addition, bifidobacteria are components of the normal flora of the human gastrointestinal tract (Ahrne et al. 1998). The lack of pathogenicity extends across all age groups and to immunocompromised individuals (Borriello et al. 2003).

B. EVALUATING THE SAFETY OF *B. LONGUM* BB536 INGESTION

The safety of *B. longum* BB536 ingestion has been extensively reviewed in GRN 268, pages 46-55, and is incorporated by reference,. In summary, *B. longum* BB536 is susceptible to antibiotics similarly to other strains of bifidobacterial species and it is unlikely to have transmissible antibiotic resistance genes. *B. longum* BB536 predominantly produces L-lactic acid, is inhibited by bile salt, contains no pathogenic or toxigenic genomic markers, has little to no hemolytic potential, and has a very low potential for infectivity. Taken together, the previously published information documenting lack of infective potential, deleterious metabolic activities, excessive immune stimulation, and gene transfer support the conclusion that *B. longum* BB536 is GRAS.

C. REVIEW OF THE SCIENTIFIC LITERATURE ON *BIFIDOBACTERIUM LONGUM* BB536

There have been many *in vitro* and *in vivo* studies performed with *B. longum* BB536 to support its safe use in the diet. In addition to the studies outlined in GRN 268, pages 56-65, Morinaga provided twelve additional published, clinical studies conducted with *B. longum* BB536 in infants and toddlers and a ChromaDex Spherix Consulting literature search in February 12, 2019

uncovered one animal study and two human clinical studies not previously reviewed. The weight of the evidence from these new studies indicates that adverse effects resulting from the use of BB536 in term infant formula at the intended use levels are not expected.

1. *In vitro* and Animal Studies of *B. longum* BB536

Since the filing of GRN 268, there has been one additional animal study. Therefore, all studies summarized in GRN 268 pages 56-65 are incorporated by reference. The LD_{50} of *B. longum* BB536 orally administered to mice was determined to be ~$5x10^{13}$ CFU/kg-bw. The LD_{50} of *B. longum* BB536 administered intraperitoneally to mice was determined to be ~$9x10^{11}$ cfu/kg-bw. Results from repeat dose studies of *B. longum* BB536 administered to rats show no intake effects on body weight, body weight gain, or feed intake at doses up to 2 x 10^{12} cfu/kg bw/day. Findings from these studies provide support for the safe use of *B. longum* BB536 under the test conditions. The new study is summarized below.

Al-Sheraji et al. (2012) studied the effect of yogurt supplemented with *B. pseudocatenulatum* G4 or *B. longum* BB536 on the lipid profile of rats fed a cholesterol-enriched diet. Thirty-two male, 4-week old Sprague-Dawley rats were divided equally amongst 4 groups and for 8 weeks fed a cholesterol-enriched diet plus: Positive control (PC) group – tap water; Group YC – milk fermented with 3% (v/v) *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*; Group YCG4 – milk fermented with 2% (v/v) *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* and 1% *B. pseudocatenulatum* G4; Group YCBB – milk fermented with 2% (v/v) *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* and 1% *B. longum* BB536. After 8 weeks, the PC group exhibited significant increases in total cholesterol, low-density lipoprotein (LDL) cholesterol, and malondialdehyde (MDA). However, either group containing *B. pseudocatenulatum* G4 or *B. longum* BB536 had significantly lower total cholesterol, LDL-C, very-low-density lipoprotein (VLDL) cholesterol, and MDA as compared to the PC group. Additionally, fecal excretion of bile acids was markedly increased in the YCG4 or YCBB groups compared to the PC group. The intakes were well tolerated as the rats in the yogurt-supplemented groups had lower food intakes, however, the body weight of the YCG4 was significantly higher compared to controls (attributed to ingestion of yogurt instead of water).

2. Clinical Studies of *B. longum* BB536

a. Healthy Adults

Seventeen clinical studies (reported in 14 papers) involving the administration of *B. longum* BB536 to healthy adults were identified, reviewed, and incorporated by reference from GRN 268, pages 60-62. The duration of *B. longum* BB536 consumption ranged from 6 days to

14 weeks. In three studies, intakes of *B. longum* BB536 were approximately 10^{11} cfu per day; participants consumed this dose for periods of 4, 13 or 14 weeks. In all other human studies reviewed, doses were in the range of approximately 10^9 to 10^{10} cfu *B. longum* BB536 per day. None of the studies reported any participant dropouts or adverse events due to the test articles. Findings from the studies provide support for the safe and well-tolerated use of *B. longum* BB536 under the test conditions.

b. Compromised Adults and Children

Since the filing of GRN 268, there have been 3 additional clinical studies in compromised adults and children. Therefore, all studies summarized in GRN 268 pages 62-63 are incorporated by reference. Eleven clinical studies involving the administration of *B. longum* BB536 to unhealthy adults or children were reviewed. Study durations ranged from 8 weeks to 1 year. Daily viable *B. longum* BB536 intakes for adults were in the range of 10^9 to10^{10} cfu in most studies, and approximately 10^{11} CFU *B. longum* BB536 per day in one study. Daily doses of *B. longum* BB536 in populations of children were approximately 10^9 cfu. None of the studies reviewed reported adverse events or patient dropouts as a result of *B. longum* BB536 supplementation. Findings from the studies provide support for the safe and well-tolerated use of *B. longum* BB536 under the test conditions. The new studies are summarized below.

