04 March 2022

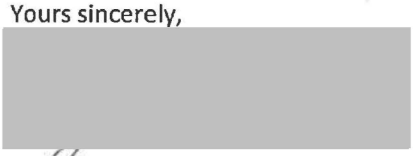
Richard Bonnette, M.S.
Consumer Safety Officer
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

Dear Mr. Bonnette

Re: GRAS Notice for *Bifidobacterium bifidum* OLB6378

Please find this attached letter authorizing Intertek Health Science Inc. as Meiji's official agent for all matters related to Meiji's GRAS notification for *Bifidobacterium bifidum* OLB6378 that was submitted to the offices of the U.S. FDA on 04 March 2022. Ryan Simon, Sr. Director, Safety & Regulatory of the Food & Nutrition Group at Intertek will be the primary contact for this notice. Mr. Simon can be reached at the following address ryan.simon@intertek.com or *via* telephone at +1 905 286-4188.

Yours sincerely,



Yoshitaka Nakamura, Ph.D.
Meiji Co., Ltd.
yoshitaka.nakamura@meiji.com

GRAS NOTICE FOR *BIFIDOBACTERIUM BIFIDUM* OLB6378

SUBMITTED TO:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
Yoshitaka Nakamura, Ph.D.
Meiji Co., Ltd.
R&D Division
Food Microbiology and Function Research Laboratories
1-29-1 Nanakuni, Hachiouji
Tokyo 192-0919, Japan

DATE:
25 February 2022

GRAS Notice for *Bifidobacterium bifidum* OLB6378

TABLE OF CONTENTS

List of Appendices

List of Figures and Tables

GRAS Notice for *Bifidobacterium bifidum* OLB6378

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Meiji Co., Ltd. (Meiji) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of freeze-dried *Bifidobacterium bifidum* OLB6378 powder, as manufactured by Meiji, in infant formula as described in Section 1.3 below, are not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on Meiji's view that these notified uses of freeze-dried *B. bifidum* OLB6378 powder are Generally Recognized as Safe (GRAS). In addition, as a responsible official of Meiji, the undersigned hereby certifies that all data and information presented in this notice represents a complete and balanced submission that is representative of the generally available literature.
Meiji considered all unfavorable as well as favorable information that is publicly available and/or known and that is pertinent to the evaluation of the safety and GRAS status of freeze-dried *B. bifidum* OLB6378 powder as a food ingredient for addition to infant formula, as described herein.

Signed,

Yoshitaka Nakamura, Ph.D.
Meiji Co., Ltd.
Yoshitaka.nakamura@meiji.com

01 March 2022
Date

1.1 Name and Address of Notifier

Yoshitaka Nakamura, Ph.D.
Meiji Co., Ltd.
R&D Division
Food Microbiology and Function Research Laboratories
1-29-1 Nanakuni, Hachiouji
Tokyo 192-0919, Japan

1.2 Common Name of Notified Substance

The subject of this GRAS notification is freeze-dried *B. bifidum* OLB6378 powder.

1.3 Conditions of Use

Meiji's freeze-dried *B. bifidum* OLB6378 powder is intended for use as a food ingredient in non-exempt term infant formula at a use level of 2.28×10^6 CFU/mL. A summary of the food uses and corresponding use levels in which freeze-dried *B. bifidum* OLB6378 powder is intended for use is provided in Table 1.3-1.

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder in the U.S.

Food Uses	Freeze-dried *Bifidobacterium bifidum* OLB6378 Powder[a] in Formula (mg/100 g)	Freeze-dried *Bifidobacterium bifidum* OLB6378 Powder[a] in Reconstituted or Ready-to-Drink Formula (mg/100 mL)	*Bifidobacterium bifidum* OLB6378 Concentration in Lyophilized Ingredient (CFU/g)	*Bifidobacterium bifidum* OLB6378 Concentration in Formula (CFU/mL)
Non-Exempt Term Infant Formula	13.5	1.82[b]	1.25×10^{11}	2.28×10^{6}

CFU = colony forming units; U.S. = United States.

[a] Containing 0.5 g of *B. bifidum* OLB6378 concentrate per gram.

[b] Dissolution ratio of 13.5% (68 kcal/100 mL).

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2019a), Meiji has concluded that the intended uses of freeze-dried *B. bifidum* OLB6378 powder as described herein are GRAS on the basis of scientific procedures.

1.5 Availability of Information

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

Meiji Co., Ltd.
R&D Division
Food Microbiology and Function Research Laboratories
1-29-1 Nanakuni, Hachiouji
Tokyo 192-0919, Japan

Should the FDA have any questions or additional information requests regarding this Notification, Meiji will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Meiji's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. § 170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

2.1.1 Taxonomic Lineage

B. bifidum OLB6378 is a substrain isolate of the *Bifidobacterium* genus (Table 2.1.1-1).

Table 2.1.1-1 Taxonomic Lineage of *Bifidobacterium bifidum* OLB6378

Rank	Scientific Name
Kingdom	*Bacteria*
Phylum	*Actinobacteria*
Class	*Actinobacteria*
Order	*Bifidobacteriales*
Family	*Bifidobacteriaceae*
Genus	*Bifidobacterium*
Species	*B. bifidum*
Strain	OLB6139
Substrain	OLB6378

2.1.2 History of *B. bifidum* OLB6378

B. bifidum OLB6378 is derived from subculture of the original parent isolate *B. bifidum* OLB6139 (Toshimitsu *et al*., 2013) that was obtained from a human infant feces sample. The substrain *B. bifidum* OLB6378 was selected as the industrial candidate from a total of 3 substrains of *B. bifidum* OLB6139 based on its superior transgenerational stability and reproductive potential over 10 generations. *B. bifidum* OLB6378 was deposited to the National Institute of Technology and Evaluation (NITE) Patent Microorganisms Depository on 26 October 2004 (Deposit number: NITE BP-31).

2.1.3 Phenotypic Identity

B. bifidum are Gram-positive, anaerobic, rod-shaped bacteria that are indigenous to the gastrointestinal tract of humans. The species is one of the first colonizers of the human gut and is a dominant member of the gut microbiota of breastfed infants (Turroni *et al*., 2014; Stewart *et al*., 2018). *B. bifidum* display a unique repertoire of glycosidic hydrolases that are predicted to be involved in the metabolism of mucin and the organism can use mucin as a sole carbon source. *B. bifidum* also play a key role in host-microbiome interactions (Turroni *et al*., 2014).

2.1.4 Genotypic Identity

The genome of strain *B. bifidum* OLB6378 has been sequenced and annotated. The complete genome sequence is 2,194,322 base pairs, 1,994 genes, has a guanine-cytosine (GC) content of 62.79%, and is absent of plasmids or prophage elements (Table 2.1.4-1). Analysis of the whole genome sequence of *B. bifidum* OLB6378 using Basic Local Alignment Search Tool (BLAST) analysis demonstrated close DNA homology to 2 known *B. bifidum* strains (S17 and PRL_2010). A polymerase chain reaction (PCR) assay was performed on *B. bifidum* OLB6378 using a primer set specific to *Bifidobacterium* targeting a specific region of 523 base

pairs, and a primer set specific to *B. bifidum* targeting a specific region of 273 base pairs. In both tests the expected PCR products were detected, thereby confirming the species identity of the strain as *B. bifidum*. A strain specific PCR primer set for *B. bifidum* OLB6378 based on a random amplified polymorphic DNA (RAPD)-PCR technique has been developed by Toshimitsu *et al*. (2013). The PCR primer set was demonstrated to discriminate *B. bifidum* OLB6378 from 47 other strains of *B. bifidum* and 20 different species of *Bifidobacterium* and, therefore, can be used for detection and quantification of *B. bifidum* OLB6378.

Table 2.1.4-1 Overview of the Genomes for *Bifidobacterium bifidum* OLB6378

Strain	OLB6378
Genome size (bp)	2,194,322
GC %	62.79
Gene (CDS)	1,994
rRNA cluster	3
tRNA	52
Plasmid	0
Prophage	0

bp = base pairs; CDS = coding sequence; GC = guanine-cytosine; rRNA = ribosomal ribonucleic acid; tRNA = transfer ribonucleic acid.

2.2 Manufacturing

2.2.1 Additives and Processing Aids

B. bifidum OLB6378 is produced by culture fermentation. The fermentation medium contains nutrient sources and ingredients that are commonly used in microbial growth media. All additives, processing aids, and food contact articles used during the manufacturing of freeze-dried *B. bifidum* OLB6378 powder are food-grade, are permitted for their respective uses by an appropriate federal regulation, have GRAS status, and/or have been the subject of an effective food contact notification (Table 2.2.1-1).

Table 2.2.1-1 Additives and Processing Aids Used in the Production of Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder

Raw Material	Use	Regulatory Status
Skim milk	Starter medium	GRAS (21 CFR §182.1)
Yeast extract	Starter and manufacturing medium	Bakers yeast extract is a direct food substance affirmed as GRAS as defined in 21 CFR §184.1983 (U.S. FDA, 2019a)
Hydrolyzed whey protein	Manufacturing medium	Whey is a direct food substance affirmed as GRAS as defined in 21 CFR §184.1979 (U.S. FDA, 2019a)
Lactose	Manufacturing medium	GRAS (21 CFR §168.122 – U.S. FDA, 2019a)
Casein	Manufacturing medium	Sodium caseinate is GRAS when used in accordance with GMP (21 CFR §182.1748 – U.S. FDA, 2019a)
Sodium hydroxide	Manufacturing medium	Used in food with no limitation other than cGMP (21 CFR §184.1763 – U.S. FDA, 2019a)
Potassium carbonate	Manufacturing medium	Used in food with no limitation other than cGMP (21 CFR §184.1619 – U.S. FDA, 2019a)
Sucrose	Carbohydrate carrier	Used in food with no limitation other than cGMP (21 CFR §184.1854 – U.S. FDA, 2019a)

Table 2.2.1-1 Additives and Processing Aids Used in the Production of Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder

Raw Material	Use	Regulatory Status
Trehalose	Carbohydrate carrier	GRAS for use in foods in general, including meat products, for multiple technical effects at levels in accordance with GMP (GRN 45 – U.S. FDA, 2000)

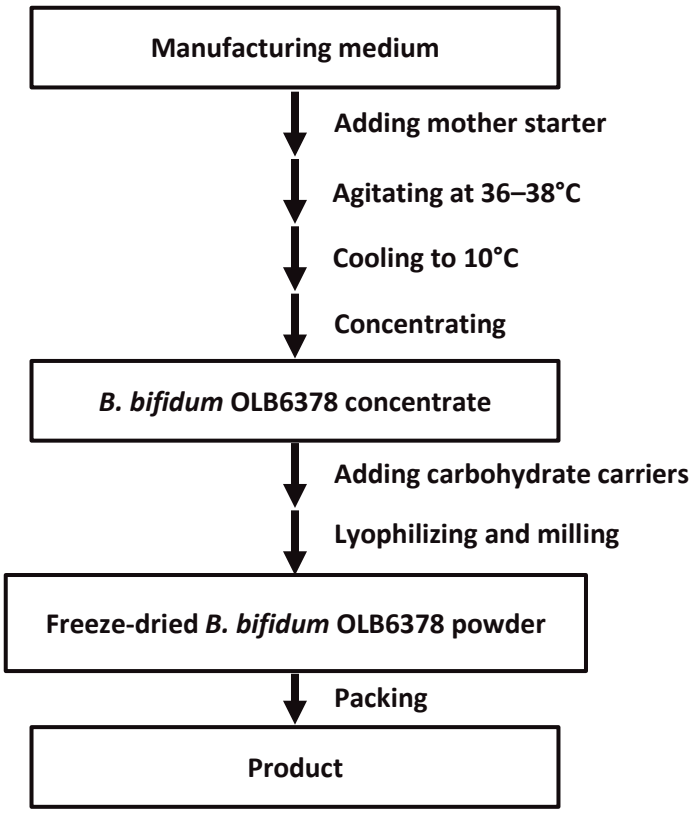
CFR = Code of Federal Regulations; cGMP = current Good Manufacturing Practice; GMP = Good Manufacturing Practice; GRAS = Generally Recognized as Safe; GRN = GRAS Notice.

2.2.2 Manufacturing Process

The manufacturing process for freeze-dried *B. bifidum* OLB6378 powder consists of culturing, concentrating, lyophilizing, and packaging and complies with current Good Manufacturing Practice (cGMP) guidelines. In preparation of the mother starter, a frozen original stock is thawed and transferred to starter medium for activation. Original stocks of *B. bifidum* OLB6378 are maintained by Food Microbiology and Function Research Laboratories of Meiji Co., Ltd. Activated *B. bifidum* OLB6378 is subcultured in 3 scale-up procedures to create the mother starter. During all subculture steps, the same starter medium is used, and the temperature is controlled at 37°C. Prior to use, the manufacturing medium is sterilized and then cooled for inoculation with the mother starter.

To begin the culturing process, the mother starter is combined with the manufacturing medium. During the culturing process, the medium is gently agitated, and the temperature is held at 36 to 38°C. After the culturing process, the culture is cooled to 10°C and analyzed by microbiological tests for quality control. The *B. bifidum* OLB6378 is then concentrated by centrifugation. Carbohydrate carriers (*e.g.,* sucrose, trehalose) are added to the *B. bifidum* OLB6378 concentrate as cryopreservation aids to facilitate the freeze-drying process and the powder is then milled and packaged into foil bags. The freeze-dried *B. bifidum* OLB6378 powder is then tested for quality control purposes. A schematic overview of the manufacturing process of freeze-dried *B. bifidum* OLB6378 powder is presented in Figure 2.2.2-1.

Figure 2.2.2-1 Schematic Overview of the Manufacturing Process of Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder



2.3 Product Specifications and Batch Analyses

2.3.1 Specifications

Meiji has established physical, chemical, and microbial specifications for freeze-dried *B. bifidum* OLB6378 powder to ensure the product is of food-grade quality. The specifications for freeze-dried *B. bifidum* OLB6378 powder are presented in Table 2.3.1-1.

Table 2.3.1-1 Specifications of Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder[a]

Parameter	Specification	Test Method
Bifidobacterium	$>1.25 \times 10^{11}$ CFU/g	BL Agar anaerobic plating method
Heavy Metals		
Arsenic	<0.1 ppm	Atomic absorption spectrometry
Lead	<0.05 ppm	Atomic absorption spectrometry
Heavy Metals (as Lead)	<5 ppm	Sodium sulfide colorimetric method
Microbial Quality		
Water activity	<0.11	Graphical interpolation method
Aerobic plate count	<1,000 CFU/g	Standard Agar plating method
Coliforms	Negative/2.22 g	BGLB broth inoculating method

Table 2.3.1-1 Specifications of Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder[a]

Parameter	Specification	Test Method
Salmonella spp.	Negative/25 g	Enrichment culture method
Bacillus cereus	Negative/0.01 g	Surface spread plating method
Staphylococcus aureus	Negative/0.01 g	Surface spread plating method
Cronobacter sakazakii	Negative/5 g	ISO/TS 22964
Molds	Negative/0.1 g	Potato Dextrose Agar plating method
Yeast	Negative/0.1 g	Potato Dextrose Agar plating method

CFU = colony forming units; ppm = parts per million.
[a] Containing 0.5 g of *B. bifidum* OLB6378 concentrate per gram.

2.3.2 Batch Analysis

Four non-consecutive lots of freeze-dried *B. bifidum* OLB6378 powder (Lot No. 130703, 141030, 150205, and 190808) were analyzed to verify that the manufacturing process produces a consistent product that meets the product specifications. A summary of the product analysis for the 4 lots of freeze-dried *B. bifidum* OLB6378 powder, demonstrating that all parameters are within the product specifications, is presented in Table 2.3.2-1.