A study of critically-ill children (1-3 years old under mechanical ventilation requiring enteral feeding) in a bicentric, randomized, double-blind, placebo-controlled method were provided with formula supplemented with pre- and probiotics (Simakachorn et al., 2011). Ninety-four patients were given enteral formula supplemented with 2 probiotic strains (*L. paracasei* NCC 2461 [5x10^6 cfu/g], *B. longum* BB536 [2x10^6 cfu/g]), prebiotics (oligofructose/inulin [2.6 g/L], Acacia gum [2.8 g/L]), and DHA [43 mg/L]. Fecal *Bifidobacteria* were increased in the test formula group and the test formula was well tolerated. There was no difference in reported adverse events between the control and test article group.

B. longum BB536 was also used to investigate its efficacy in inducing remission of ulcerative colitis (UC) (Tamaki et al., 2016). The randomized, double-blinded, placebo-controlled trial enrolled fifty-six Japanese patients with mild to moderate UC and randomly treated them with placebo (28 patients) or 2-3x10^{11} freeze-dried viable *B. longum* BB536 (28 patients) (3x daily in individual dose sachets) for 8 weeks. At the end of the study, there was a significant decrease in UC disease activity index (UCDAI) scores in the BB536 group compared to baseline, which was not seen in the placebo group. Specifically, the BB536 group had a reduction in rectal bleeding and mucosal findings compared to baseline. This reduction was not seen in the placebo group. The intake of BB536 was well tolerated as there was no major adverse event reported in either group and only one patient complained of a dry cough after 2 weeks of receiving BB536.

Del Giudice et al. (2017) utilized a randomized, double-blind, placebo-controlled study to examine the effect of probiotics on children with seasonal allergenic rhinitis and intermittent asthma. Forty children (ages 6-11) were treated with an oral supplementation containing *Bifidobacteria* mixture, *B. longum* BB536 ($3x10^9$ cfu/day), *B. infantis* M-63 ($1x10^9$ cfu/day), and *B. breve* M-16V ($1x10^9$ cfu/day) as powder in 3 mg sachet for 4 weeks. Adverse events were monitored and there were no events related to the clinical intervention. Children treated with probiotic mixture achieved a significant improvement of symptoms and quality of life (QoL).

c. Infants and Toddlers

In addition to the two infant studies outlined in GRN 268, page 64, there have been 12 additional studies conducted on infants (10) or toddlers (2) with *B. longum* BB536 since the filing of GRN 268, which are summarized in Table 3. Mah et al. (2007) conducted a double-blind, placebo-controlled study on fecal microbiota of thirty-seven Asian infants administered probiotic formula ($2x10^7$ cfu/g of *L. rhamnosus* GG and $1x10^7$ cfu/g *B. longum* BB536) for 6 months. While Bifidobacteria significantly increased in the first year of life, there was no major difference between the control group and probiotic group. In the probiotic group, *L. rhamnosus* and *B. longum* could be detected more frequently during supplementation, however, there was no difference after administration had ended. There were 2 withdrawals from each group, however, no reason was provided, and adverse events were not monitored or reported.

Puccio et al. (2007) assessed the safety and tolerability of formula supplemented with a total of $2x10^7$ cfu of *B. longum* BB536 and 90:10 galacto-oligosaccharides (GOS)/fructo-oligosaccharides (FOS). One hundred thirty-eight infants were enrolled in a 7-month prospective, randomized, reference-controlled, double-blinded study who were not breast fed after day 14 of birth. Results indicate the intake was well tolerated, as there was no statistically significant difference in stool consistency, odor, frequency of crying, restlessness, colic, spitting, and vomiting between the two groups, while the supplemented group had fewer incidents of constipation. There was also no statistically significant difference in frequency or severity of adverse events reported, and no event was associated with the supplemented formula.

In another prospective, randomized, controlled, double-blind study of infant formula supplemented with a total of 10^9 cfu *B. longum* BB536 and *L. rhamnosus* LPR, and 90:10 GOS/FOS, two hundred eighty-four healthy infants were given supplemented formula for 16 weeks (Chouraqui et al., 2008). Similar to the Puccio study, supplementation was well tolerated and there was no statistically significant difference stool consistency, flatulence, colic, spitting up, or vomiting between the two groups. There was also no statistically significant difference in frequency or severity of adverse events reported, and no event was associated with the experimental formula.

In a study of ninety-four low-birth weight preterm infants (<1500g and <32 weeks), capsules of a total of 10^8 cfu *B. longum* BB536 and *L. rhamnosus* GG were administered by enteral feeding through opening the capsules and mixing with 1mL sterile water in a bicentric, double-blind, randomized, controlled trial (Rouge et al., 2009). While there was no difference in the primary endpoint of tolerance to enteral feeding in preterm infants, there was a significant decrease in the time to reach full enteral feeding in infants who weighed >1000g in the probiotic group. While 6 deaths were observed during the course of the trial (2 in probiotic group and 4 in the control group), none were associated with the test article and was an expected outcome in a trial when working with preterm infants. Additionally, no adverse events were observed.