Table 2.3.2-1 Batch Analysis of Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder[a]

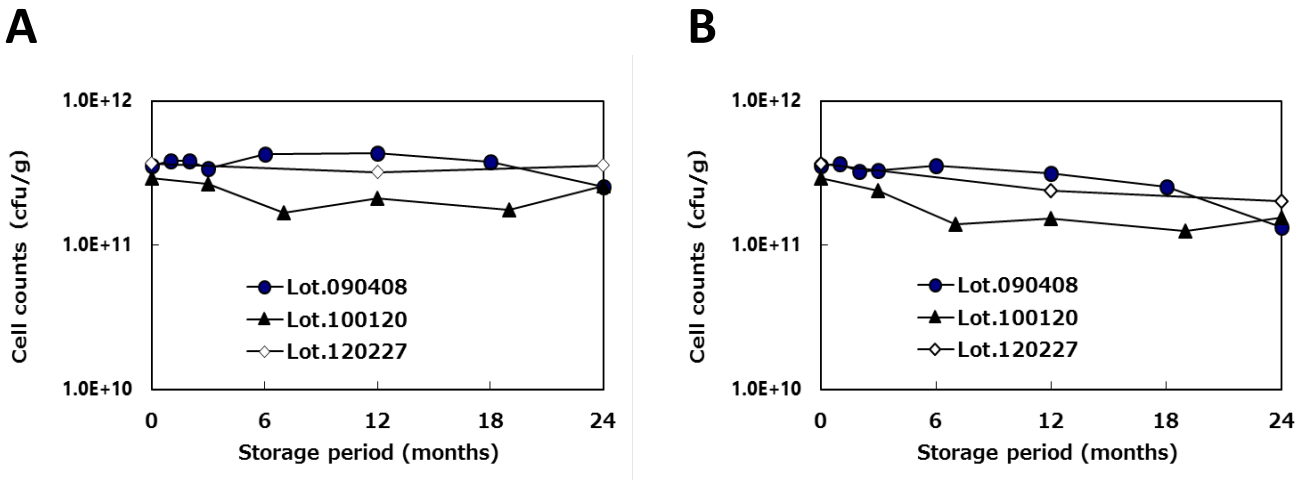
Parameter	Specification	Lot No.			
		130703	141030	150205	190808
Bifidobacterium	$>1.25 \times 10^{11}$ CFU/g	3.9×10^{11}	3.9×10^{11}	5.8×10^{11}	3.7×10^{11}
Heavy Metals					
Arsenic	<0.1 ppm	<0.1	<0.1	<0.1	<0.1
Lead	<0.05 ppm	NM	<0.05	<0.05	<0.05
Heavy Metals (as Lead)	<5 ppm	<5	<5	<5	<5
Microbial Quality					
Water activity	<0.11	<0.11	<0.11	<0.11	<0.11
Coliforms	Negative/2.22 g	Negative	Negative	Negative	Negative
Aerobic plate count	<1,000 CFU/g	<300	<300	<300	<300
Coliforms	Negative/2.22 g	Negative	Negative	Negative	Negative
Salmonella spp.	Negative/25 g	Negative	Negative	Negative	Negative
Bacillus cereus	Negative/0.01 g	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative/0.01 g	Negative	Negative	Negative	Negative
Cronobacter sakazakii	Negative/5 g	Negative	Negative	Negative	Negative
Molds	Negative/0.1 g	Negative	Negative	Negative	Negative
Yeast	Negative/0.1 g	Negative	Negative	Negative	Negative

CFU = colony forming units; NM = not measured; No. = number; ppm = parts per million.
[a] Containing 0.5 g of *B. bifidum* OLB6378 concentrate per gram.

2.4 Stability

Meiji provided stability data demonstrating that freeze-dried *B. bifidum* OLB6378 powder (Lot No. 090408, 100120, and 120227) stored at -20°C and 10°C is stable for 24 months (Figure 2.4-1). Freeze-dried *B. bifidum* OLB6378 powder stored at -20°C experienced no significant losses in viability (CFU/g) over 24 months. Although the viability of *B. bifidum* OLB6378 stored at 10°C experienced a slight downward trend over 24 months compared to baseline values, at no timepoint over the 24-month period did the viability of *B. bifidum* OLB6378 stored at either temperature fall below 1.25×10^{11} CFU *B. bifidum*/g.

Figure 2.4-1 Viability of *Bifidobacterium bifidum* OLB6378 in Lyophilized Concentrate over 24 Months at (A) -20°C and (B) 10°C



Part 3. §170.235 Dietary Exposure

3.1 History of Use of *B. bifidum* OLB6378

3.1.1 Natural Occurrence of *B. bifidum*

Bifidobacterium sp. colonize the human infant intestine within the first weeks of life from maternal transfer and dominant growth supported by bifidogenic agents provided by human breast milk (Peirotén *et al*., 2018). The dominant bifidobacterial species in breastfed infants are *B. bifidum, Bifidobacterium breve, Bifidobacterium catenulatum* group, and *Bifidobacterium longum* subsp. *infantis* (Turroni *et al*., 2014; Kato *et al*., 2017; Nagpal *et al*., 2017; Stewart *et al*., 2018). Therefore, the presence of *B. bifidum* in the intestine of infants is considered normal.

3.1.2 *B. bifidum* as a Food Ingredient

Several bifidobacteria species are consumed globally as a component of a variety of fermented foods and are widely used as food ingredients in the U.S. and other countries. The safe use of various bifidobacteria species in food, including *B. bifidum*, is supported by several GRAS Notifications that have been reviewed by the FDA and have received "no questions" letters from the Agency. These GRAS Notifications are further detailed in Section 6.2.

3.2 Estimated Intake of Freeze-Dried *B. bifidum* OLB6378 Powder

3.2.1 Methods

Estimates for the intake of freeze-dried *B. bifidum* OLB6378 powder were based on the proposed use level (2.28×10^6 CFU/mL) for freeze-dried *B. bifidum* OLB6378 powder in non-exempt term infant formula and in conjunction with food consumption data included in the U.S. National Center for Health Statistics' National Health and Nutrition Examination Surveys (NHANES) 2015-2016 (CDC, 2018a,b; USDA, 2018). Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for use in non-exempt term infant formula and the percentage of consumers were determined. The per person and per kilogram body weight intakes were reported for the following population groups:

- Younger Infants, aged 0 to <6 months;
- Older Infants, 6 to <12 months;
- Young Children, 12 to <24 months; and
- Younger Infants to Young Children, aged 0 to <24 months.

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of freeze-dried *B. bifidum* OLB6378 powder by the U.S. population[1]. Estimates for the daily intake of freeze-dried *B. bifidum* OLB6378 powder represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2015-2016; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population.

"*Per capita*" intake refers to the estimated intake of freeze-dried *B. bifidum* OLB6378 powder averaged over all individuals surveyed, regardless of whether they consumed non-exempt term infant formula, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of non-exempt term infant formula during the 2 survey days). "Consumer-only" intake refers to the estimated intake of freeze-dried *B. bifidum* OLB6378 powder by those individuals who reported consuming non-exempt term infant formula. Individuals were considered "consumers" if they reported consumption of non-exempt term infant formula on either Day 1 or Day 2 of the survey.

The estimates for the intake of freeze-dried *B. bifidum* OLB6378 powder was generated using the maximum use level indicated for non-exempt term infant formula, as presented in Table 1.3-1, together with food consumption data available from the 2015-2016 NHANES datasets. The results for freeze-dried *B. bifidum* OLB6378 powder are presented in Section 3.2.2.

3.2.2 Intake Estimates for Freeze-Dried B. bifidum OLB6378 Powder

Approximately 55.3% and 63.1% of younger infants and older infants were consumers of infant formula, respectively. The percentage of consumers was low in young children (3.7%). The consumer-only estimates

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (younger infants to young children), the mean and 90th percentile consumer-only intakes on an absolute basis of freeze-dried *B. bifidum* OLB6378 powder were determined to be 13.3 and 20.8 mg/person/day, respectively. Younger infants were determined to have the greatest mean consumer-only intakes of freeze-dried *B. bifidum* OLB6378 powder at 13.8 mg/person/day and older infants had the highest 90th percentile consumer-only intakes of 22.1 mg/person/day (Table 3.2.2-1).

Based on the freeze-dried *B. bifidum* OLB6378 powder concentration in the lyophilized ingredient of 1.25×10^{11} CFU/g, the estimated daily of intake of freeze-dried *B. bifidum* OLB6378 powder in CFU per day is provided in Table 3.2.2-2. Among the total population (younger infants to young children), the mean and 90th percentile consumer-only intakes of freeze-dried *B. bifidum* OLB6378 powder were determined to be 1.66×10^9 and 2.60×10^9 CFU/person/day, respectively. The greatest mean and 90th percentile consumer-only intakes of freeze-dried *B. bifidum* OLB6378 powder were 1.73×10^9 CFU/person/day (in younger infants) and 2.76×10^9 CFU/person/day (in older infants), respectively.

Table 3.2.2-1 Summary of the Estimated Daily Intake (in Milligrams Per Day) of Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (months)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Younger Infants	0 to <6	7.6	18.7	55.3	101	13.8	20.8
Older Infants	6 to <12	8.6	20.0	63.1	95	13.6	22.1
Young Children	12 to <24	0.3*	na	3.7	6	7.2*	9.5*
Total (Younger Infants to Young Children)	0 to <24	4.4	16.7	32.9	202	13.3	20.8

n = sample size; na = not applicable; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table 3.2.2-2 Summary of the Estimated Daily Intake (in CFU Per Day) of Freeze-Dried *Bifidobacterium bifidum* OLB6378 Powder from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (months)	*Per Capita* Intake (CFU/day)		Consumer-Only Intake (CFU/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Younger Infants	0 to <6	0.95×10^9	2.34×10^9	55.3	101	1.73×10^9	2.60×10^9
Older Infants	6 to <12	1.08×10^9	2.50×10^9	63.1	95	1.70×10^9	2.76×10^9
Young Children	12 to <24	0.04×10^9*	na	3.7	6	0.90×10^9*	1.19×10^9*
Total (Younger Infants to Young Children)	0 to <24	0.55×10^9	2.09×10^9	32.9	202	1.66×10^9	2.60×10^9

CFU = colony forming units; n = sample size; na = not applicable; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

3.2.3 Summary and Conclusions

Consumption data and information pertaining to the proposed use levels of freeze-dried *B. bifidum* OLB6378 powder in non-exempt term infant formula were used to estimate the *per capita* and consumer-only intakes of freeze-dried *B. bifidum* OLB6378 powder for infants and young children. There were several assumptions included in the assessment which render exposure estimates that may be considered suitably conservative. For example, it has been assumed in the exposure assessment that all infant formula would contain freeze-dried *B. bifidum* OLB6378 powder at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that freeze-dried *B. bifidum* OLB6378 powder will have 100% market penetration in all identified food categories.

In summary, on a consumer-only basis, the estimated intakes of freeze-dried *B. bifidum* OLB6378 powder were similar in younger and older infants, while young children had much lower intakes. In younger infants (0 to <6 months), the mean and 90th percentile intakes of freeze-dried *B. bifidum* OLB6378 powder were 13.8 mg/day (1.73×10^9 CFU/day) and 20.8 mg/day (2.60×10^9 CFU/day), respectively. In older infants (6 to <12 months), the mean and 90th percentile intakes of freeze-dried *B. bifidum* OLB6378 powder were 13.6 mg/day (1.70×10^9 CFU/day) and 22.1 mg/day (2.76×10^9 CFU/day), respectively. Intake estimates for young children (12 to <24 months) may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with freeze-dried *Bifidobacterium bifidum* OLB6378 powder.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative and Safety Information

6.1 Narrative

Freeze-dried *B. bifidum* OLB6378 powder is a microbial ingredient intended for use in infant formula products in the U.S. *B. bifidum* are gram-positive anaerobic bacteria that are indigenous to the gastrointestinal tract of humans. *Bifidobacterium* sp. colonize the human infant intestine within the first weeks of life from maternal transfer and dominant growth supported by bifidogenic agents provided by human breast milk (Peirotén *et al*., 2018). *B. bifidum* are reported to be among the first colonizers of the infant gut, an occurrence mediated by the capacity of bifidobacteria to utilize HMOs present in breast milk as energy sources (Gotoh *et al*., 2018). The presence of *B. bifidum* in the intestine of infants is considered normal. *B. bifidum* OLB6378 is derived from subculture of the original parent isolate *B. bifidum* OLB6139 (Toshimitsu *et al*., 2013) that was obtained from a human infant feces sample. To establish the safety of freeze-dried *B. bifidum* OLB6378 powder for its intended uses, species and strain specific data on toxigenicity, antibiotic resistance, nonclinical and clinical toxicology, metabolic fate and colonization, and bacterial translocation and pathogenicity were assessed. To identify published scientific literature relevant to the safety of *B. bifidum* OLB6378, a comprehensive literature search was conducted using the electronic search tool ProQuest Dialog™. The search was conducted on 21 August 2019 using databases including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and ToxFile®. An update to this search was conducted on 23 February 2022 utilizing identical parameters and databases. Consistent with the requirements of the GRAS standard, conclusions on the GRAS status of freeze-dried *B. bifidum* OLB6378 powder have considered all publicly available sources of information including favorable and potentially unfavorable information. An overall assessment on the safety of freeze-dried *B. bifidum* OLB6378 powder for use as an ingredient in infant formula was conducted using the aforementioned data and information in conjunction with the Pariza decision tree (Pariza *et al.,* 2015) for determining the safety of microbial cultures used for human consumption and it was concluded, "*The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption".* All information used to establish the safety of freeze-dried *B. bifidum* OLB6378 powder is available in the public domain and, as such, there are no data that are exempt from disclosure under the Freedom of Information Act.

6.2 History of Safe Use

Several bifidobacteria species are consumed globally as a component of a variety of fermented foods and are widely used as food ingredients in the U.S. and other countries. The safe use of various bifidobacteria species in food, including *B. bifidum*, is supported by several GRAS Notifications that have been reviewed by the FDA and have received "no questions" letters from the Agency (Table 6.2-1). Of note, GRN 758 and 814 have concluded that other strains of *B. bifidum,* R0071 and BGN4, are GRAS for use in non-exempt term infant formula (U.S. FDA, 2018; U.S. FDA, 2019d).

Table 6.2-1 Bifidobacterial Strains that have been GRAS Notified to the U.S. FDA for Specified Food Uses

GRN[a]	GRAS Substance	Intended Use
Bifidobacterium bifidum		
758 (U.S. FDA, 2018)	*Bifidobacterium bifidum* R0071, *Lactobacillus helveticus* R0052, and *Bifidobacterium longum* subsp. *infantis* R0033	For use, both individually and in combination, as an ingredient of powdered infant formulas at 5×10^7 CFU/g of powder in formulas with hydration rates of 12.5 to 13.5 g/100 mL.
814 (U.S. FDA, 2019b)	*Bifidobacterium bifidum* BGN4	For use as an ingredient in powdered non-exempt term infant formula at up to 10^8 CFU per gram of powdered formula. Also, for use in fermented milk; includes buttermilk and kefir; flavored milk beverages mixes, dried milk powder; imitation milk; yogurt; baby cereals and foods, powder form; meal replacement powder and nutrition drink mix powder; and sugar substitute, powder form at up to 10^9 CFU per serving.
Other bifidobacterial spp.		
49 (U.S. FDA, 2002)	*Bifidobacterium lactis* strain Bb12 and *Streptococcus thermophilus* strain Th4	Ingredients in milk-based infant formula that is intended for consumption by infants 4 months and older, at levels not to exceed good manufacturing practice.
268 (U.S. FDA, 2009)	*Bifidobacterium longum* strain BB536	Ingredient in breads/baked goods, cereals, dairy products/dairy-based foods and dairy substitutes, fruit products, candy, chewing gum, cocoa powder, condiment sauces, flavored beverage syrups, fruit flavored powder beverage mixes, gelatin desserts, gravies, margarine, peanut and other nut butter/spreads, snack foods, weaning foods at a maximum level of 1×10^{10} CFU per serving and in milk based powdered infant formula at a level of 1×10^{10} CFU per gram of infant formula powder that is intended for consumption for term infants aged 9 months and older.
377 (U.S. FDA, 2011)	*Bifidobacterium animalis* subsp. *lactis* strain Bf-6	As an ingredient in foods.
445 (U.S. FDA, 2013a)	*Bifidobacterium animalis* subsp. *lactis* strains HN019, Bi-07, BI-04, and B420	As ingredients in ready-to-eat breakfast cereals, bars, cheeses, milk drinks and milk products, bottled water and teas, fruit juices, fruit nectars, fruit 'ades' and fruit drinks, chewing gum, and confections at a maximum level of 2×10^{11} CFU per serving.
453 (U.S. FDA, 2013b)	*Bifidobacterium breve* M-16V	As an ingredient in baked goods, breakfast cereals, fruit juices and nectars, fruit ices, vegetable juices, milk-based drinks and powders, dairy product analogs, frozen dairy desserts, processed cheese, imitation cheese, cheese spreads, butter-type products, snack foods, gelatin, pudding, fillings, meal replacements, snack bars, nut and peanut spreads, hard and soft candies, cocoa-type powder, and condiment sauces at levels up to 5×10^9 CFU per serving.
454 (U.S. FDA, 2013c)		As an ingredient in non-exempt powdered term infant formulas (milk- or soy-based) and exempt powdered term infant formula containing partially-hydrolyzed milk or soy proteins, at levels up to 10^8 CFU per gram of infant formula powder.
(U.S. FDA, 2013d)		As an ingredient in exempt term powdered amino acid-based formulas, at levels providing 10^8 colony forming units per gram of infant formula powder.