In a randomized, double-blind, controlled trial of three hundred and ninety-three healthy Indonesian toddlers (age 1), growth and cognitive function (by the Bayley Scale of Infant and Toddler Development) was assessed after being fed formula supplemented with *B. longum* BB536, *L. rhamonosus* LPR, prebiotics (inulin and fructo-oligosaccharides), and long-chain polyunsaturated fatty acids (LCPUFA) for 12 months (does not provided) (Firmansyah et al., 2011). After 12 months, the synbiotic group weighed significantly more than the control group, closer to the World Health Organization Child Growth Standard. There was no difference in stool frequency or any stool characteristics between the groups. While there were some serious adverse events (such as typhoid fever), they occurred in both groups at a similar rate and were not considered related to the test material.

Hascoet et al. (2011) utilized a randomized, double-blind, controlled trial to investigate probiotic effect on infant gut microbiota on one hundred ninety healthy infants who were given formula or formula supplemented with $2x10^7$ cfu/g *B. longum* BB536. After 4 months of intake, there was no statistically significant difference in stool frequency, vomiting, spitting up, crying, fussiness, colic, or flatulence between any of the groups. While the most common adverse events affected the GI or upper respiratory tract, there was no difference in reported adverse events between any of the study groups.

In an open trial by Morinaga Milk Industry of *Bifidobacterium* supplementation on allergy development in healthy infants, one hundred sixty-six healthy infants were provided no probiotic (n=36) or $5x10^9$ cfu/day *B. longum* BB536 and *B. breve* M-16V (n=130) for six months (Enomoto et al., 2014). The results of that trial determined that the risk of developing eczema/atopic dermatitis during the first 18 months of life was significantly decreased in the probiotic group compared to the control. Furthermore, no adverse events reported were related to the intake.

Lau et al. (2018) evaluated the effects of *B. longum* BB536 on diarrhea and/or upper respiratory illnesses in five hundred twenty healthy Malaysian children (age 2-6 years) in a randomized, double-blind, parallel, placebo-controlled study. Subjects received $5x10^9$ cfu BB536 or placebo daily for 10 months. A significant decrease in the number of respiratory illnesses

occurred in the probiotic group compared to control as well as a reduction in duration of sore throat. There was no difference in incidents of diarrhea, vomiting, or fever between the groups.

Lastly, four other studies that administered *B. longum* BB536 probiotic supplementation in doses ranging from $2.8x10^8$ to $1x10^9$ cfu/day to infants did not monitor or report adverse events (Soh et al., 2009; Grzeskowiak et al., 2012; Ishizeki et al., 2013; Wu et al., 2016). Taken together, all clinical studies suggest *B. longum* BB536 is well tolerated and safe for consumption in infants and toddlers.

Table 3. Studies of *B. longum* BB536 Ingestion in Infants and Toddlers

Reference	Study Design and Population	Treatments; Numbers of Subjects	Duration	Safety Parameters
Bennet et al., 1992	Controlled trial of orally administered *B. longum* BB536, *B. breve* BB576, and/or *L. acidophilus* LAC-343 on full-term infants previously treated with antibiotics	Of 14 infants: 3 received no probiotics (control) 3 received *B. breve* BB576 3 received *B. longum* BB536 3 received *L. acidophilus* LAC343 2 received a mix of all three strains	5 days	Adverse Events: • Adverse events were not reported or monitored
Akiyama et al., 1994	Controlled trial of orally administered *B. longum* on extremely premature infants (gestational age 26.4±0.2 weeks)	Group 1 (Control): Maltodextrin; n= 5 Group 2: 5x10^8 cells/day; n=5	8 weeks	Adverse Events: • Adverse events were not reported or monitored
Mah et al., 2007	Double-blind, placebo-controlled trial of probiotic formula (*L. rhamnosus GG* and *B. longum* BB536) on infants at risk of atopic disease	Group 1 (Control): Commercial infant formula (Nestle) without probiotics; n=17 Group 2: Commercial infant formula supplemented with 1x10^7 cfu/g BB536 and 2x10^7 cfu/g GG, n=20	6 months	Withdrawals: • 2 withdrawals from each group (reason was not reported) Adverse Events: • Adverse events were not reported or monitored

Table 3. Studies of *B. longum* BB536 Ingestion in Infants and Toddlers

Reference	Study Design and Population	Treatments; Numbers of Subjects	Duration	Safety Parameters
Puccio et al., 2007	Prospective, randomized, reference-controlled, double-blinded trial of starter formula (supplemented with *B. longum* BB536 and prebiotic mixture (90% GOS and 10% FOS)) on infants not breastfed after 14 days of birth	Group 1 (Control): starter formula (Nestle) without supplementation; n=69 Group 2: starter formula supplemented with 2x10^7 cfu total *B. longum* BB536 and 4 g/L prebiotic mixture (90% GOS and 10% FOS); n=55 Provided *ad libitum*	98 days	Withdrawals: • 14 participants withdrew from the control group and 23 withdrew from the experimental group • There was no statistically significant difference in dropout rate between the two groups Tolerance: • Intake was well tolerated as there was no statistically significant difference in stool consistency, odor, frequency of crying, restlessness, colic, spitting, and vomiting between the two groups Adverse Events: • There was no statistically significant difference in frequency or severity of adverse events reported. • Additionally, no event was associated with the experimental formula Other Parameters: • There was no statistically significant difference in recumbent length, head circumference, and absolute weight gain between groups

Table 3. Studies of *B. longum* BB536 Ingestion in Infants and Toddlers				
Reference	**Study Design and Population**	**Treatments; Numbers of Subjects**	**Duration**	**Safety Parameters**
Chouraqui et al., 2008	Prospective, randomized, controlled, double-blind, trial of infant formula supplemented with pre- and probiotics on healthy full-term infants (≥ 38 weeks)	Group 1 (Control): control formula without supplementation; n=70 Group 2: formula with 10^9 cfu total *B. longum* BB536 and *L. rhamnosus* LPR; n=70 Group 3: formula with 10^9 cfu total BB536, LPR, and 4g/L of 90% GOS/10% FOS; n=70 Group 4: formula with 10^9 cfu total BB536, *L. paracasei* ST11, and 4g/L of 90% GOS/10% FOS; n=74	16 weeks	Withdrawals: • A total of 57 infants dropped out by end of intake period • There was no statistically significant difference in dropout rate between the groups Tolerance: • There was no statistically significant difference in non-liquid stool consistency, flatulence, colic, spitting up, and vomiting between the groups • During the intake period, stool frequency was significantly higher in Group 3 compared to the control group (p=0.03), but no difference in the other groups and the control group • During the intake period, liquid stool frequency was significantly higher in Group 4 compared to the control group (p=0.005) and Group 2 (p=0.008) Adverse Events: • There was no statistically significant difference in frequency or severity of adverse events reported. • Additionally, no significant adverse event was associated with the experimental formula

Table 3. Studies of *B. longum* BB536 Ingestion in Infants and Toddlers				
Reference	**Study Design and Population**	**Treatments; Numbers of Subjects**	**Duration**	**Safety Parameters**
				Other Parameters: • There was no statistically significant difference in recumbent length, head circumference, and weight gain between groups
Rouge et al., 2009	Bicentric, double-blind, randomized, controlled trial stratified for center and birth weight of enteral probiotics (*B. longum* BB536, *L. rhamnosus* GG) on low-birth-weight preterm infants (<1500g and <32 weeks)	Group 1 (Control): placebo capsule (maltodextrin); n=49 Group 2: capsule 4x daily of 10^8 cfu total lyophilized *B. longum* BB536 and *L. rhamnosus* GG, n=45	14 days	Adverse Events • 6 deaths were observed but were not associated with intake (2 in probiotic group and 4 in control group) • No unexpected adverse events were observed
Soh et al., 2009	Randomized, double-blind, placebo-controlled trial of probiotic supplementation on Asian infants with family history of allergic disease	Group 1 (Control): commercially-available cow's milk formula (Nestle), n=126 Group 2: commercially-available cow's milk formula supplemented with 2.8x10^8 cfu/day total of *B. longum* BB536 and *L. rhamnosus* LPR (1:2), n=127	6 months	Withdrawals: • 8 participants withdrew before follow-up visits (5 from control and 3 from intake group) unrelated to study formula • 10 participants were lost post follow-up (8 from control and 2 from intake group) Adverse Events: • Adverse events were not reported or monitored

Table 3. Studies of *B. longum* BB536 Ingestion in Infants and Toddlers				
Reference	**Study Design and Population**	**Treatments; Numbers of Subjects**	**Duration**	**Safety Parameters**
Firmansyah et al., 2011	Randomized, double-blind, controlled trial of prebiotics, probiotics and long-chain polyunsaturated fatty acids in healthy Indonesian toddlers (≥ 12 months)	Group 1 (Control): cow's milk formula, n=194 Group 2: cow's milk formula supplemented with *B. longum* BB536, *L. rhamonosus* LPR, prebiotics (inulin and fructo-oligosaccharides, 30:70), and long-chain polyunsaturated fatty acids (LCPUFA), n=199 (dose not provided)	12 months	Withdrawals: • Similar dropout rate between groups (41 from control and 38 from experimental group) Tolerance: • No significant difference in stool frequency or stool characteristics between the two groups Adverse Events: • Two adverse events reported in experimental group and four in the control group, however, none were considered related to the test material • The risk of diarrhea was higher in the synbiotics group compared with the control group, however there was no difference in frequency in any other adverse events between the two groups
Hascoet et al., 2011	Randomized, double-blind, controlled trial of study formula with and without *B. longum* BB536 on healthy, full-term infants	Group 1 (Control): control formula, n=38 Group 2: study formula, n=39 Group 3: study formula + 2x10^7 cfu/g *B. longum* BB536, n=40 Reference group: breast-fed, n=70	4 months	Withdrawals: • There were 49 infant withdrawals, however, data from these infants were used when available Tolerance: • There was no statistically significant difference in stool frequency, vomiting, spitting up, crying, fussiness, colic, or flatulence between any groups