Table 6.2-1 Bifidobacterial Strains that have been GRAS Notified to the U.S. FDA for Specified Food Uses

GRN[a]	GRAS Substance	Intended Use
813 (U.S. FDA, 2019c)	*Bifidobacterium longum* BORI	For use as an ingredient in powdered non-exempt term infant formula at up to 10^8 CFU per gram of powdered formula. Also for use in fermented milk; includes buttermilk and kefir; flavored milk beverages mixes, dried milk powder; imitation milk; yogurt; baby cereals and foods, powder form; meal replacement powder and nutrition drink mix powder; and sugar substitute, powder form at up to 10^9 CFU per serving.
855 (U.S. FDA, 2019d)	*Bifidobacterium animalis* ssp. *lactis* strain R0421	Intended for use as an ingredient in non-exempt powdered milk-based infant formula intended for healthy term infants at a level to provide 5×10^9 CFU/800 mL of formula as prepared.
856 (U.S. FDA, 2019e)	*Bifidobacterium animalis* ssp. *lactis* strain BB-12	For use as an ingredient in conventional foods for use by the general population, excluding foods subject to regulation by the USDA, at levels intended to provide 5×10^{11} CFU/serving.
872 (U.S. FDA, 2019f)	*Bifidobacterium animalis* ssp. *lactis* UABla-12	Intended for use as an ingredient in foods generally, excluding infant formula and foods under the authority of USDA at levels intended to provide 10^9 to 10^{11} CFU per serving.
877 (U.S. FDA, 2019g)	*Bifidobacterium longum* BB536	Intended for use as an ingredient in term infant formula at a level of 1×10^8 CFU per gram of product.
950 (U.S. FDA, 2021a)	*Bifidobacterium longum* ssp. *infantis* DSM 33361	For use as an ingredient in cow milk-, soy-, and partially hydrolyzed protein-based, non-exempt infant formula for term infants at a level up to 1×10^{10} colony forming units (CFU)/g, and in conventional foods, including but not limited to milk and dairy products; plant-based dairy alternatives; beverages; bars; confectionery; and cereals and at a use level up to 2.8×10^{10} CFU/serving.
952 (U.S. FDA, 2021b)	*Bifidobacterium animalis* ssp. *lactis* strain AD011	Ingredient in non-exempt infant formula (milk- and soy-based) for term infants at levels up to 10^8 colony forming units (CFU)/g of powdered formula; and, in fermented milk, including buttermilk and kefir, flavored milk beverage mixes, dried milk powder, imitation milk, yogurt, powdered baby cereals and foods, meal replacement and nutritional drink mix powders, and powdered sugar substitutes at levels up to 10^{10} CFU *B. lactis* AD011/serving.
985 (U.S. FDA, 2021c)	*Bifidobacterium longum* ssp. *infantis* strain ATCC SD 6720	Intended for use as an ingredient in non-exempt powdered infant formula for term infants and powdered toddler formula at a level of 1×10^8 colony forming units/g of powdered formula.
1002[b] (U.S. FDA, 2021d)	*Bifidobacterium breve* strain MCC1274	Intended for use as an ingredient in conventional foods including baked goods, breakfast cereals, fruit juices and nectars, fruit ices, vegetable juices, milk-based drinks and powders, yogurt, dairy product analogs, frozen dairy desserts, cheeses, condiments and spreads, nut and peanut spreads, gelatins and puddings, milk and non-milk meal replacements, soft and hard candies and snack foods, and infant and toddler foods at a maximum level of 5×10^{10} colony forming units (CFU)/serving.
1003[b] (U.S. FDA, 2021e)	*Bifidobacterium longum* ssp. *infantis* M-63	Intended for use as an ingredient in non-exempt cow milk- and soy-based infant formula for term infants at a level up to 1×10^8 colony forming units (CFU)/g of powdered formula, and in certain conventional foods in the following categories at a level up to 1.25×10^{10} CFU per serving: breads and baked goods; ready-to-eat and hot breakfast cereals; fruit juices, nectars, and blends; dairy products and dairy substitutes; candy; condiment sauces; gelatin desserts; peanut and other nut butters and spreads; snack foods; and infant and toddler foods.

CFU = colony forming units; GRAS = Generally Recognized as Safe; USDA = United States Department of Agriculture.

[a] https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&sort=GRN_No&order=DESC&startrow=1&type=basic&search=bifido

[b] Pending review by the U.S. FDA.

The European Food Safety Authority (EFSA) has granted Qualified Presumption of Safety (QPS) status to the *B. bifidum* species, thereby excluding any strain of the microbial species from the need for further safety assessment, other than the generic qualification that "*the strains should not harbor any acquired antimicrobial resistance genes to clinically relevant antimicrobials*" (EFSA, 2007). The QPS approach was developed by EFSA Panel on Biological Hazards to aid in the safety assessment of microorganisms that are commonly used in food. Granting of and maintaining QPS status indicates that the evaluation of a defined taxonomic group for use in human food and animal feed production by EFSA was not associated with safety concerns or, if safety concerns existed, they could be defined and excluded. With respect to *B. bifidum*, EFSA concluded, "*There are apparently no specific safety concerns regarding the genus Bifidobacterium (especially concerning B. animalis; B. longum, B. breve, B. adolescentis, and B. bifidum) with the exception of the species associated with dental caries, B. dentium*" (EFSA, 2007), and QPS status for the genus has been maintained upon subsequent reviews by the Panel (EFSA, 2019). The *B. bifidum* species is also part of the International Dairy Federation and European Food and Feed Cultures Association joint authoritative inventory of microorganisms with a documented history of safe use in fermented milk as a microbial ingredient since 1970 (Mogensen *et al.*, 2002). Recent updates to the inventory confirm that *B. bifidum* has been safely used in the dairy industry for decades (IDF, 2018).

6.3 Toxigenicity

6.3.1 Hemolytic Potential

The hemolytic activity of *B. bifidum* OLB6378 cells (2.3×10^{11} cells/mL) was measured after anaerobic culture at 37°C for 24 hours using an Anaeropack-kenki (gas producing pouch) on BL agar medium with defibrinated horse blood. The hemolytic activity of the positive and negative controls (*Bacillus cereus* NBRC15305^{T} and *Bacillus subtilis* NBRC 3936, respectively) was measured by the erythrocyte suspension test. *B. bifidum* OLB6378 did not cause a zone of hemolysis around the colony in the BL agar plate. The negative control in the erythrocyte suspension test demonstrated sedimented erythrocytes after centrifugation and no red color in the supernatant, while the positive control demonstrated no sedimented erythrocytes after centrifugation and a red-colored supernatant. These results demonstrate that *B. bifidum* OLB6378 has no hemolytic potential.

6.3.2 Bioinformatic Assessment for Undesirable Gene Products

The complete genome of *B. bifidum* OLB6378 has been sequenced and annotated. A bioinformatic evaluation of the annotated sequence was conducted to identify potential undesirable gene expression products. No plasmid DNA was reported and no chromosomal DNA originating from a plasmid or phage was identified. Putative genes associated with hemolysis (n=3), virulence (n=1), enzymes involved in the activation of carcinogenic compounds (n=4), antibiotic resistance (n=2), efflux pumps (n=6), and conjugative transfer (n=6) were identified in the *B. bifidum* OLB6378 genome (Table 6.3.2-1). These putative genes were then compared to annotated sequences reported for other *B. bifidum* strains whose genomes have been completely sequenced (*B. bifidum* S17 and PRL2010) and that have been extensively investigated as microbial ingredients and have an apparent history of safe human consumption (NCBI, 2014a,b). The hits to the "undesirable genes" in Table 6.3.2-1 were determined to be homologous to genes that are characteristic of the *Bifidobacterium* genus and therefore were concluded not to be of safety concern. Conjugative transfer genes were identified as being potentially unique to *B. bifidum* OLB6378; however, no drug resistant genes in the integrative and conjugative elements-like region were reported. Based on these data, there are no gene elements in the *B. bifidum* OLB6378 genome that are associated with safety concerns.

Table 6.3.2-1 Genomic Analysis for Potential Risk Factors in the *Bifidobacterium bifidum* OLB6378 Genome

Risk Factor		Correlated Genes and Traits	Number of Genes	Remarks
Pathogenic Factor	Tissue (Intestinal cells) infiltration	Lysin (hemolysin, endolysin, *etc*.)	3	Hemolysin homolog. Also found in *Bifidobacterium bifidum* S17 and PRL2010.
		Leukocidin	0	
		Gelatinise	0	
		Mucinase	0	
	Toxin Production	Toxin*	0	--
	Other	Virulence Factor	1	Conserved hypothetical membrane spanning protein with virulence factor MviN domain. Also found in *Bifidobacterium bifidum* S17 and PRL2010.
Amine Production		Amino acid decarboxylase	0	--
		Biogenic amine (histamine, tyramine, *etc*.)	0	
Carcinogenicity		Nitroreductase	3	Also found in *Bifidobacterium bifidum* S17 and PRL2010.
		Nitrate reductase	0	
		Azoreductase	0	
		Beta-glucuronidase	0	
		Beta-glucosidase	1	6-phospho-beta-glucosidase or beta-glucosidase. Also found in *Bifidobacterium bifidum* S17 and PRL2010.
		Urease	0	
		Dehydroxylase	0	
Effect on Intestinal Bacteria		Antibiotics production	0	--
		Bacteriocin production	0	
Transmission of Drug Resistance		Antibiotic resistance	2	Bacitracin resistance protein, Glyoxalase/bleomycin resistance protein/dioxygenase. Also found in *Bifidobacterium bifidum* S17 and PRL2010.
		Efflux pump	6	Macrolide or multidrug efflux pump. Also found in *Bifidobacterium bifidum* S17 and PRL2010.
		Conjugative transfer	6	ICE-like region present. However, there are no drug resistant genes in the ICE-like region.

ICE = integrative and conjugative elements.
* Not including toxin-antitoxin systems.

6.4 Antibiotic Resistance

Antibiotic resistance was evaluated in accordance with the "*Final report from the Committee on Antimicrobial Susceptibility Testing, Japanese Society of Chemotherapy, on the agar dilution method (2007)"* (Nagayama *et al*., 2008). A total of 29 antibiotics used for infusion or intravenous injection in neonates was studied. 0.1 mL of the *B. bifidum* OLB6378 suspension (approx. 10^3 CFU/mL) was applied to a BL agar plate containing 29 antibiotics at concentrations of 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1,024, 2,048, 4,096, and 8,192 µg/mL, followed by anaerobic culture in an Anaero Pack for 3 days at 37°C. The lowest concentration at which bacterial growth was completely inhibited after culture was defined as the minimum inhibitory concentration (MIC). *B. bifidum* OLB6378 displayed high susceptibility to penicillin derivatives, cephems, carbapenems, macrolides, glycopeptide derivatives and oxazolidinone antibiotics (MIC of ≤1 µg/mL) (Table 6.4-1). Resistance to aminoglycosides was reported (MIC of 128 to 512 µg/mL); however, this resistance phenotype is considered an intrinsic characteristic of members of the *Bifidobacterium* genus (Mayrhofer *et al*., 2011). Therefore, these results did not identify evidence of any acquired antibiotic resistance traits that would be of safety concern for use of this strain as an ingredient in infant formula.

Table 6.4-1 Antibiotic Resistance Testing of *Bifidobacterium bifidum* OLB6378

Antibiotics	MIC µg/mL	Antibiotics	MIC µg/mL
Penicillins		**Carbapenems**	
- Penicillin formulation		Imipenem + Cilastatin sodium	≤1
Benzylpenicillin potassium	≤1	Meropenem Hydrate	≤1
- Synthetic penicillin formulation		**Aminoglycosides**	
Ampicillin	≤1	Gentamicin sulfate	256
Doyle	≤1	Amikacin sulfate	128
Pentcillin	≤1	Kanamycin sulfate	512
Cephems		Streptomycin	128
- First generation		**Tetracyclines**	
Cefazolin sodium	≤1	Minocycline hydrochloride	2
- Second generation		Tetracycline	2
Cefotiam hydrochloride	≤1	**Macrolides**	
Cefmetazole sodium	≤1	Erythromycin	≤1
- Third generation		**Lincomycins**	
Cefotaxime sodium	≤1	Clindamycin phosphate	32
Cefoperazone sodium	≤1	**Fosfomycins**	
Hydrochloric acid cefmenoxime	≤1	Fosfomycin sodium	16
Cefozopran hydrochloride	≤1	**Chloramphenicols**	
- After third generation		Chloramphenicol	32
Ceftazidime	≤1	**Glycopeptides**	
Ceftriaxone sodium	≤1	Vancomycin	≤1
Flomoxef sodium	≤1	**Oxazolidinones**	
Monobactam class		Linezolid	≤1
Aztreonam	≤1		

MIC = minimum inhibitory concentration.

6.5 Toxicological Studies

The physiological effects of ingested microorganisms are typically host species-specific, with the high degree of variability in these effects often precluding extrapolation of the results of animal studies to humans (Salminen *et al.*, 1998). As *B. bifidum* OLB6378 was isolated from the feces of a healthy human infant, toxicity studies conducted using rodents or other animal species administered viable populations of *B. bifidum* OLB6378 at high dietary concentrations are of limited relevance to humans (Verschuren, 1995). A similar viewpoint was emphasized by a panel of experts who stated that "*for the safety related endpoints important in assessment of probiotics, validated animal models do not exist and, as a result, the determination of safety rests primary on human studies*" (Shane *et al*., 2010). Although human studies conducted with the *B. bifidum* OLB6378 were considered pivotal to the GRAS evaluation, safety-related information obtained from animal studies can provide corroborative evidence that the strain is safe for food use. Unpublished acute toxicity, 90-day toxicity, and genotoxicity studies have been conducted with Meiji's viable and heat-killed *B. bifidum* OLB6378 preparations. As no safety concerns were reported from any of the studies on heat-killed *B. bifidum* OLB6378, and the GRAS evaluation was for use of viable *B. bifidum* OLB6378, studies conducted with the heat-killed product are not discussed further. Additional published studies evaluating the consumption of *B. bifidum* OLB6378 in animal models with compromised gastrointestinal function (*i.e.*, ileal damage, NEC) demonstrate that *B. bifidum* OLB6378 displays low

opportunistic potential for infection and does not worsen the pathological state in the animals. The results of these animal studies, as summarized in the sections that follow, demonstrate that *B. bifidum* OLB6378 is well tolerated and not associated with toxigenicity or pathogenicity and provide corroborating evidence for the safe use of *B. bifidum* OLB6378 as a human food ingredient.

6.5.1 Acute Toxicity Studies

An unpublished acute toxicity study of viable *B. bifidum* OLB6378 (Lot No. BB050126; milky white freeze-dried powder; 7.1×10^{11} cells/g) was performed in Sprague-Dawley rats [Crj:CD(SD)IGS]. This study was conducted at Kobuchisawa Research Laboratories, Fuji Biomedix Co., Ltd., in compliance with Good Laboratory Practice (GLP) (OECD, 1998) regulations (Ordinance No. 21 of MHW, Japan) (MHLW, 1997) and toxicity study guidelines (Notification No. 24 and 88, MHW, Japan) (MHLW, 1989, 1993). The study consisted of 4 groups (n=5/sex/group) that received *B. bifidum* OLB6378 by gavage at doses of 1×10^{10}, 3×10^{10}, or 1×10^{11} cells/kg body weight, and a control suspension containing a freeze-dried bacterial powder having the same weight as the test substance. No deaths were reported in either sex at any of the doses tested; therefore, it was estimated that the minimum lethal dose of viable *B. bifidum* OLB6378 was greater than 1×10^{11} cells/kg body weight.