Table 3. Studies of *B. longum* BB536 Ingestion in Infants and Toddlers				
Reference	**Study Design and Population**	**Treatments; Numbers of Subjects**	**Duration**	**Safety Parameters**
				Adverse Events: • There was no statistically significant difference in adverse events reported among groups (most common ones affected GI or upper respiratory tract)
Grzeskowiak et al., 2012	Bicentric, Randomized, double-blind, placebo-controlled trial of probiotics on infants involved in an allergy prevention study	Finland Group 1 (Control): control formula (Nestle Pro Natal), n=22 Group 2: formula + 10^9 cfu/day *L. rhamnosus* LPR and 10^9 cfu/day *B. longum* BB536, n=28 Group 3: formula + 10^9 cfu/day *L. paracasei* ST11 and 10^9 cfu/day *B. longum* BB536, n=29 Germany Group 1 (Control): partially hydrolyzed 100% whey formula (Nestle Beba-HA), n=32 Group 2: formula + 10^9 cfu/day *L. rhamnosus* LPR and 10^9 cfu/day *B. longum* BB536, n=24 Group 3: formula + 10^9 cfu/day *B. longum* BB536, n=25	Finland: 2 months Germany: 4 months	Adverse Events: • Adverse events were not reported or monitored

Table 3. Studies of *B. longum* BB536 Ingestion in Infants and Toddlers

Reference	Study Design and Population	Treatments; Numbers of Subjects	Duration	Safety Parameters
Ishizeki et al., 2013	Controlled trial of bifidobacterial on intestinal microbiota in low-birth weight infants (1000-2000g)	Group 1 (Control): No probiotic, n=16 Group 2: 5x10^8 cfu/day *B. breve* M-16V n=15 Group 3: 5x10^8 cfu/day each of *B. breve* M-16V, *B. infantis* M-63, and *B. longum* BB536 n=13	6 weeks	Adverse Events: • Adverse events were not reported or monitored
Enomoto et al., 2014	Open trial of *Bifidobacterium* supplementation on allergy development in healthy infants	Group 1 (Control): No probiotic, n=36 Group 2: 5x10^9 cfu/day each of *B. longum* BB536 and *B. breve* M-16V, n=130	6 months	Adverse Events: • No adverse events reported were related to intake
Wu et al., 2016	Randomized, double-blind, controlled trial of *Bifidobacterium* supplementation on healthy infants	Group 1 (Control): commercial formula, n=148 Group 2: commercial formula supplemented with 1x10^7 cfu/g *B. longum* BB536 , n=153	6 months	Withdrawals: • Total of 36 dropouts (19 in control group and 17 in the intake group) • None were associated with the intake Adverse Events: • Adverse events were not reported or monitored
Lau et al., 2018	Randomized, double-blind, parallel, placebo-controlled study of *B. longum* BB536 in healthy Malaysian children (age 2-6 years)	Group 1 (Control): placebo, n=261 Group 2: 5x10^9 cfu/day *B. longum* BB536 (Morinaga), n=259	10 months	Tolerance: • No difference in diarrhea, vomiting, or fever between the groups • There was a significant reduction in the occurrence of respiratory illnesses and a reduction in duration of sore throat (46% fewer days) in the experimental group compared to the control and.

D. REVIEW OF SCIENTIFIC LITERATURE ON OTHER STRAINS OF *B. LONGUM*

No new published studies on other strains of *B. longum* were retrieved from the Pubmed on February 12, 2019. Therefore, all studies discussed in GRN 268 are incorporated by reference, pages 65-66. Animal and clinical studies conducted on other strains of *B. longum* provide corroborative evidence for the safety of human consumption of *B. longum* BB536. Rats administered $1x10^9$ cfu/kg-bw/day of *B. longum* SP1205 showed no adverse effects on general health, growth, blood biochemistry, or histological parameters. In a 7-day restricted diet study on mice, *B. longum* OLL6001 resulted in no body weight change. Results from healthy adults consuming $4x10^9$ cfu/day of *B. longum* 46 and *B. longum* 2C showed no safety-related adverse events and were well-tolerated. These results provide corroborative evidence to support the evidence that *B. longum* BB536 is safe for human consumption.

E. CORROBORATIVE EVIDENCE FOR THE SAFETY OF *B. LONGUM* BB536: ANIMAL TOXICITY STUDIES USING OTHER SPECIES OF *BIFIDOBACTERIA*

No new published studies that provide corroborative evidence on the safety of *B. longum* BB536 were retrieved from the Pubmed on February 12, 2019. Therefore, all studies discussed in GRN 268 are incorporated by reference, pages 67-74. While other bifidobacterial strains are not identical to *B. longum* BB536, they share many common characteristics. Therefore, studies on other bifidobacteria species commonly used as probiotics or in food production, such as *B. breve* and *B. infantis*, can be used to provide corroborative evidence for the safety of *B. longum* BB536. Results from acute studies in rats demonstrate that under conditions of the tests, neither *B. breve* nor *B. infantis* presented toxicological concerns at the highest doses tested. Results from subchronic toxicity studies of *B. infantis* and *B. breve* in rats demonstrate that under conditions of the tests, NOAELs were determined to be the doses tested. The doses tested were $2.3x10^{11}$ cfu *B. breve*/kg-bw/day and $7.6x10^{10}$ CFU *B. infantis*/kg-bw/day. Results from these studies of *B. breve* and *B. infantis* provide corroborative data to support the available evidence that *B. longum* BB536 is safe for human consumption.

VII. SUPPORTING DATA AND INFORMATION

A. REFERENCES

All information included in the following list of references is generally available.

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B. EXPERT PANEL STATEMENT

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of the use of *Bifidobacterium longum* BB536 as an ingredient for use in infant formula. This GRAS determination for the use of *B. longum* BB536 as an ingredient in term infant formulas at a maximum level of 1×10^8 CFU *B. longum* BB536 per gram of product at the end of product shelf-life is based upon scientific procedures as described under 21 CFR §170.30(b). The intake of *B. longum* BB536 from the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). To demonstrate that *B. longum* BB536 is safe, and GRAS, under the intended conditions of use, the safety of the intake of *B. longum* BB536 has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The safety of the intake of *Bifidobacterium longum* BB536 has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to infant formula, and is based on generally available and accepted information.

The proposed use of *B. longum* BB536 as an ingredient in term infant formula has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1) Bifidobacteria are naturally occurring bacteria that contribute to the composition of the gut microflora of humans. Bifidobacteria represent up to 25% of the cultivatable fecal bacteria in adults and 80% in infants. *Bifidobacterium longum* has been detected in feces from infants and adults.

2) *Bifidobacterium longum* BB536, a strain of *B. longum*, is a Gram-positive anaerobic bacterium. *B. longum* BB536 was originally isolated from a healthy infant in 1969. The bacterium has been deposited with the American Type Culture Collection (ATCC) and is designated BAA-999™.

3) The maintenance of the original frozen culture has been tightly controlled to ensure purity and stability of the strain.

4) Finished products made with *B. longum* BB536 cultures reproducibly meet compositional standards and comply with limits on contaminants appropriate for food-grade ingredients. Product specifications are set to assure that *B. longum* BB536 is suitable for use in infant formula.

5) Bifidobacteria are commonly consumed in fermented foods throughout the world. *B. longum* BB536 was first commercially available in Japan in 1977 and the availability of *B. longum* BB536 on the European market began in 1986.

6) *B. longum* BB536 has been tested for parameters outlined in the Food and Agriculture Organization of the United Nations/World Health Organization's (FAO/WHO) guidelines for the evaluation for microbes for probiotic use in foods. Results from these tests provide evidence that *B. longum* BB536 is safe for use in foods, namely:

   - Available antibiotic resistance pattern suggests that *B. longum* BB536 does not present concerns for antibiotic resistance in humans.
   - *B. longum* BB536 produces predominantly L-lactic acid, while production of D- lactic acid is negligible.
   - *B. longum* BB536 has been reported to deconjugate bile salts. The production of deconjugated bile salts was concurrent with bacterial growth, and deconjugated bile salts were the only compound produced.
   - Results from comparisons of amino acid sequences of known bacterial toxins with sequences of the predicted proteins from the genomic sequence of *B. longum* BB536 and genomic sequences of three known pathogens with sequences of the predicted proteins from the genomic sequence of *B. longum* BB536 indicate that there is no significant homology.
   - *B. longum* BB536 was not observed to have hemolytic activity.

7) The LD_{50} of *B. longum* BB536 orally administered to mice was determined to be ~$5x10^{13}$ cfu/kg-bw. The LD_{50} of *B. longum* BB536 administered intraperitoneally to mice was determined to be ~$9x10^{11}$ cfu/kg-bw.

8) Results from repeat dose studies of *B. longum* BB536 administered to rats show no intake effects on body weight, body weight gain, or feed intake at doses up to 2 x 10^{12} cfu/kg bw/day. Findings for the studies provide support for the safe use of *B. longum* BB536 under the test conditions.

9) Seventeen clinical studies (reported in 14 papers) involving the administration of *B. longum* BB536 to healthy adults were identified and reviewed. The duration of *B. longum* BB536 consumption ranged from 6 days to 14 weeks. In three studies, intakes of *B. longum* BB536 were approximately 10^{11} cfu per day; participants consumed this dose for periods of 4, 13 or 14 weeks. In all other human studies reviewed, doses were in the range of approximately 10^{9} to 10^{10} cfu *B. longum* BB536 per day. None of the studies reported any participant dropouts or adverse events due to the test articles. Findings from the studies provide support for the safe and well-tolerated use of *B. longum* BB536 under the test conditions.

10) Eleven clinical studies involving the administration of *B. longum* BB536 to unhealthy adults or children were identified and reviewed. Study durations ranged from 8 weeks to 1 year. Daily viable *B. longum* BB536 intakes for adults were in the range of 10^{9} to10^{10} cfu in most studies, and approximately 10^{11} cfu *B. longum* BB536 per day in one study. Daily doses of *B. longum* BB536 in populations of children were approximately 10^{9} cfu. None of the studies reviewed reported adverse events or patient dropouts as a result of *B. longum* BB536 supplementation. Findings from the studies provide support for the safe and well-tolerated use of *B. longum* BB536 under the test conditions.