6.5.2 Subchronic Toxicity Studies

An unpublished 90-day toxicity study of viable *B. bifidum* OLB6378 (Lot No. BB050126; milky white freeze-dried powder; 7.1×10^{11} cells/g) was performed in Sprague-Dawley rats [Crj:CD(SD)IGS]. This study was conducted at Kobuchisawa Research Laboratories, Fuji Biomedix Co., Ltd., in compliance with GLP regulations (Ordinance No. 21 of MHW, Japan) and toxicity study guidelines (Notification No. 24 and 88, MHW, Japan). The study consisted of 4 groups that received *B. bifidum* OLB6378 at doses of 3×10^{10} or 1×10^{11} cells/kg body weight/day (high dose based on the highest dose of the acute toxicity study), a control suspension containing a freeze-dried bacterial powder protective substance (whey degradation medium and dispersion medium) having the same weight as the test substance, or saline by gavage. The *B. bifidum* OLB6378 3×10^{10} cells/kg body weight group consisted of 6 animals/sex and the other 3 groups consisted of 12 animals/sex/group. A recovery period of 4 weeks was assessed for the 3 test groups (6 of the 12 animals/sex/group), excluding the *B. bifidum* OLB6378 3×10^{10} cells/kg body weight group. The endpoints evaluated included clinical observations, body weight, food consumption, urinalysis, ophthalmology, hematology, blood chemistry, organ weights, gross pathology, and histopathology. No test substance-related changes were reported in either sex at any of the doses tested. The no-observed-adverse-effect level (NOAEL) of viable *B. bifidum* OLB6378 in this 90-day toxicity study was considered 1×10^{11} cells/kg body weight/day, the highest dose tested.

6.5.3 Genotoxicity Studies

An unpublished *in vivo* micronucleus study of viable *B. bifidum* OLB6378 (Lot No. BB050126; milky white freeze-dried powder; 7.1×10^{11} cells/g) was performed in ICR male mice [Crlj:CD1(ICR)]. This study was conducted at Kobuchisawa Research Laboratories, Fuji Biomedix Co., Ltd., in compliance with GLP regulations (Ordinance No. 21 of MHW, Japan) and toxicity study guidelines (Notification No. 1604, MHW, Japan). No deaths and no test substance related effects on clinical signs or body weights were reported in this test, and the incidence of micronucleated polychromatic erythrocytes (MNPCE) and polychromatic erythrocytes (PCE) did not show statistical significance in any test substance group compared with the negative control. It was concluded that viable *B. bifidum* OLB6378 has no potential for genotoxicity.

6.5.4 Other Animal Studies

Several other studies focusing on efficacy evaluations in different animal models (*i.e.,* ileal damage, NEC incidence, cytokine regulation) with *B. bifidum* OLB6378 were identified in the literature (Agah *et al.,* 2019; Khailova *et al.,* 2009, 2010; Underwood *et al.,* 2012; Wang *et al.,* 2020). Since limited safety endpoints were measured in these studies, they are only considered corroborative to the safety of *B. bifidum* OLB6378. Meiji notes these studies represent pathologic states of impaired gastrointestinal barrier health and therefore findings from these studies also corroborate the non-pathogenic nature of *B. bifidum* OLB6378 as no evidence of sepsis or worsening of the induced pathologies were reported.

6.6 Human Studies

As described in Section 6.5, the physiological effects of ingested microorganisms are typically host species-specific. As described by Pariza *et al.* (2015), for microbial strains without a history of safe use to be used in human food, "*Experimental evidence of safety is required. Such evidence may include, but is not necessarily limited to, studies in appropriate animal models, and clinical trials in humans*". Therefore, as *B. bifidum* OLB6378 was isolated from the feces of a healthy human infant and proposed for use in human infant formula, clinical strain specific studies are essential to provide evidence that the strain is safe for use in human food. Published and unpublished human studies involving consumption of *B. bifidum* OLB6378 by infants and adults are described below to demonstrate the safety of the strain. Additionally, published studies evaluating the consumption of other *B. bifidum* strains, alone or in combination with other microbial species, by human infants are described below to demonstrate the safety of the *B. bifidum* species and corroborate the safety of *B. bifidum* OLB6378.

6.6.1 Studies Conducted with *B. bifidum* OLB6378

6.6.1.1 Infant Studies

The safety of *B. bifidum* OLB6378 was evaluated in 3 published clinical studies in preterm infants (Yamasaki *et al*., 2012; Totsu *et al*., 2014; Tanaka *et al*., 2017), and 1 follow-up study (Totsu *et al*., 2018). These studies included preterm infant populations administered *B. bifidum* OLB6378 during the first 2 weeks of life for durations of up to 6 months (Table 6.6.1.1-1). The findings from these studies demonstrate that *B. bifidum* OLB6378 is safe and well tolerated for use by preterm infants. No evidence of increased mortality or effects on anthropometric measures were reported in any of these studies. Since these studies were conducted in preterm infants, which are a vulnerable population group, conclusions from these studies can be extended to term infants. This conclusion is further corroborated by a published clinical study in term infants (Terahara *et al*., 2021). These data demonstrate the safety of the proposed use of *B. bifidum* OLB6378 as an ingredient in infant formula.

An additional unpublished clinical study in preterm infants and an unpublished secondary analysis of Totsu *et al*. (2014) are also summarized in Table 6.6.1.1-1. The findings from these unpublished studies support the conclusion that *B. bifidum* OLB6378 is safe and well tolerated for use by preterm and term infants. No evidence of increased mortality or effects on anthropometric measures were reported in any of these studies. These data support the safety of the proposed use of *B. bifidum* OLB6378 as an ingredient in infant formula.

Table 6.6.1.1-1 Studies in Infants Administered *Bifidobacterium bifidum* OLB6378

Author	Study Population; Design	Treatments	Duration of Exposure	Treatment Initiated ≤2 Weeks of Birth	Relevant Safety Related Findings
Published Studies					
Yamasaki *et al*. (2012)	VLBW infants (<1,500 g) R	(1) *B. bifidum* OLB6378 (2.5×10^9 CFU); ≤48 hrs after birth (n=18) (2) *B. bifidum* OLB6378 (2.5×10^9 CFU); ≥48 hrs after birth (n=18) Test article suspended in water, breast milk or infant formula	Reported as time to reach 2,000 g	Yes	• No difference in duration of hospitalization and body weight at 40 weeks • No differences in morbidity or mortality • No difference in time to enteral feeding (100 mL/kg)
Totsu *et al*. (2014)	VLBW infants (<1,500 g) DB, R, PC, MCT	(1) Placebo (dextrin); ≤48 hrs after birth (n=119) (2) *B. bifidum* OLB6378 (2.5×10^9 CFU); ≤48 hrs after birth (n=114)	Reported as time to reach 2,000 g	Yes	• No differences in length of hospital stay, body weight at discharge, body weight gain, or head circumference measures • No difference in morbidity or mortality between groups
Tanaka *et al*. (2017)	LBW infants (1,500–2,500 g) SB, NR	(1) Control (no intervention; n=31) (2) Viable *B. bifidum* OLB6378 ($>2.5 \times 10^9$ CFU); ≤48 hrs after birth (n=30) (3) Heat-killed *B. bifidum* OLB6378 ($>2.5 \times 10^9$ cells); ≤48 hrs after birth (n=37) Test article suspended in water, breast milk or infant formula	6 mo	Yes	• No difference in body weight between groups at 6 mo • No reports of mortality, sepsis, or severe digestive symptoms in any of the groups • No side-effects or subject withdrawals
Totsu *et al*. (2018)	Follow-up to Totsu *et al*. (2014)		Follow-up at 18 mo	-	• No differences between groups in body weight, body length, head circumference, or use of O_2 at 18 mo

Table 6.6.1.1-1 Studies in Infants Administered *Bifidobacterium bifidum* OLB6378

Author	Study Population; Design	Treatments	Duration of Exposure	Treatment Initiated ≤2 Weeks of Birth	Relevant Safety Related Findings
Terahara *et al.* (2021)	Full term infants with normal body weight DB, R, PC	(1) Placebo (infant formula; n=49) (2) Infant formula containing 40 mg/100 g heat-treated *B. bifidum* OLB6378 concentrate (n=45)	Up to 8 weeks of age	Yes	• No safety concerns associated with administration of heat-treated *B. bifidum* OLB6378 concentrate • No side effects in either group • No difference in adverse events between both groups
Unpublished Studies					
Unpublished internal secondary analysis of Totsu *et al*. (2014) Terahara *et al.* (2012) [unpublished]			Reported as time to reach 2,000 g	Yes	• No adverse events reported in both groups • Mortality rate was not significantly different between groups • No safety concerns associated with administration of *B. bifidum* OLB6378
Unpublished internal clinical study Terahara *et al.* (2020) [unpublished]	LBW infants (1,500–2,500 g) DB, R, PC	(1) "Meiji LW" formula while in hospital, then "Meiji Hohoemi" formula until 6 mo (n=37) (2) "Meiji LW" formula containing 70 mg/100 g heat-treated *B. bifidum* OLB6378 concentrate while in hospital, then "Meiji Hohoemi" formula containing 40 mg/100 g heat-treated *B. bifidum* OLB6378 concentrate until 6 mo n=39)	6 mo	Yes	• Physical evaluations and blood tests in both groups were unremarkable • No safety concerns associated with administration of heat-treated *B. bifidum* OLB6378 concentrate

CFU = colony forming units; DB = double blind; hrs = hours; LBW = low birth weight; MCT = multicenter trial; mo = month(s); NR = nonrandomized; OLB6378 = *Bifidobacterium bifidum* OLB6378; PC = placebo controlled; R = randomized; SB = single blind; VLBW = very low birth weight.

6.6.1.2 Adult Studies

Meiji conducted an unpublished randomized, double-blind, placebo-controlled study titled, "*Investigation of ingestion of Bifidobacterium bifidum OLB6378 powder (human study)*", evaluating the safety and tolerance of *B. bifidum* OLB6378 in a group of 30 healthy adult female subjects. Participants were randomized to receive placebo (dextrin) or *B. bifidum* OLB6378 (4×10^{10} CFU/day) provided as powdered sachets containing dextrin as an excipient. Treatments were ingested daily by the participants for 38 days. Subjects were monitored using questionnaire survey data for measures of gastrointestinal tolerance, overall health, and oral malodor. Blood was collected at the baseline and at the end of the 38-day treatment period for analyses of hematology and clinical chemistry parameters. The authors reported that *B. bifidum* OLB6378 was safe and well tolerated and did not adversely affect blood safety measures. There were no reported findings in this study to suggest that *B. bifidum* OLB6378 would be unsafe for use as a food ingredient, corroborating the safety of the strain.

6.6.2 Studies Conducted in Infants with Other Strains of *B. bifidum*

6.6.2.1 Single Strain Studies

A comprehensive search of the published literature was performed to identify infant studies involving other strains of *B. bifidum* administered alone (*i.e.,* single strain studies) that reported safety-related endpoints. Two relevant studies were identified in the search and are summarized below. There were no reported findings in these studies to suggest that the species *B. bifidum* is unsafe for use in infant formula.

Manzano *et al*. (2017) performed a multi-center, randomized, double-blind, placebo-controlled trial to determine the safety and tolerance of 3 microbial strains in healthy infants (≥37 weeks gestation at birth; aged 3 to 12 months). Infants received either a placebo supplement (potato starch excipient also used in the microbial supplements; n=52) or 1 of the 3 microbial strains (*B. longum* subsp. *infantis* R0033, N=53; *B. bifidum* R0071, n=51; or *Lactobacillus helveticus* R0052, n=52) at a level of 3×10^9 CFU/day in 10 mL of water, breast milk, or infant formula for 8 weeks (2 weeks run-in, 8 weeks treatment, and 2 weeks follow-up). The safety evaluation included: growth (weight, height, and head circumference), adverse events, concentrations of D-lactic acid in urine samples, characteristics of the stools, and use of medication. The authors concluded that "*the use of B. infantis R0033, L. helveticus R0052 and B. bifidum R0071 in infancy is safe, and well tolerated*", as there were no significant differences between groups regarding the parameters measured in the safety evaluation.

6.6.2.2 Mixed Microbial Studies

A comprehensive search of the published literature was conducted to identify infant studies conducted with other strains of *B. bifidum* administered in combination with other species of bacteria (*i.e.,* mixed microbial studies) that reported safety-related endpoints. Six relevant studies were identified in the initial search (5 full text and 1 abstract) and five relevant studies were identified in the updated search (4 full text and 1 abstract) the details of each are summarized in Table 6.6.2.2-1. Reported data from these studies do not provide any evidence to demonstrate a potential safety concern of *B. bifidum* when administered to infants in combination with other microbial ingredients.

Table 6.6.2.2-1 Studies in Infants Administered *Bifidobacterium bifidum* with Other Species of Bacteria

Author	Study Population; Design	Treatments	Duration of Exposure	Treatment Initiated ≤2 Weeks of Birth	Relevant Safety Related Findings
Kowalska-Duplaga *et al*. (2004) – Abstract only	Infants and children (1 mo to 4 yrs) with acute diarrhea DB, R, PC, MCT	(1) Trilac® (3 strains of lactic acid bacteria: *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*) (1.6×10^9 CFU) (2) Comparable placebo (1) and (2) n=176	Twice daily for 5 d	No	• No adverse effects of the treatment were noted
Lin *et al*. (2008)	VLBW infants (<1,500 g) SB, R, C, MCT	(1) Infloran [*Bifidobacterium bifidum* NCDO 1748 (1×10^9 CFU) and *Lactobacillus acidophilus* NCDO 1453 (1×10^9 CFU)] added to breast milk or mixed feeding (breast milk and formula) (n=217) (2) Breast milk or mixed feeding (breast milk and formula) (n=217)	Twice daily for 6 wks	Yes	• No adverse effect, such as sepsis, flatulence, or diarrhea, was noted
Underwood *et al*. (2009)	Premature infants (750 to 2,000 g) DB, R, PC	(1) Culturelle® (*Lactobacillus rhamnosus* GG as well as inulin; 5×10^9 CFU) in saline (n=30) (2) ProBioPlus DDS® (*Bifidobacterium bifidum*, *Lactobacillus acidophilus, Bifidobacterium longum,* and *Bifidobacterium infantis* as well as inulin; 5×10^9 CFU of each organism) in saline (n=31) (3) Placebo consisting of a 1:30 dilution of an elemental formula (Pregestamil) (n=29)	Twice daily for 28 d or until discharge if earlier	Yes	• No significant effect on growth of either microbial treatment was observed • No adverse reactions were noted
Samanta *et al*. (2009)	Preterm infants (gestational age <32 wks) and VLBW infants (<1,500 g) DB, R, C	(1) *Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium longum*, and *Lactobacillus acidophilus*; strains NR; 2.5×10^9 CFU in breast milk (n=91) (2) Breast milk (n=95)	Twice daily for the duration of hospital stay (≤ ~24 d)	Yes	• No compound-related adverse effects • Blood cultures did not contain any of the administered microbial ingredients

Table 6.6.2.2-1 Studies in Infants Administered *Bifidobacterium bifidum* with Other Species of Bacteria

Author	Study Population; Design	Treatments	Duration of Exposure	Treatment Initiated ≤2 Weeks of Birth	Relevant Safety Related Findings
Allen *et al*. (2010)	Pregnant women (≥16 yrs; singleton pregnancy; last month of pregnancy) and their infants DB, R, PC	(1) Capsules containing *Bifidobacterium bifidum* CUL20 (1.25×10^9 CFU), *Lactobacillus salivarius* CUL61 (6.25×10^9 CFU), *Lactobacillus paracasei* CUL08 (1.25×10^9 CFU), and *Bifidobacterium animalis subsp. lactis* CUL34 (1.25×10^9 CFU) (n=220) (2) Identical placebo capsules containing maltodextrin (n=234) Mothers: ingested capsule by mouth or sprinkled contents of capsule onto food Infants: contents of capsule mixed with formula or expressed breast milk or sprinkled directly into mouth	Once daily for women in last month of pregnancy and for infants from birth to 6 mo	Yes	• Severe adverse events occurred in 18 mothers and 63 infants with a similar frequency in each group • None of the adverse events were attributed to the intervention
Saengtawesin *et al*. (2014)	VLBW infants (<1,500 g) R, C	(1) Infloran [*Bifidobacterium bifidum* NCDO 1748 (1×10^9 CFU) and *Lactobacillus acidophilus* NCDO 1453 (1×10^9 CFU)] added to breast milk or mixed feeding (breast milk and formula) (n=31) (2) Breast milk or mixed feeding (breast milk and formula) (n=29)	Twice daily for 6 wks or until discharge if earlier	Yes	• No deaths during the study period • No adverse effects such as sepsis, flatulence or diarrhea were noted