11) Fourteen studies involving the administration of *B. longum* BB536 to infants or toddlers were identified and reviewed. Study durations ranged from 5 days to 12 months. Infants and toddlers received formula supplemented with *B. longum* BB536 ranging from 10^{7}-10^{9} cfu. None of the studies reported adverse events due to *B. longum* BB536 supplementation. Findings from the studies provide support for the safe and well-tolerated use of *B. longum* BB536 in infants and toddlers under the test conditions.

12) Research on bifidobacteria has been conducted on several strains of *Bifidobacterium longum*; results from studies of other strains of *B. longum* provide corroborative evidence for the safety of human consumption of *B. longum* BB536.

13) While other bifidobacterial strains are not identical to *B. longum* BB536, they share many common characteristics. Therefore, studies on other bifidobacteria species

commonly used as probiotics or in food production, such as *B. breve* and *B. infantis,* can be used to provide corroborative evidence for the safety of *B. longum* BB536. Results from acute studies in rats demonstrate that under conditions of the tests, neither *B. breve* nor *B. infantis* presented toxicological concerns at the highest doses tested. Results from subchronic toxicity studies of *B. infantis* and *B. breve* in rats demonstrate that under conditions of the tests, No Observed Adverse Effect Levels (NOAELs) were determined to be the doses tested. The doses tested were $2.3x10^{11}$ cfu *B. breve*/kg-bw/day and $7.6x10^{10}$ cfu *B. infantis*/kg-bw/day. Results from these studies of *B. breve* and *B. infantis* provide corroborative data to support the available evidence that *B. longum* BB536 is safe for human consumption.

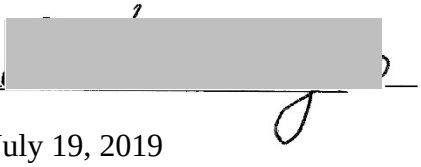
14) Assuming addition of $1x10^8$ cfu of *B. longum* BB536 per gram of infant formula, the formula is the sole source of nutrition, and the caloric requirements of a 1-month-old infant and 6-month-old infant are 472 kcal/day and 645 kcal/day, the estimated intakes of *B. longum* BB536 from infant formula are $9.9x10^9$ and $1.35x10^{10}$ cfu, respectively.

Determination of the GRAS status of *B. longum* BB536 under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, FACN, FASN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, and Thomas E. Sox, PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of *B. longum* BB536 and the potential human exposure to *B. longum* BB536 resulting from its intended use as an ingredient in term infant formula and have concluded:

There is no evidence in the available information on B. longum BB536 that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when B. longum BB536 is used at levels that might reasonably be expected from the proposed applications. B. longum BB536 is GRAS for use in infant formula as proposed by Morinaga Milk Industry Co, Ltd.

Therefore, *B. longum* BB536 is safe and GRAS at the proposed levels of addition to term infant formula. *B. longum* BB536 is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

Roger Clemens, DrPH, CNS, FACN, FIFT Signature: 
GRAS Expert Panel Member
School of Pharmacy Date: July 19, 2019
University of Southern California

A. Wallace Hayes PhD, DATS, FACT, FACN Signature: 
GRAS Expert Panel Member
University of South Florida Date: July 19, 2019
College of Public Health

Thomas E. Sox, PhD, JD Signature: 
GRAS Expert Panel Member
Principal, Pondview Consulting LLC Date: July 19, 2019

October 28, 2019

Stephanie Hice, Ph.D.
Staff Fellow (Biologist)
Division of Food Ingredients
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RE: Response to FDA Questions on GRN 000877 - *Bifidobacterium longum* BB536 in Infant Formula

Dear Dr. Hice:

In response to your e-mail of October 10, 2019, following are our responses to FDA's questions on GRN 877. FDA's questions are in italics and our responses are in plain text

> *1. Please state whether any of the raw materials used in the fermentation media and during production of Bifidobacterium longum BB536 are major allergens or derived from major allergens. Please state whether the final ingredient contains any major allergens.*

None of the raw materials used during the production of *Bifidobacterium longum* BB536 are major allergens or derived from major allergens. The final ingredient also does not contain any major allergens.

> *2. The notice does not mention the type of formula the notifier intends to add the ingredient to. Please indicate the intended source of the protein base (e. g., milk, soy, whey) of infant formula.*

The notifier intends for the ingredient to be added to all protein-based and amino acid-based infant formulas. Please add "milk, soy, whey, or amino acid-based term infant formulas" under Section E. Intended Use on page 1 of the GRN 877 dossier.

> *3. The notifier indicated that B. longum BB536 is a white to slightly brown powder. Please include a statement indicating that B. longum BB536 is not intended to be used as a color additive.*

B. longum BB536 produced by Morinaga Milk Industry Co., Ltd. is not intended to be used as a color additive.

> *4. The notifier should indicate that all analytical methods used to analyze the batches for conformance with the stated specifications have been validated for that particular purpose.*

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Morinaga Milk Industry Co., Ltd. have validated all of their analytical methods for qualification of each batch with the product specifications.