Table 6.6.2.2-1 Studies in Infants Administered *Bifidobacterium bifidum* with Other Species of Bacteria

Author	Study Population; Design	Treatments	Duration of Exposure	Treatment Initiated ≤2 Weeks of Birth	Relevant Safety Related Findings
Robertson *et al*. (2019)	Preterm infants (gestational age <32 wks) and VLBW infants (<1,500 g) at 32-36 weeks gestation Single Center Retrospective Observational Study	(1) Infloran capsules (125 mg) [*Bifidobacterium bifidum* (1×10^9 CFU) and *Lactobacillus acidophilus* (1×10^9 CFU)] added to breast milk or mixed feeding (breast milk and formula) (2) Four Labinic Drops once daily [(~0.5×10^9 CFU dosage each of *L. acidophilus, B. bifidum* and *B. longum* subspecies *infantis* daily] added to breast milk or mixed feeding (breast milk and formula) (3) Pre-probiotic period 01 January 2008 to 31 December 2012 (n=469) Treatment (1) occurred from 01 Jan 2013 to April 2016. Treatment (2) occurred from April 2016 to 31 December 2017 (n=513).	Half capsule twice daily until 34 weeks postmenstrual age Four drops once daily until 34 weeks postmenstrual age	Yes	• No adverse effects of the treatment were noted • No significant differences in death before discharge between groups
Xiao *et al.* (2019)	Term infants (single birth at gestational age ≥37 weeks) and birth weight >2,500 g R, DB, PC	(1) One probiotics sachet (1.5 g), which contained 1.425×10^8 CFU of each *B. infantis* R0033 and *B. bifidum* R0071, with 9.6×10^9 CFU of *L. helveticus R0052*, was diluted in the feeding bottle (n=48) (2) Identical placebo sachets containing mainly potato starch (n=57)	Once daily for four weeks	No	• No statistical differences in anthropometric measures (weight, height, body mass index, and head circumference) • No significant difference between adverse events between groups; all adverse events declared mild and unrelated to product or research

Table 6.6.2.2-1 Studies in Infants Administered *Bifidobacterium bifidum* with Other Species of Bacteria

Author	Study Population; Design	Treatments	Duration of Exposure	Treatment Initiated ≤2 Weeks of Birth	Relevant Safety Related Findings
Bommer *et al*. (2020)	Infants with gestational age between 30 to 38 weeks Single Center Retrospective Regression Discontinuity Analysis (n=1734)	(1) Infloran capsules [*Bifidobacterium bifidum* (1×10^9 CFU) and *Lactobacillus acidophilus* (1×10^9 CFU)], or (2) BactoFlor [3×10^9 CFU/g containing *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, and *Streptococcus thermophilus*; no information on individual probiotic strain concentrations supplied by the manufacturer]	Once capsule per day (1) or 0.5 g per day (2) for 42 days after cessation of antibiotic treatment or after tolerating at least 4 mL per feed of enteral feeding	NR	• No adverse effects of the treatment were noted
Meyer *et al.* (2020)	Preterm infants (gestational age <32 wks) and VLBW infants (<1,500 g) MCT Retrospective Study	(1) Infloran capsules [*Bifidobacterium bifidum* (1×10^9 CFU) and *Lactobacillus acidophilus* (1×10^9 CFU)], or (2) *Lactobacillus GG (*6×10^9 CFU) with bovine lactoferrin (100 mg) (3) Pre-probiotic period (2007-2010) (n=2,556) All units using Infloran gave one capsule daily for infants <1,500 g; in one unit this was given as a half capsule twice daily while in another unit infants >1,500 g received 2 capsules daily. Treatment (1) and (2) occurred from 2013 to 2015 (n=1,937).	4–6 weeks or until 34 weeks corrected gestation in 4 of the 6 units; in the other 2 it continued until 36 weeks or discharge.	Yes	• No adverse effects of the treatment were noted

Table 6.6.2.2-1 Studies in Infants Administered *Bifidobacterium bifidum* with Other Species of Bacteria

Author	Study Population; Design	Treatments	Duration of Exposure	Treatment Initiated ≤2 Weeks of Birth	Relevant Safety Related Findings
Uberos *et al*. (2021) Abstract only	VLBW infants (<1,500 g) Prospective Observational Study	(1) Infloran capsules [*Bifidobacterium bifidum* (1×10^9 CFU) and *Lactobacillus acidophilus* (1×10^9 CFU)] (n=65) (2) Bivos [*Lacticaseibacillus rhamnnosus* (10^9 CFU)] (n=108) (3) No treatment (n=72)	NR	Yes	• No adverse effects of the treatment were noted

C = controlled; CFU = colony forming units; d = day; DB = double blind; MCT = multicenter trial; mo = month(s); NR = not reported; PC = placebo controlled; R = randomized; SB = single blind; VLBW = very low birth weight; wks = weeks; yrs = years.

6.7 Metabolic Fate and Colonization

A microorganism that survives ingestion may be a transient passenger or may establish itself for a variable time in the gastrointestinal system. Tanaka *et al.* (2019) demonstrated that low birth weight infants orally administered viable *B. bifidum* OLB6378 (>2.5 $\times$ 10^9 CFU/day) for 1 month had a significantly higher proportion of feces rich in bifidobacteria compared to control infants (see Table 6.6.1.1-1 for further study details). While this study demonstrates an increase in bifidobacteria following oral ingestion of viable *B. bifidum* OLB6378, the indigenous microflora profiles of most animals are intrinsically highly stable and resistant to colonization by exogenous microorganisms. Permanent lifelong colonization by ingested microorganisms is rare, however certain strains can be recovered in the feces and colonic mucosa for weeks after discontinuation of oral administration (Codex Alimentarius, 2009). Microorganisms are adapted for survival within specific niches to which they have become adapted, and therefore long-term colonization of *B. bifidum* OLB6378 within the gastrointestinal tract of infants is not expected. Organisms not surviving gastrointestinal transit would be metabolized by human digestive enzymes and the cellular components (proteins, lipids, carbohydrates) used as a source of nutrients, whereas non-nutritive components would be further metabolized by the resident microflora of the colon and/or excreted in the feces.

6.8 Bacterial Translocation and Pathogenicity

The translocation of live bacteria from the gastrointestinal tract to extraintestinal sites is a rare event that may occur in cases of compromised gastrointestinal integrity. The resulting transport of bacteria to the mesenteric lymph nodes, liver, spleen, kidney, and systemic circulation may lead to the development of bacteremia, sepsis, and/or multiple organ failure (Ishibashi and Yamazaki, 2001; Lichtman, 2001; MacFie, 2004; Liong, 2008). Typically, in healthy individuals, the gastrointestinal mucosa is impermeable to bacteria and studies in which microbial ingredients were administered at high quantities to healthy subjects have generally found an absence of microbial translocation (Liong, 2008). However, there have been rare case reports of lactic acid bacteria (including bifidobacteria) entering the systemic circulation following oral administration (Gasser, 1994; Weber *et al*., 2015).

Gasser reviewed the safety of lactic acid bacteria and their role in human infection and identified 1 publication that reported 9 cases of bloodstream infections by *Bifidobacterium* (Bourne *et al*., 1978). The species was identified as *Bifidobacterium eriksonii* (reclassified as *Bifidobacterium dentium*) in 5/9 cases, *Bifidobacterium adolescentis* in 1/9 cases, and general *Bifidobacterium* sp. in 3/9 cases. In all cases the origin of infection was either the digestive tract or was attributed to obstetrical issues and all patients had some sort of pre-existing underlying disease or condition. It was concluded that these cases "*represent infectious problems of extreme rarity*" (Gasser, 1994). Weber *et al.* (2015) assessed the risk factors associated with *Bifidobacterium* spp. bacteremia and identified 21 cases in adults and infants in a search of the PubMed database. The species most frequently identified were *B. longum* and *B. dentium*/*B. eriksonii;* no cases were associated with *B. bifidum.* As part of an evidence-based overview of the safety of lactobacilli and bifidobacteria used as microbial ingredients in foods, Borriello *et al*. (2003) stated, "*Current evidence suggests that the risk of infection with probiotic lactobacilli or bifidobacteria is similar to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers* [...]". Boyle *et al*. (2006) reviewed the use of microbial ingredients in clinical practice and associated risks and concluded, "*We are not aware of any reports of Bifidobacterium sepsis related to probiotic use*". Based on the above data and information, it is therefore highly unlikely that bacterial translocation of *B. bifidum* OLB6378 will occur from its consumption as an ingredient in infant formula.

A pathogenic organism is a microorganism that has the ability to cause disease in a host organism. Determinants conferring pathogenic phenotypes to members of the *Bifidobacterium* genus with a history of food use have never been reported, and it is generally recognized that *B. bifidum* is non-pathogenic and non-toxicogenic. The ability of *B. bifidum* OLB6378 to act as an opportunistic pathogen was evaluated in 3 unpublished mouse studies, including an acute oral dosing study in gnotobiotic infant mice, an acute oral dosing study in germ-free adult mice, and an acute oral dosing study in immunodeficient gnotobiotic adult mice (gnotobiotic NOG mice). The results from these animal studies described below, demonstrate that *B. bifidum* OLB6378 is of low pathogenic potential, including in cases of extreme immunodeficiency.

Pregnant gnotobiotic (germ-free) BALB/c mice were administered *B. bifidum* OLB6378 or *B. adolescentis* JCM 1275^T (2×10^8 CFU/0.2 mL) by gastric tube (14 days after mating) and the survival of the neonatal mice was reported. Necropsy of the neonates (n=7/sex/group) was performed at 8 weeks of age (Post-natal Day 56) and the mesenteric lymph node (MLN), liver, and cecum were isolated. Viable cell counts in the cecum, MLN, and liver was determined. No deaths of the neonatal mice were reported up to the day of necropsy. Viable *Bifidobacterium* were reported in the cecum of all neonates necropsied, confirming that the test strain migrated from the mother to the neonate. The viable cell count in the cecum of the strain *B. bifidum* OLB6378 group was $\geq 1.0 \times 10^8$ CFU/g of cecum. Bacterial translocation to the mesenteric lymph nodes and the liver was reported in 14% of the mice from each group (2/14 animals). The study reported that this translocation incidence was consistent with the translocation rate reported with *Lactobacillus* GG, a microbial strain that has an extensive history of safe use in preterm infants.

B. bifidum OLB6378 (dose not reported) was administered orally (method of administration not reported) to germ-free adult BALB/c mice (age and sex not reported; n=5–6/group) and necropsied at 3, 7, 14, and 28 days post-treatment to evaluate bacterial translocation. The weights of the spleen, kidneys, liver, and lungs did not significantly differ between the untreated group and the *B. bifidum* OLB6378 treated group. No bacterial translocation was reported in the mesenteric lymph nodes, spleen, kidney, or liver after the single administration of *B. bifidum* OLB6378 at all post-treatment time points.

B. bifidum OLB6378 and *E. coli* (10^8 CFU/0.2 mL) were administered by gastric tube to female gnotobiotic NOG mice (9 weeks of age; n=3/group) and necropsied at 13 days post-treatment to evaluate bacterial translocation. The NOG mouse is an immunodeficient animal model that is absent of T and B lymphocytes and therefore is highly susceptible to invasion by pathogenic bacteria. Abdominal aorta blood, spleen, MLN, liver, and gastrointestinal tract were isolated at necropsy. Viable cell counts in the blood, spleen, MLN, liver, and gastrointestinal tract were determined. No deaths were reported up to the day of necropsy. The *B. bifidum* OLB6378 count in the cecum of all 3 *B. bifidum* OLB6378 administered mice was 10^9 cells/g of cecum. Bacterial translocation was only reported in the *E. coli* monoassociated mice (n=2/3 in mesenteric lymph node and n=1/3 in liver).

6.9 Undesirable Metabolic Activities

B. bifidum OLB6378 was tested for phenotypic properties that are generally considered undesirable for microbial ingredients. The strain did not produce secondary bile acids or D-lactic acid. Secondary bile acids have been associated with colon cancer (Ajouz *et al.*, 2014). An increase in D-lactic acid can lead to D-lactic acidosis and severe neurologic impairment (Yilmaz *et al*., 2018).

To test for the ability to produce secondary bile acids, test strains [viable *B. bifidum* OLB6378; Positive control = *Clostridium scindens* JCM6567^T (ATCC35704); and Negative control = *B. longum* subsp. *longum* JCM1217 (ATCC15707)] were incubated with bile acid-containing media in an anaerobic chamber at 37°C for 48 hours. The resulting culture was analyzed for primary and secondary bile acids. Viable *B. bifidum* OLB6378 culture and the negative control culture only produced primary bile acids (cholic acid and chenodeoxycholic acid), with no secondary bile acids detected. The positive control culture produced bile acid metabolites including secondary bile acids (deoxycholic acid, 7-oxo-deoxycholic acid, and 7-oxo-lithocholic acid) at concentrations markedly higher than those of primary bile acids detected.

To test for the ability to produce D-lactic acid, test strains (viable *B. bifidum* OLB6378; Positive control = *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL2038) were cultured and then assessed for D- and L-lactic acid concentrations using F-kit (Roche/R-Biopharm). Viable *B. bifidum* OLB6378 culture only accumulated L-lactic acid, with the concentration of D-lactic acid below the detection limit (0.063 g/L). The positive control accumulated both D- and L-lactic acid.

6.10 Application of the Decision Tree Approach (Pariza *et al.,* 2015)

The decision tree for determining the safety of microbial cultures to be consumed by humans or animals published by Pariza *et al.* (2015) was applied as follows to evaluate the safety of *B. bifidum* OLB6378 for use as an ingredient in infant formula:

1. Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? (If YES, go to 2. If NO, the strain must be characterized and unambiguously identified before proceeding).

   Answer: YES

   Analysis of the whole genome of B. bifidum OLB6378 demonstrated close DNA homology to 2 known B. bifidum strains (S17 and PRL_2010) (Section 2.1). Additionally, PCR was performed on B. bifidum OLB6378 using a primer set specific to Bifidobacterium and a primer set specific to B. bifidum, thereby confirming the identity of the strain as B. bifidum (Section 2.1).

2. Has the strain genome been sequenced? (If YES, go to 3. If NO, the genome must be sequenced before proceeding to 3.)

   Answer: YES

   The genome of B. bifidum OLB6378 has been sequenced and annotated (Section 2.1).

3. Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? (If YES, go to 4. If NO, go to 15.)

   Answer: YES

   A bioinformatic evaluation of the annotated sequence was conducted to identity potential undesirable gene expression products and it was concluded that there are no gene elements in the B. bifidum OLB6378 genome associated with safety concerns (Section 6.3.2).

4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA? (If YES, go to 5. If NO, go to 15.)

   Answer: YES

   The results of in vitro MIC testing using 29 clinically relevant antibiotics did not identify evidence of any acquired antibiotic resistance traits of B. bifidum OLB6378 that would be of safety concern for use of the strain as an ingredient in infant formula (Section 6.4).

5. Does the strain produce antimicrobial substances? (If NO, go to 6. If YES, go to 15.)

   Answer: NO

   The B. bifidum species is not commonly associated with the production of antibiotics used in medical or veterinary medicine.

6. Has the strain been genetically modified using rDNA techniques? (If YES, go to 7a or 7b. If NO, go to 8a or 8b.)

   Answer: NO

8a. For strains to be used in human food: Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? (If YES, go to 9a. If NO, go to 13a.)

   Answer: NO

   B. bifidum OLB6378 is a substrain isolate derived from subculture of the original parent isolate B. bifidum OLB6139 (Toshimitsu et al., 2013) that was obtained from a human infant feces sample.