> *5. In the product specifications, the notifier should provide a specification for Escherichia coli and data from 3 non-consecutive batches demonstrating conformance with the provided specification.*

The microbiological specifications include a specification for *Enterobacteriaceae* of not detected in 10g which is inclusive of *Escherichia coli*. However, in order to address this question, the notifier has tested their batches for *E. coli* and all are negative (see data below). The notifier will periodically check for *E. coli* as a quality control check.

Parameter	**Method**	**Lot No.**					
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Escherichia coli	ISO 7251:2005	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Should you have additional questions, please let us know.

Sincerely,

[redacted]

Claire L, Kruger, Ph.D., D.A.B.T.
President

ChromaDex Spherix Consulting
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November 05, 2019

Questions/Comments Regarding GRN 000877:

On October 10, 2019, FDA asked the following:

- The notice does not mention the type of formula the notifier intends to add the ingredient to. Please indicate the intended source of the protein base (e. g., milk, soy, whey) of infant formula.

On October 28, 2019, the notifier provided the following answer:

- "The notifier intends for the ingredient to be added to all protein-based and amino acid-based infant formulas. Please add "milk, soy, whey, or amino acid-based term infant formulas" under Section E. Intended Use on page 1 of the GRN 877 dossier."

The GRAS notice stated the intended use as: "... term non-exempt infant formulas for healthy infants at 1 x 10^8 CFU/g infant formula throughout the product shelf-life." FDA asked the notifier about the intended source of protein base (ex. milk, soy, whey). However, the notifier is now adding amino acid-based infant formulas; amino-acid based infant formulas are exempt infant formulas, which changes the intended use of this ingredient.

Amino acid-based infant formulas are considered exempt infant formulas, which are formulas represented and labeled for use by infants who have inborn errors of metabolism or low birth weight, or who otherwise have unusual medical or dietary problems.

1. Does the notifier intend to keep the new inclusion of amino acid-based formulas, or remove it? Should the notifier want to include amino acid-based formulas, they would need to provide the basis for the safe use of the ingredient in the new intended population.

2. Please clarify the intended use of *Bifidobacterium longum* BB536 and the intended source of the protein base of infant formula.

From:	kbrailer@spherixgroup.com
To:	Hice, Stephanie
Cc:	ckruger@spherixgroup.com; dconze@spherixgroup.com; "Fred Lozy"
Subject:	RE: GRN 000877 - Additional Questions for Notifier
Date:	Friday, November 8, 2019 1:58:27 PM

Dear Dr. Hice,

The notifier intends for the ingredient to be added to all protein-based non-exempt infant formulas. Please add “milk, soy, and whey term infant formulas” and remove amino-acid based under Section E. Intended Use on page 1 of the GRN 877 dossier.

Please let us know if this response is sufficient or if you require additional information.

Best regards,

Kathy Brailer
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kbrailer@spherixgroup.com
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> **From:** "Hice, Stephanie" <Stephanie.Hice@fda.hhs.gov>
> **Date:** November 5, 2019 at 2:51:30 PM EST
> **To:** "ckruger@spherixgroup.com" <ckruger@spherixgroup.com>
> **Subject: RE: GRN 000877 - Additional Questions for Notifier**
>
> Dear Dr. Kruger,
>
> During our review of GRAS Notice No. 000877 and your October 28 response to our clarifying questions, we noted further questions that need to be addressed and are attached to this email.
>
> We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.
>
> If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.
>
> Sincerely,
>
> Stephanie Hice
>
> **Stephanie Hice, PhD**
> *Staff Fellow (Biologist)*

Division of Food Ingredients

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

From:	kbrailer@spherixgroup.com
To:	Hice, Stephanie
Cc:	ckruger@spherixgroup.com; dconze@spherixgroup.com; "Fred Lozy"
Subject:	RE: GRN 000877 - Additional Questions for Notifier
Date:	Wednesday, November 13, 2019 4:23:08 PM
Attachments:	image001.png

Dear Dr. Hice,

Yes, milk, soy, and whey are the only protein sources of infant formula the ingredient would be added to.

Best regards,

Kathy Brailer
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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Sent: Wednesday, November 13, 2019 3:14 PM
To: kbrailer@spherixgroup.com
Cc: ckruger@spherixgroup.com; dconze@spherixgroup.com; 'Fred Lozy' <flozy@spherixgroup.com>
Subject: RE: GRN 000877 - Additional Questions for Notifier

Dear Ms. Brailer,

Thank you for your prompt reply to our questions.

To clarify, when the notifier states that they intend "… for the ingredient to be added to all protein-based non-exempt infant formulas", are milk, soy and whey the only protein sources that the ingredient will be added to?

Thank you, and please let me know if you have any questions.

Sincerely,

Stephanie Hice

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients

Center for Food Safety and Applied Nutrition

Office of Food Additive Safety
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

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Sent: Friday, November 8, 2019 1:58 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Cc: ckruger@spherixgroup.com; dconze@spherixgroup.com; 'Fred Lozy' <flozy@spherixgroup.com>
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Please let us know if this response is sufficient or if you require additional information.

Best regards,

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If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

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Staff Fellow (Biologist)
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