13a. For strains to be used in human food: Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? (If YES, go to 15. If NO, go to 14a.)

   Answer: NO

   The safety of viable B. bifidum OLB6378 *was evaluated in 3 clinical studies in preterm infants (Yamasaki et al., 2012; Totsu et al., 2014; Tanaka et al., 2017). The results of these studies support that the administration of B. bifidum OLB6378 to preterm infants at up to* 2.5×10^9 *CFU/day for up to 6 months is not associated with undesirable physiological effects (Section 6.6.1.1).*

14a. The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.

6.11 GRAS Panel Evaluation

Meiji has concluded that freeze-dried *B. bifidum* OLB6378 powder is GRAS for use in infant formula, as described in Section 1.3, on the basis of scientific procedures. This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of *B. bifidum* OLB6378, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Dennis M. Bier, M.D. (Professor of Pediatrics, Baylor College of Medicine), Joseph F. Borzelleca, Ph.D. (Professor Emeritus, Virginia Commonwealth University School of Medicine), and Michael W. Pariza, Ph.D. (Professor Emeritus, University of Wisconsin, Madison).

The GRAS Panel, convened by Meiji, independently and critically evaluated all data and information presented herein, and also concluded that freeze-dried *B. bifidum* OLB6378 powder is GRAS for use in infant formula as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel, and evaluation of such data as it pertains to the proposed GRAS uses of freeze-dried *B. bifidum* OLB6378 powder is presented in Appendix A. Note that the estimated intake values in the GRAS panel consensus statement were calculated assuming intended uses of freeze-dried *B. bifidum* OLB6378 powder in exempt preterm and non-exempt term infant formula. The estimated intake values in this GRAS notice were calculated based on the intended use of freeze-dried *B. bifidum* OLB6378 powder only in non-exempt term infant formula and are therefore slightly different; these differences are not meaningful to the safety conclusions.

6.12 Conclusion

Based on the above data and information presented herein, Meiji has concluded that freeze-dried *Bifidobacterium bifidum* OLB6378 powder is GRAS, on the basis of scientific procedures, for use in infant formula as described in Section 1.3. General recognition of Meiji's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of freeze-dried *Bifidobacterium bifidum* OLB6378 powder in food, who similarly concluded that the proposed uses of freeze-dried *Bifidobacterium bifidum* OLB6378 powder are GRAS on the basis of scientific procedures.

Freeze-dried *Bifidobacterium bifidum* OLB6378 powder therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

Part 7. §170.255 List of Supporting Data and Information

Agah S, Alizadeh AM, Mosavi M, Ranji P, Khavari-Daneshvar H, Ghasemian F, et al. (2019). More Protection of Lactobacillus acidophilus Than Bifidobacterium bifidum Probiotics on Azoxymethane-Induced Mouse Colon Cancer. Probiotics & Antimicro Prot 11: 857-864. DOI:10.1007/s12602-018-9425-8.

Ajouz H, Mukherji D, Shamseddine A (2014). Secondary bile acids: an underrecognized cause of colon cancer. World J Surg Oncol 12:164 [5pp]. DOI:10.1186/1477-7819-12-164.

AlFaleh K, Anabrees J (2014). Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev (4):CD005496. DOI:10.1002/14651858.CD005496.

Allen SJ, Jordan S, Storey M, Thornton CA, Gravenor M, Garaiova I, et al. (2010). Dietary supplementation with lactobacilli and bifidobacteria is well tolerated and not associated with adverse events during late pregnancy and early infancy. J Nutr 140(3):483-488. DOI:10.3945/jn.109.117093.

Athalye-Jape G, Patole S (2019). Probiotics for preterm infants - time to end all controversies. Microb Biotechnol 12(2):249-253. DOI:10.1111/1751-7915.13357.

Bommer C, Horn S, Vollmer S. (2020). The effect of routine probiotics supplementation on preterm newborn health: a regression discontinuity analysis. Am J Clin Nutr 112:1219–1227. DOI:10.1093/ajcn/nqaa196.

Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M, et al. (2003). Safety of probiotics that contain lactobacilli or bifidobacteria. Clin Infect Dis 36(6):775-780. DOI:10.1086/368080.

Bourne KA, Beebe JL, Lue YA, Ellner PD (1978). Bacteremia due to Bifidobacterium, Eubacterium or Lactobacillus; twenty-one cases and review of the literature. Yale J Biol Med 51(5):505-512.

Boyle RJ, Robins-Browne RM, Tang ML (2006). Probiotic use in clinical practice: what are the risks? Am J Clin Nutr 83(6):1256-1264; quiz 1446-1447. DOI:10.1093/ajcn/83.6.1256.

CDC (2018a). *National Health and Nutrition Examination Survey (NHANES): 2015-2016*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2015 [NHANES Home Page last reviewed: October 30, 2018].

CDC (2018b). *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: July 2018].

Codex Alimentarius (2009). *Foods Derived from Modern Biotechnology, 2nd edition*. (Codex Alimentarius). Geneva, Switz.: World Health Organization (WHO) / Rome, Italy: Food and Agriculture Organization of the United Nations (FAO). Available at: http://www.fao.org/docrep/011/a1554e/a1554e00.htm.

Dazult Ltd. (2018). *DaDiet - The Dietary Intake Evaluation Tool [Software]*. (Version 17.04). Straffan, Ireland: Dazult Ltd. Available online: http://dadiet.daanalysis.com.

EFSA (2007). Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA (Question no EFSA-Q-2005-293, adopted: 19 November 2007 by European Food Safety Authority). EFSA J 587:1-16. DOI:10.2903/j.efsa.2007.587. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/587.

EFSA (2019). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 10: Suitability of taxonomic units notified to EFSA until March 2019. (EFSA Panel on Biological Hazards/BIOHAZ) (Question no: EFSA-Q-2016-00831; adopted: 5 June 2019 by European Food Safety Authority). EFSA J 17(7):5753 [79 pp.]. DOI:10.2903/j.efsa.2019.5753. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/5753.

Gasser F (1994). Safety of lactic acid bacteria and their occurrence in human clinical infections. Bull Inst Pasteur 92(1):45-67.

Gotoh A, Katoh T, Sakanaka M, Ling Y, Yamada C, Asakuma S, et al. (2018). Sharing of human milk oligosaccharides degradants within bifidobacterial communities in faecal cultures supplemented with *Bifidobacterium bifidum*. Sci Rep 8(1):13958 [14pp]. DOI:10.1038/s41598-018-32080-3.

IDF (2018). *Inventory of Microbial Food Cultures with Safety Demonstration in Fermented Food Products*. (Bulletin (IDF) No. 495). Brussels, Belgium: International Dairy Federation (IDF). Available at: https://store.fil-idf.org/product/bulletin-idf-n-495-2018-inventory-microbial-food-cultures-safety-demonstration-fermented-food-products/.

Ishibashi N, Yamazaki S (2001). Probiotics and safety. Am J Clin Nutr 73(2, Suppl.):465S-470S. DOI:10.1093/ajcn/73.2.465s.

Kato K, Odamaki T, Mitsuyama E, Sugahara H, Xiao JZ, Osawa R (2017). Age-related changes in the composition of gut *Bifidobacterium* species. Curr Microbiol 74(8):987-995. DOI:10.1007/s00284-017-1272-4.

Khailova L, Dvorak K, Arganbright KM, Halpern MD, Kinouchi T, Yajima M, et al. (2009). *Bifidobacterium bifidum* improves intestinal integrity in a rat model of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 297(5):G940-G949. DOI:10.1152/ajpgi.00141.2009.

Khailova L, Mount Patrick SK, Arganbright KM, Halpern MD, Kinouchi T, Dvorak B (2010). *Bifidobacterium bifidum* reduces apoptosis in the intestinal epithelium in necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 299(5):G1118-G1127. DOI:10.1152/ajpgi.00131.2010.

Klein CJ (2002). Nutrient requirements for preterm infant formulas. J Nutr 132(6, Suppl. 1):1395S-1577S. DOI:10.1093/jn/132.6.1395S.

Kowalska-Duplaga K, Fyderek K, Szajewska H, Janiak R (2004). Efficacy of Trilac® in the treatment of acute diarrhoea in infants and young children - A multicentre, randomized, double-blind placebo-controlled study. Pediatr Wspolczesna 6(3):295-299 [abstract only].

Lichtman SM (2001). Ba[c]terial translocation in humans. J Pediatr Gastroenterol Nutr 33(1):1-10. DOI:10.1097/00005176-200107000-00001.

Lin HC, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RI, et al. (2008). Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. Pediatrics 122(4):693-700. DOI:10.1542/peds.2007-3007.

Liong MT (2008). Safety of probiotics: translocation and infection. Nutr Rev 66(4):192-202. DOI:10.1111/j.1753-4887.2008.00024.x.

MacFie J (2004). Current status of bacterial translocation as a cause of surgical sepsis. Br Med Bull 71(1):1-11. DOI:10.1093/bmb/ldh029.

Manzano S, De Andrés J, Castro I, Rodríguez JM, Jiménez E, Espinosa-Martos I (2017). Safety and tolerance of three probiotic strains in healthy infants: a multi-centre randomized, double-blind, placebo-controlled trial. Benef Microbes 8(4):569-578. DOI:10.3920/BM2017.0009.

Mayrhofer S., Mair C., Kneifel W., Domig K. J. (2011). Susceptibility of bifidobacteria of animal origin to selected antimicrobial agents. *Chemother. Res. Pract.* 989520 10.1155/2011/989520

Meyer MP, Chow SSW, Alsweiler J, Bourchier D., Broadbent R, Knight D, et al. (2020) Probiotics for prevention of severe necrotizing enterocolitis: experience of New Zealand neonatal intensive care units. Front. Pediatr. 8:119. DOI:10.3389/fped.2020.00119.

MHLW (1989). *Guidelines for Toxicity Studies on Drugs*. (Notification no 24, September 11, 1989). Tokyo, Japan: Ministry of Health, Labour and Welfare, Japan (MHLW), Pharmaceuticals and Cosmetics Division, Pharmaceuticals Affairs Bureau.

MHLW (1993). *Revised Guidelines for Single and Repeated Dose Toxicity Studies*. (Notification no 88 of the Pharmaceuticals and Cosmetics Division, PAD dated August 10, 1993). Tokyo, Japan: Ministry of Health, Labour and Welfare, Japan (MHLW).

MHLW (1997). *Good Laboratory Practice [GLP] Standard Ordinance for Non-Clinical Laboratory Studies on Safety of Drugs]*. (MHW Ordinance no 21, March 26, 1997). Tokyo, Japan: Ministry of Health, Labour and Welfare, Japan (MHLW). Available at: http://law.e-gov.go.jp/htmldata/H09/H09F03601000021.html [Last updated: July 30, 2014].

Mogensen G, Salminen S, O'Brien J, Ouwehand A, Holzapfel W, Shortt C et al. (2002). Inventory of microorganisms with a documented history of use in food. In: *Health Benefits and Safety Evaluation of Certain Food Components*. (Bulletin of the International Dairy Federation, No. 377). Brussels, Belgium: International Dairy Federation (IDF), pp. 10-19.

Nagayama A, Yamaguchi K, Watanabe K, Tanaka M, Kobayashi I, Nagasawa Z (2008). Final report from the Committee on Antimicrobial Susceptibility Testing, Japanese Society of Chemotherapy, on the agar dilution method (2007). J Infect Chemother 14(5):383-392. DOI:10.1007/s10156-008-0634-z.

Nagpal R, Kurakawa T, Tsuji H, Takahashi T, Kawashima K, Nagata S, et al. (2017). Evolution of gut *Bifidobacterium* population in healthy Japanese infants over the first three years of life: a quantitative assessment. Sci Rep 7(1):10097 [11pp, plus supplementary data]. DOI:10.1038/s41598-017-10711-5.

NCBI (2014a). Search results for 'Bifidobacterium bifidum S17, complete genome'. (GenBank: CP002220.1). In: *NCBI Nucleotide Database*. Bethesda (MD): National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine (NLM). Available at: https://www.ncbi.nlm.nih.gov/nuccore/CP002220 [Circular BCT 31-Jan-2014].

NCBI (2014b). Search results for 'Bifidobacterium bifidum PRL2010, complete genome'. (GenBank: CP001840.1). In: *NCBI Nucleotide Database*. Bethesda (MD): National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine (NLM). Available at: https://www.ncbi.nlm.nih.gov/nuccore/CP001840 [Circular BCT 31-Jan-2014].

OECD (1998). *OECD Principles of Good Laboratory Practice*. (Series on Principles of Good Laboratory Practice and Compliance Monitoring, no. 1 [ENV/MC/CHEM(98)17]). Paris, France: Organisation for Economic Co-Operation & Development (OECD), Environment Directorate, Chemicals Group and Management Committee, OECD Environmental Health and Safety Publications. Available at: http://www.oecd-ilibrary.org/environment/oecd-principles-on-good-laboratory-practice_9789264078536-en [As revised in 1997].

Olsen R, Greisen G, Schrøder M, Brok J (2016). Prophylactic probiotics for preterm infants: a systematic review and meta-analysis of observational studies. Neonatology 109(2):105-112. DOI:10.1159/000441274.

Pariza MW, Gillies KO, Kraak-Ripple SF, Leyer G, Smith AB (2015). Determining the safety of microbial cultures for consumption by humans and animals. Regul Toxicol Pharmacol 73(1):164-171. DOI:10.1016/j.yrtph.2015.07.003.

Peirotén A, Arqués JL, Medina M, Rodríguez-Mínguez E (2018). Bifidobacterial strains shared by mother and child as source of probiotics. Benef Microbes 9(2):231-238. DOI:10.3920/BM2017.0133.

Rao SC, Athalye-Jape GK, Deshpande GC, Simmer KN, Patole SK (2016). Probiotic supplementation and late-onset sepsis in preterm infants: a meta-analysis. Pediatrics 137(3):e20153684 [16pp]. DOI:10.1542/peds.2015-3684.

Robertson C, Savva GM, Clapuci R, Jones J, Maimouni H, Brown E, et al. (2019). Incidence of necrotising enterocolitis before and after introducing routine prophylactic *Lactobacillus* and *Bifidobacterium* probiotics. Arch Dis Child Fetal Neonatal Ed 105:F380–F386. DOI:10.1136/archdischild-2019-317346.

Robinson J (2014). Cochrane in context: probiotics for prevention of necrotizing enterocolitis in preterm infants. Evid Based Child Health 9(3):672-674. DOI:10.1002/ebch.1977.

Saengtawesin V, Tangpolkaiwalsak R, Kanjanapattankul W (2014). Effect of oral probiotics supplementation in the prevention of necrotizing enterocolitis among very low birth weight preterm infants. J Med Assoc Thai 97(Suppl. 6):S20-S25.

Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM et al. (1998). Demonstration of safety of probiotics - a review. Int J Food Microbiol 44(1/2):93-106. DOI:10.1016/S0168-1605(98)00128-7.

Samanta M, Sarkar M, Ghosh P, Ghosh JK, Sinha Mk, Chatterjee S (2009). Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. J Trop Pediatr 55(2):128-131. DOI:10.1093/tropej/fmn091.

Shane AL, Cabana MD, Vidry S, Merenstein D, Hummelen R, Ellis CL, et al. (2010). Guide to designing, conducting, publishing and communicating results of clinical studies involving probiotic applications in human participants. Gut Microbes 1(4):243-253. DOI:10.4161/gmic.1.4.12707.

Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, et al. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature 562(7728):583-588. DOI:10.1038/s41586-018-0617-x.

Tanaka K, Tsukahara T, Yanagi T, Nakahara S, Furukawa O, Tsutsui H, et al. (2017). *Bifidobacterium bifidum* OLB6378 simultaneously enhances systemic and mucosal humoral immunity in low birth weight infants: a non-randomized study. Nutrients 9(3):195 [10pp]. DOI:10.3390/nu9030195.

Terahara M, Shibata T, Kinouchi T, Toshimitsu T, Ikegami S (2012) [unpublished]. *Study on Effectiveness of Bifidobacterium bifidum Administration to Very Low Birth Weight Infants (Multicenter Study).* Food Science Research Laboratories, Meiji Co., Ltd.

Terahara M, Nakamura Y, Tsuboi M, Jinno S, Tsukahara T, Miyake T, Shimoko N (2021). Effects of the intake of non-live Bifidobacterium bifidum on the faecal IgA of full-term infants: a double-blind, randomised, placebo-controlled study. Biosci Microbiota Food Health 40(4):196–203. DOI:10.12938/bmfh.2021-018.

Terahara M, Tsuchiya K, Tsuboi M, Jinno S, Nakamura Y (2020) [unpublished]. *Effect of Heat-Treated Bifidobacterium bifidum OLB6378 Administration on Fecal IgA and Fecal Bifidobacterium Counts in Low Birth Weight Infants: A Randomized Placebo-Controlled Double-Blind Parallel-Group Trial.* Food Science Research Laboratories, Meiji Co., Ltd.

Toshimitsu T, Nakamura M, Ikegami S, Terahara M, Itou H (2013). Strain-specific identification of *Bifidobacterium bifidum* OLB6378 by PCR. Biosci Biotechnol Biochem 77(3):572-576. DOI:10.1271/bbb.120803.

Totsu S, Yamasaki C, Terahara M, Uchiyama A, Kusuda S (2014). Probiotics Study Group in Japan. Bifidobacterium and enteral feeding in preterm infants: cluster-randomized trial. Pediatr Int 56(5):714-719. DOI:10.1111/ped.12330.

Totsu S, Terahara M, Kusuda S (2018). Probiotics and the development of very low birthweight infants: follow-up study of a randomised trial. BMJ Paediatr Open 2(1):e000256 [7pp]. DOI:10.1136/bmjpo-2018-000256.

Turroni F, Duranti S, Bottacini F, Guglielmetti S, Van Sinderen D, Ventura M (2014). *Bifidobacterium bifidum* as an example of a specialized human gut commensal. Front Microbiol 5:437 [8pp]. DOI:10.3389/fmicb.2014.00437.

U.S. FDA (2000). *Agency Response Letter GRAS Notice No. GRN 45 [Trehalose, Westminister (CO): Hayashibara International Inc.]*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Premarket Approval. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=45 [Oct. 5, 2000 - FDA response - no questions].

U.S. FDA (2002). *Agency Response Letter GRAS Notice No. GRN 49 [Bifidobacterium lactis strain Bb12 and Streptococcus thermophilus strain Th4, Glendale (CA): Nestle USA]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=49 [Mar. 19, 2002 - FDA response - no questions; additional correspondence available].

U.S. FDA (2009). *Agency Response Letter GRAS Notice No. GRN 268 [Bifidobacterium longum strain BB536, Tokyo, Japan: Morinaga Milk Industry Co., Ltd.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=268 [Jul. 8, 2009 - FDA response - no questions].

U.S. FDA (2011). *Agency Response Letter GRAS Notice No. GRN 377 [Bifidobacterium animalis subsp. lactis strain Bf-6, Wayzata (MN): Cargill Inc.]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=377 [Sep. 29, 2011 - FDA response - no questions].

U.S. FDA (2013a). *Agency Response Letter GRAS Notice No. GRN 445 [Bifidobacterium animalis subsp. lactis strains HN019, Bi-07, Bl-04 and B420, Madison (WI): Danisco USA, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=445 [Apr. 10, 2013 - FDA response - no questions].

U.S. FDA (2013b). *Agency Response Letter GRAS Notice No. GRN 453 [Bifidobacterium breve M-16V, Tokyo, Japan: Morinaga Milk Industry Co., Ltd]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=453 [Sep. 27, 2013 - FDA response - no questions].

U.S. FDA (2013c). *Agency Response Letter GRAS Notice No. GRN 454 [Bifidobacterium breve M-16V, Tokyo, Japan: Morinaga Milk Industry Co., Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=454 [Sep. 27, 2013 - FDA response - no questions].

U.S. FDA (2013d). *Agency Response Letter GRAS Notice No. GRN 455 [Bifidobacterium breve M-16V, The Netherlands: Danone Trading B.V.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=455 [Sep. 30, 2013 - FDA response - no questions].

U.S. FDA (2018). *Agency Response Letter GRAS Notice No. GRN 758 [Lactobacillus helveticus R0052, Bifidobacterium longum subsp. infantis R0033, and Bifidobacterium bifidum R0071, Mirabel (QC): Lallemand Health Solutions]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=758 [Aug 20, 2018 - FDA response - no questions].

U.S. FDA (2019a). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs* (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
168—Sweeteners and table sirups	168.122	Lactose
170—Food Additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe
	182.1748	Sodium caseinate
184—Direct food substances affirmed as generally recognized as safe	184.1619	Potassium carbonate
	184.1763	Sodium hydroxide
	184.1854	Sucrose
	184.1979	Whey
	184.1983	Bakers yeast extract

U.S. FDA (2019b). *Agency Response Letter GRAS Notice No. GRN 814 [Bifidobacterium bifidum BGN4, Republic of Korea: Bifico Co., Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=814 [Jun. 25, 2019 - FDA response - no questions].

U.S. FDA (2019c). *Agency Response Letter GRAS Notice No. GRN 813 [Bifidobacterium longum BORI, Republic of Korea: Bifico Co., Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=813 [Jun. 21, 2019 - FDA response - no questions].

U.S. FDA (2019d). *Agency Response Letter GRAS Notice No. GRN 855 [Bifidobacterium animalis subsp. lactis strain R0421, Mirabel (QC): Lallemand Health Solutions]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=855 [Feb. 5, 2020 – FDA response – no questions].

U.S. FDA (2019e). *Agency Response Letter GRAS Notice No. GRN 856 [Bifidobacterium animalis subsp. lactis strain BB-12, Milwaukee (WI): Chr. Hansen, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=856 [Dec. 9, 2019 - FDA response - no questions].

U.S. FDA (2019f). *Agency Response Letter GRAS Notice No. 872 [Bifidobacterium animalis subsp. lactis UABla-12, Windsor (WI): UAS Laboratories, LLC.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=872 [Dec. 9, 2019 - FDA response - no questions].

U.S. FDA (2019g). *Agency Response Letter GRAS Notice No. 877 [Bifidobacterium longum BB536, Tokyo, Japan: Morinaga Milk Industry Co., Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=877 [Dec. 26, 2019 - FDA response - no questions].

U.S. FDA (2021a). *Agency Response Letter GRAS Notice No. 950 [Bifidobacterium longum subsp. infantis DSM 33361, Milwaukee (WI): Chr. Hansen, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=950 [Mar. 1, 2021 - FDA response - no questions].

U.S. FDA (2021b). *Agency Response Letter GRAS Notice No. 952 [Bifidobacterium animalis subsp. lactis strain AD011, Republic of Korea: BIFIDO Co. Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=952 [Mar. 17, 2021 - FDA response - no questions].

U.S. FDA (2021c). *Agency Response Letter GRAS Notice No. 985 [Bifidobacterium longum subsp. infantis strain ATCC SD 6720, Wilmington (DE): Danisco, USA Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=985 [Dec. 21, 2021 - FDA response - no questions].

U.S. FDA (2021d). *Agency Response Letter GRAS Notice No. 1002 [Bifidobacterium breve strain MCC1274, Tokyo, Japan: Morinaga Milk Industry Co., Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=1002 [Pending as of Feb. 21, 2022].

U.S. FDA (2021e). *Agency Response Letter GRAS Notice No. 1003 [Bifidobacterium longum subsp. infantis M-63, Tokyo, Japan: Morinaga Milk Industry Co., Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=1003 [Pending as of Feb. 21, 2022].

Uberos J, Campos-Martinez A, Fernandez-Marin E, Millan IC, Lopez AR, Blanca-Jover E. (2021). Effectiveness of two probiotics in preventing necrotising enterocolitis in a cohort of very-low-birth-weight premature new-borns. Beneficial Microbes 0(0):1-8. DOI:10.3920/BM2021.0088. [abstract only].

USDA (2018). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2015-2016.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: July 31, 2018].

Underwood MA, Salzman NH, Bennett SH, Barman M, Mills DA, Marcobal A, et al. (2009). A randomized placebo-controlled comparison of 2 prebiotic/probiotic combinations in preterm infants: impact on weight gain, intestinal microbiota, and fecal short-chain fatty acids. J Pediatr Gastroenterol Nutr 48(2):216-225. DOI:10.1097/MPG.0b013e31818de195.

Underwood MA, Kananurak A, Coursodon CF, Adkins-Reick CK, Chu H, Bennett SH, et al. (2012). *Bifidobacterium bifidum* in a rat model of necrotizing enterocolitis: antimicrobial peptide and protein responses. Pediatr Res 71(5):546-551. DOI:10.1038/pr.2012.11.

Verschuren PM (Foreword) (1995). A scientific basis for regulations on pathogenic microorganisms in foods. Dairy Food Environ Sanit 15(5):301-308.

Wang Q, Wang, K, Wu W, Lv L, Bian X, Yang L, et al. (2020). Administration of *Bifidobacterium bifidum* CGMCC 15068 modulates gut microbiota and metabolome in azoxymethane (AOM)/dextran sulphate sodium (DSS)-induced colitis-associated colon cancer (CAC) in mice. Appl Microbiol Biotechnol 104:5915–5928. DOI: 10.1007/s00253-020-10621-z.

Weber E, Reynaud Q, Suy F, Gagneux-Brunon A, Carricajo A, Guillot A, Botelho-Nevers E (2015). *Bifidobacterium* species bacteremia: risk factors in adults and infants. Clin Infect Dis 61(3):482-484. DOI:10.1093/cid/civ347.

Xiao L, Gong C, Ding Y, Ding G, Xu X, Deng C et al. (2019). Probiotics maintain intestinal secretory immunoglobulin A levels in healthy formula-fed infants: a randomised, double-blind, placebo-controlled study. Beneficial Microbes 10(7):729-739. DOI:10.3920/BM2019.0025.

Yamasaki C, Totsu S, Uchiyama A, Nakanishi H, Masumoto K, Washio Y, et al. (2012). Effect of *Bifidobacterium* administration on very-low-birthweight infants. Pediatr Int 54(5):651-656. DOI:10.1111/j.1442-200X.2012.03649.x.

Yilmaz B, Schibli S, Macpherson AJ, Sokollik C (2018). D-lactic acidosis: successful suppression of D-lactate-producing *Lactobacillus* by probiotics. Pediatrics 142(3):e20180337 [5pp]. DOI:10.1542/peds.2018-0337.

From: yoshitaka.nakamura@meiji.com
To: Morissette, Rachel
Cc: ryan.simon@intertek.com
Subject: [EXTERNAL] RE: questions for GRN 001090
Date: Wednesday, February 22, 2023 3:49:09 AM
Attachments: image008.png
image010.png
image012.png
image014.png
image016.png
image018.png
GRN 001090 - Response to FDA Questions - FINAL 21Feb2023 .pdf

---

Dear Dr. Morissette,

Please see the attached responses to the United States (U.S.) Food and Drug Administration (FDA)'s letter dated 08 February 2023 pertaining to information provided within Meiji Co., Ltd. (Meiji)'s Generally Recognized as Safe (GRAS) Notice for the intended use of ***Bifidobacterium bifidum*** strain NITE BP-31 filed by the Agency under GRN 001090.

We hope this information adequately addresses the Agency's questions regarding GRN 001090. If the Agency requires any additional information or further clarification, Meiji will be happy to provide it upon request.

Sincerely,

Yoshitaka Nakamura, Ph.D.
R&D Division
Food Microbiology and Function Research Laboratories
Meiji Co., Ltd.

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, February 9, 2023 6:05 AM
To: 中村 吉孝 <yoshitaka.nakamura@meiji.com>
Cc: Ryan Simon Intertek <ryan.simon@intertek.com>
Subject: questions for GRN 001090

Dear Dr. Nakamura,

Please see attached our questions for GRN 001090. Let me know if you have any questions at this time.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

Meiji Co., Ltd.
R&D Division
Food Microbiology and Function Research Laboratories
1-29-1 Nanakuni, Hachiouji, Tokyo 192-0919, JAPAN
TEL:+81-42-632-5803/FAX:+81-42-637-3011

21 February 2023

Rachel Morissette, Ph.D.
Regulatory Review Scientist
Division of Food Ingredients
Center for Food Safety & Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Re: GRAS Notice No. GRN 001090

Dear Dr. Morissette,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s letter dated 08 February 2023 pertaining to information provided within Meiji Co., Ltd. (Meiji)'s Generally Recognized as Safe (GRAS) Notice for the intended use of *Bifidobacterium bifidum* strain NITE BP-31 filed by the Agency under GRN 001090.

FDA.1. *On p.8, Meiji states that sucrose and trehalose are used as cryoprotectants in the manufacturing process. We have no concerns regarding Meiji's use of sucrose as a cryoprotectant. However, while trehalose has been evaluated under GRN 000045 as a multipurpose ingredient for use in food in general, it has not been evaluated for use in infant formula. Therefore, we request that Meiji removes the use of trehalose as a cryoprotectant.*

Meiji has removed the use of trehalose as a cryoprotectant. Additionally, trehalose has been removed from Table 2.2.1-1 (see updated table below).

Table 2.2.1-1 Additives and Processing Aids Used in the Production of Freeze-dried *Bifidobacterium bifidum* OLB6378 Powder

Raw Material	Use	Regulatory Status
Skim milk	Starter medium	GRAS (21 CFR §182.1)
Yeast extract	Starter and manufacturing medium	Bakers yeast extract is a direct food substance affirmed as GRAS as defined in 21 CFR §184.1983 (U.S. FDA, 2019)
Hydrolyzed whey protein	Manufacturing medium	Whey is a direct food substance affirmed as GRAS as defined in 21 CFR §184.1979 (U.S. FDA, 2019)
Lactose	Manufacturing medium	GRAS (21 CFR §168.122 – U.S. FDA, 2019)
Casein	Manufacturing medium	Sodium caseinate is GRAS when used in accordance with GMP (21 CFR §182.1748 – U.S. FDA, 2019)
Sodium hydroxide	Manufacturing medium	Used in food with no limitation other than cGMP (21 CFR §184.1763 – U.S. FDA, 2019)
Potassium carbonate	Manufacturing medium	Used in food with no limitation other than cGMP (21 CFR §184.1619 – U.S. FDA, 2019)
Sucrose	Carbohydrate carrier	Used in food with no limitation other than cGMP (21 CFR §184.1854 – U.S. FDA, 2019)

CFR = *Code of Federal Regulations*; cGMP = current Good Manufacturing Practice; GMP = Good Manufacturing Practice; GRAS = Generally Recognized as Safe.

FDA.2. *Please specify the protein base(s) of the non-exempt infant formula(s) (e.g., cow milk, soy, etc) to which Meiji intends to add B. bifidum strain NITE BP-31.*

Meiji intends to add *B. bifidum* strain NITE BP-31 to a cow milk protein base.

FDA.3. *In describing the test methods used for specifications, please provide complete citations for any published or compendial methods used and provide a statement to confirm that all methods, including any internally developed methods, are validated and appropriate for the intended purposes.*

All test methods used for specifications are developed by an external laboratory testing company and Meiji confirms that all test methods have been validated and are appropriate for their intended purpose.

FDA.4. *Please confirm that B. bifidum strain NITE BP-31 is non-pathogenic and non-toxigenic.*

Meiji confirms that *B. bifidum* strain NITE BP-31 is non-pathogenic and non-toxigenic.

FDA.5. *Please briefly describe the in-process controls in place during the fermentation process and clarify how contamination is controlled for during the manufacturing process. Additionally, please state whether the fermentation process is conducted in a contained, sterile environment.*

Meiji routinely evaluates the quality of the *B. bifidum* strain NITE BP-31 products using in-process controls to ensure that the finished products are not contaminated. The timing and parameters measured during the culturing and concentrating process are provided in Table FDA.5-1. Meiji confirms that the fermentation process is conducted in a contained, sterile environment.

Table FDA.5-1 Quality Control Parameters Monitored During the Production of *Bifidobacterium bifidum* OLB6378 Powder

	Process		
Parameter*	**Culturing**	**Concentrating**	**Freeze-dried Powder/ Finished Product**
Culture pH	X		
Water activity			X
Bifidobacterium	X	X	X
Aerobic plate count		X	X
Coliforms	X	X	X
Salmonella spp.		X	X
Bacillus cereus	X	X	X
Staphylococcus aureus		X	X
Cronobacter sakazakii		X	X
Molds	X	X	X
Yeast	X	X	X
Arsenic			X
Lead			X
Heavy Metals (as Lead)			X
Appearance		X	X
Odor and Taste		X	X

"X" denotes that the parameter is measured.
* Methods are validated and are the same as those described in the product specifications.

FDA.6. *Please briefly describe how the stability of B. bifidum strain NITE BP-31 is ensured.*

The stability of *B. bifidum* strain NITE BP-31 (OLB6378) is discussed in Section 2.4 of the Notice (*Stability*). *B. bifidum* strain NITE BP-31 demonstrates high viability when stored at -20°C. Therefore, Meiji packs the manufactured *B. bifidum* strain NITE BP-31 in aluminum bags and stores them at -20°C or below to ensure their stability.

FDA.7. *Please briefly specify how the purity of B. bifidum strain NITE BP-31 is ensured.*

Meiji routinely evaluates the genotype of the *B. bifidum* strain NITE BP-31 using strain-specific identification methods by PCR (Toshimitsu *et al*., 2013) in the finished product and ensures it is consistent with that of the original stock.

FDA.8. *On p.34, Meiji states that the results of MIC testing did not identify any evidence of acquired antibiotic resistance genes that would pose a safety concern. Please state whether B. bifidum strain NITE BP-31 is capable of DNA transfer to other organisms.*

As discussed in Section 6.3.2 of the Notice (*Bioinformatic Assessment for Undesirable Gene Products*), no plasmid DNA was reported in *B. bifidum* strain NITE BP-31 (OLB6378). Therefore, Meiji confirms that *B. bifidum* strain NITE BP-31 does not transfer DNA to other organisms.

FDA.9. *Please identify if any of the raw materials used in the fermentation process are major allergens or are derived from major allergens and discuss whether these pose a safety concern. If none of the raw materials used in the manufacturing process is a major allergen or is derived from major allergens, please provide a statement of affirmation. As of January 1, 2023, under the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act, sesame is now also considered a major food allergen: (https://www.fda.gov/food/cfsan-constituent-updates/faster-act-video-food-industry-and-other-stakeholders).*

Raw materials used in the manufacturing process contain the major allergen “milk.” *B. bifidum* strain NITE BP-31 is used in a cow's milk-based non-exempt infant formula, so there are no additional allergenicity issues arising from the inclusion of “milk” in raw materials used in the manufacturing process.

FDA.10. *On p.10, Meiji lists a specification for Cronobacter sakazakii and states that the method used is ISO/TS 22964. The current version of this method is ISO 22964:2017, which corresponds to "Microbiology of the Food Chain - Horizontal Method for the Detection of Cronobacter spp." Please clarify whether Meiji tests for the presence of Cronobacter spp. or C. sakazakii, specifically. If it is the former, please state whether presumptive positives are further analyzed to determine if the isolate is C. sakazakii.*

Additionally, the referenced method, ISO 22964, recommends a sample size of 10 g. On p.10, the specification provided for C. sakazakii is "Negative/5 g." Please clarify whether Meiji has validated this method for a sample size of 5 g.

Meiji currently uses ISO 22964:2017 to detect *C. sakazakii*. Additionally, the analytical methods used to detect *C. sakazakii* have been validated and are fit for purpose. Meiji has validated this method for a sample size of 5 g.

FDA.11. *On p.10, Meiji provides the following specifications:*
– coliforms: negative/2.22 g
– B. cereus: negative/0.01 g
– S. aureus: negative/0.01 g
– yeast: negative/0.1 g
– molds: negative/0.1 g
Please clarify the sample sizes used for the methods cited for these microbial analyses, and state whether these methods (and corresponding sample sizes) have been validated for their intended purpose.

The sample sizes used for the methods are as below:

- Coliforms: 2.22 g
- *B. cereus*: 0.01 g
- *S. aureus*: 0.01 g
- Yeast: 0.1 g
- Molds: 0.1 g

Meiji confirms the reported sample sizes as accurate for each parameter. The methods and sample sizes have been validated and are appropriate for their intended purpose.

FDA.12. *On p.19, Meiji states, "Resistance to aminoglycosides was reported (MIC of 128 to 512 µg/mL); however, this resistance phenotype is considered an intrinsic characteristic of members of the Bifidobacterium genus (Mayrhofer et al., 2011)."*

Please briefly summarize the reference in the quoted passage, focusing the discussion on the intrinsic resistance to aminoglycosides that members of the genera have.

Mayrhofer *et al.* (2011) state that most bifidobacteria have been reported as resistant to aminoglycosides due to the absence of a cytochrome-mediated drug transport system, such that aminoglycosides cannot reach their target. The authors cite Bryan *et al.* (1979) who reported a mechanism of aminoglycoside resistance in the anaerobic bacteria *Clostridium perfringens* and *Bacteroides fragilis*. Bryan *et al.* (1979) reported that cytochromes involved in electron transport chain and redox catalysis were not detected in aminoglycoside-resistant *C. perfringens*. Cytochrome b was detected in *B. fragilis*, in which some degree of aminoglycoside entry was reported following induction of fumarate-dependent electron transport, and there is evidence that adenosine trisphosphate (ATP) synthesis may be coupled with electron transport using fumarate as a terminal electron acceptor in this bacterium. The authors concluded that, "[…] *these results demonstrate that anaerobic bacteria unable to carry out oxygen- or nitrate-dependent electron transport are resistant to streptomycin and gentamicin because of failure to transport aminoglycosides*" (Bryan *et al.*, 1979).

In addition to the reports above, it is well-established that aminoglycoside transport responds to the magnitude of the proton-motive force generated by respiration. Bacteria with absent or limited electron transport systems and with relatively inefficient yields of ATP and other membrane energy from this source are therefore resistant to aminoglycosides as they are unable to transport them (Mingeot-Leclercq *et al.*, 1999; Krause *et al.*, 2016).

Therefore, members of the anaerobic genus *Bifidobacterium*, with absent electron transport systems, are resistant to aminoglycosides because of poor aminoglycoside transport.

FDA.13. *On p.16, Meiji states that an updated literature search was performed in February 2022. Please confirm that no new information that may appear counter to your GRAS conclusion has been published since February 2022.*

An updated literature search was performed in February 2023 according to the methods in the Notice, and abstracts of relevant publications were reviewed. Several publications were identified as relevant to the safety of *B. bifidum* strain NITE BP-31 (Table FDA.13-1). Meiji identified 1 publication raising potential adverse effects following administration of *B. bifidum*. Springer *et al.* (2022) presented a poster at the Perinatal Society of Australia and New Zealand 2022 Annual Congress, "Better Together: Collaborative Care, Research and Guidelines," held in May 2022. The abstract was published in the supplement to the Journal of Paediatrics and Child Health and describes a retrospective observational cohort study. The study authors identified 3 occurrences of neonatal bacteremia with bifidobacteria species in the cohort that was administered routine probiotic prophylaxis (consisting of *B. bifidum* and *Lactobacillus acidophilus*). One case underwent comparative genomic testing showing a match between the organism in the probiotic and in the blood culture isolate. No other details regarding the poster abstract are publicly available. Given the absence of these data, including the method of

identification and the strain identity of the *Bifidobacterium*, Meiji considers the discussion in Section 6.8 of the Notice (*Bacterial Translocation and Pathogenicity*) as sufficient in addressing potential risk of bacterial translocation and pathogenicity of *B. bifidum* strain NITE BP-31. Therefore, Meiji confirms that no new information that may appear to counter their GRAS conclusion has been published since February 2022.

Table FDA.13-1 Relevant Publications Identified in Literature Since February 2022

Title	Author	Study Design – Population	DOI
Effect of a Multi-Strain Probiotic on Growth and Time to Reach Full Feeds in Preterm Neonates	Sowden *et al.* (2022a)	Clinical trial administering *Bifidobacterium bifidum* with other microorganisms – Preterm infants	doi.org/10.3390/nu14214658
Effect of a Multi-Strain Probiotic on the Incidence and Severity of Necrotizing Enterocolitis and Feeding Intolerances in Preterm Neonates	Sowden *et al.* (2022b)	Clinical trial administering *B. bifidum* with other microorganisms – Preterm infants	doi.org/10.3390/nu14163305
Multispecies Probiotic for the Prevention of Antibiotic-Associated Diarrhea in Children: A Randomized Clinical Trial	Łukasik *et al.* (2022)	Clinical trial administering *B. bifidum* with other microorganisms – Children of age 3 months to 18 years	doi.org/10.1001/jamapediatrics.2022.1973
Probiotic supplementation and systemic inflammation in relapsing-remitting multiple sclerosis: A randomized, double-blind, placebo-controlled trial	Rahimlou *et al.* (2022)	Clinical trial administering *B. bifidum* with other microorganisms – Adults with multiple sclerosis	doi.org/10.3389/fnins.2022.901846
Effectiveness of two probiotics in preventing necrotising enterocolitis in a cohort of very-low-birth-weight premature new-borns	Uberos *et al.* (2022)	Observational study evaluating *B. bifidum* with other microorganisms – Preterm infants	doi.org/10.3920/BM2021.0088
Exploring the long-term colonisation and persistence of probiotic-prophylaxis species on the gut microbiome of preterm infants: a pilot study	Westaway *et al.* (2022)	Observational study evaluating *B. bifidum* with other microorganisms – Preterm infants	doi.org/10.1007/s00431-022-04548-y
Strain-specific impacts of probiotics are a significant driver of gut microbiome development in very preterm infants	Beck *et al.* (2022)	Retrospective study evaluating *B. bifidum* with other microorganisms – Preterm infants	doi.org/10.1038/s41564-022-01213-w
Enteral supplementation with probiotics in preterm infants: A retrospective cohort study and 6-year follow-up	Brown *et al.* (2022)	Retrospective study evaluating *B. bifidum* with other microorganisms – Preterm infants	doi.org/10.3389/fnut.2022.1063121
Neonatal bacteraemia with bifidobaceteria or	Springer *et al.* (2022)*	Retrospective study evaluating *B. bifidum* with	doi.org/10.1111/jpc.15946

Table FDA.13-1 Relevant Publications Identified in Literature Since February 2022

Title	Author	Study Design – Population	DOI
lactobacillus species after the introduction of prophylactic probiotics: A retrospective observational cohort study		other microorganisms – Preterm infants	
Effects of Bifidobacterium with the Ability of 2'-Fucosyllactose Utilization on Intestinal Microecology of Mice	Mao *et al.* (2022)	Rodent efficacy study administering *B. bifidum* in isolation – NA	doi.org/10.3390%2Fnu14245392
Early life administration of Bifidobacterium bifidum BD-1 alleviates long-term colitis by remodeling the gut microbiota and promoting intestinal barrier development	Peng *et al.* (2022)	Rodent efficacy study administering *B. bifidum* in isolation – NA	doi.org/10.3389/fmicb.2022.916824

NA = not applicable.
* Only poster abstract available.

--

We hope this information adequately addresses the Agency's questions regarding GRN 001090. If the Agency requires any additional information or further clarification, Meiji will be happy to provide it upon request.

Sincerely,

[redacted]

Yoshitaka Nakamura, Ph.D.
R&D Division
Food Microbiology and Function Research Laboratories
Meiji Co., Ltd.

REFERENCES

Beck LC, Masi AC, Young GR, Vatanen T, Lamb CA, Smith R, et al. (2022). Strain-specific impacts of probiotics are a significant driver of gut microbiome development in very preterm infants. Nat Microbiol 7(10):1525-1535. DOI:10.1038/s41564-022-01213-w.

Brown BE, Huisman E, Miller MR, Ulrich C, Reid G, da Silva O (2022). Enteral supplementation with probiotics in preterm infants: A retrospective cohort study and 6-year follow-up. Front Nutr 9:Article 1063121 [7pp]. DOI:10.3389/fnut.2022.1063121.

Bryan LE, Kowand SK, Van Den Elzen HM (1979). Mechanism of aminoglycoside antibiotic resistance in anaerobic bacteria: *Clostridium perfringens* and *Bacteroides fragilis*. Antimicrob Agents Chemother 15(1):7-13. DOI:10.1128/aac.15.1.7.

Krause KM, Serio AW, Kane TR, Connolly LE (2016). Aminoglycosides: An overview. Cold Spring Harb Perspect Med 6(6):a027029 [18pp]. DOI:10.1101/cshperspect.a027029.

Lukasik J, Dierikx T, Besseling-van der Vaart I, de Meij T, Szajewska H (2022). Multispecies probiotic for the prevention of antibiotic-associated diarrhea in children: a randomized clinical trial. JAMA Pediatr 176(9):860-866. DOI:10.1001/jamapediatrics.2022.1973.

Mao B, He Z, Chen Y, Stanton C, Ross RP, Zhao J, et al. (2022). Effects of *Bifidobacterium* with the ability of 2' fucosyllactose utilization on intestinal microecology of mice. Nutrients 14(24):5392 [17pp]. DOI:10.3390/nu14245392.

Mayrhofer S, Mair C, Kneifel W, Domig KJ (2011). Susceptibility of bifidobacteria of animal origin to selected antimicrobial agents. Chemother Res Pract 2011:Article ID 89520 [6pp]. DOI:10.1155/2011/989520.

Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM (1999). Aminoglycosides: activity and resistance. Antimicrob Agents Chemother 43(4):727-737. DOI:10.1128/aac.43.4.727.

Peng C, Li J, Miao Z, Wang Y, Wu S, Wang Y, et al. (2022). Early life administration of *Bifidobacterium bifidum* BD-1 alleviates long-term colitis by remodeling the gut microbiota and promoting intestinal barrier development. Front Microbiol 13:Article 916824. DOI:10.3389/fmicb.2022.916824.

Rahimlou M, Nematollahi S, Husain D, Banaei-Jahromi N, Majdinasab N, Hosseini SA (2022). Probiotic supplementation and systemic inflammation in relapsing-remitting multiple sclerosis: A randomized, double-blind, placebo-controlled trial. Front Neurosci 16:Article 901846 [Corrigendum: 1085572]. DOI:10.3389/fnins.2022.901846.

Sowden M, van Niekerk E, Bulabula ANH, Twisk J, van Weissenbruch MM (2022a). Effect of a multi-strain probiotic on growth and time to reach full feeds in preterm neonates. Nutrients 14(21):4658 [12pp]. DOI:10.3390/nu14214658.

Sowden M, van Weissenbruch MM, Bulabula ANH, van Wyk L, Twisk J, van Niekerk E (2022b). Effect of a multi-strain probiotic on the incidence and severity of necrotizing enterocolitis and feeding intolerances in preterm neonates. Nutrients 14(16):3305 [10pp]. DOI:10.3390/nu14163305.

Springer S, Voss L, Knight D, Alsweiler J (2022). Neonatal bacteraemia with bifidobaceteria or lactobacillus species after the introduction of prophylactic probiotics: A retrospective observational cohort study. J Paediatr Child Health 58(Suppl. 2):90-91. DOI:10.1111/jpc.15946.

Toshimitsu T, Nakamura M, Ikegami S, Terahara M, Itou H (2013). Strain-specific identification of *Bifidobacterium bifidum* OLB6378 by PCR. Biosci Biotechnol Biochem 77(3):572-576. DOI:10.1271/bbb.120803.

U.S. FDA (2019). *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs* (Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/2019/.

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
168—Sweeteners and table sirups	168.122	Lactose
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe
	182.1748	Sodium caseinate
184—Direct food substances affirmed as generally recognized as safe	184.1619	Potassium carbonate
	184.1763	Sodium hydroxide
	184.1854	Sucrose
	184.1979	Whey
	184.1983	Bakers yeast extract

Uberos J, Campos-Martinez A, Fernandez-Marín E, Millan IC, Lopez AR, Blanca-Jover E (2022). Effectiveness of two probiotics in preventing necrotising enterocolitis in a cohort of very-low-birth-weight premature new-borns. Benef Microbes 13(1):25-31. DOI:10.3920/bm2021.0088.

Westaway JAF, Huerlimann R, Kandasamy Y, Miller CM, Norton R, Watson D, et al. (2022). Exploring the long-term colonisation and persistence of probiotic-prophylaxis species on the gut microbiome of preterm infants: a pilot study. Eur J Pediatr 181(9):3389-3400. DOI:10.1007/s00431-022-04548-y.