GRAS Notification for Use of *Bifidobacterium infantis* M-63 in General Foods and Cow's Milk- and Soy-Based, Non-Exempt Infant Formula

Prepared for:

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LIST OF ABBREVIATIONS

ADI: acceptable daily intake

ADP: adenosine diphosphate

ALP: alkaline phosphatase

ALT: alanine aminotransferase

BGLB: brilliant green bile lactose broth

B. infantis: *Bifidobacterium longum* subspecies *infantis*

BLG: β-lactoglobulin

BMO: bovine milk oligosaccharides

CFP: carbohydrate fermentation pattern

CFR: United States Code of Federal Regulations

CFU: colony forming units

CIP: clean in place

COA: Certificate of Analysis

EDI: Estimated Daily Intake

EFFCA: European Food & Feed Cultures Association

EFSA: European Food Safety Authority

FAO/WHO: Food and Agriculture Organization of the United Nations/World Health Organisation

FCC: Food Chemical Codex

FD: functional dyspepsia

FDA: United States Food and Drug Administration

FFDCA: Federal Food, Drug, and Cosmetic Act

FNDDS: Food and Nutrition Database for Dietary Studies

FOS: fructo-oligosaccharides

FSSC: Food Safety System Certification

GI: gastrointestinal

GMO: genetically modified organism

GOS: galacto-oligosaccharides

GRAS: Generally Recognized As Safe

GRN: GRAS Notification

HACCP: Hazard Analysis and Critical Control Point

IBS: irritable bowel syndrome

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

IDF: International Dairy Foundation

ISO: International Organization for Standardization

KO: knock out

LLDPE: linear low-density polyethylene

LOQ: Limit of quantification

MEC: mobile examination center

MIC: minimum inhibitory concentration

NASH: non-alcoholic steatohepatitis

NCBI: National Center for Biotechnology Information

ND: not detected

NEC: necrotizing enterocolitis

NHANES: National Health and Nutrition Examination Survey

NITE: National Institute of Technology (Japan)

NOAEL: no observed adverse effect level

NR: not required

OVA: ovalbumin

PEG: polyethylene glycol

MM: microbial mixture containing *B. longum* BB536, *B. infantis* M-63, and *B. breve* M-16V

ppm: parts per million

PSU: primary sampling unit

QPS: qualified presumption of safety

RAPD PCR: random amplification of polymorphic DNA polymerase chain reaction

RO: reverse osmosis

SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

TC: total cholesterol

TG: triglycerides

WT: wild-type

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

A. SUBMISSION OF GRAS NOTICE

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

B. NAME AND ADDRESS OF THE SPONSOR

Morinaga Milk Industry Co., Ltd.
33-1, Shiba 5-Chome, Minato-ku
Tokyo 108-8384
JAPAN

C. COMMON OR USUAL NAME

Bifidobacterium longum subsp. *infantis* M-63*; Bifidobacterium infantis* M-63; *B. infantis* M-63; M-63

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

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

E. INTENDED USE

B. infantis M-63 will be used as an ingredient in general foods and cow's milk- and soy-based, non-exempt term infant formula.

F. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of *B. infantis* M-63 as an ingredient in conventional foods at a maximum level of 1.25×10^{10} CFU per serving and powdered, cow's milk- and soy-based, non-exempt, term infant formula at a maximum level of 1×10^{8} CFU/g is based upon scientific procedures as described under 21 CFR §170.30(b). The intake of *B. infantis* M-63 from the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). To demonstrate that *B. infantis* M-63 is safe, and GRAS, under the intended conditions of use, the safety of the intake of *B. infantis* M-63 has been determined to be GRAS by demonstrating that

the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of *B. infantis* M-63 as an ingredient in foods and non-exempt infant formula has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

- Bifidobacteria are naturally occurring bacteria that contribute to the composition of the gut microflora of humans. *Bifidobacterium longum* species have been detected in feces from infants and adults.
- *Bifidobacterium infantis* M-63, a strain of *B. longum* subspecies *infantis*, is a Gram-positive anaerobic bacterium that was originally isolated from a healthy infant in 1963. The bacterium has been deposited with the National Institute of Technology and Evaluation (NITE, Japan) and is designated NITE BP-02623.
- *B. infantis* M-63 was first commercially available in 2006 and has since been sold in a variety of markets including China, France, Japan, Indonesia, Italy, and Spain.
- The original stock culture of *B. infantis* M-63 has been maintained at −80°C since it was obtained by Morinaga Milk in the 1970s, and no selective pressures have been applied.
- *B. infantis* M-63 cultures are used to produce two product formulations: 1) M-63, using tapioca starch, for use in general foods, and 2) M-63 type-C, using cornstarch, for use in general foods and infant formula.
- Finished products made with *B. infantis* M-63 consistently comply with established, food-grade product specifications. Specifications are in place to control anaerobic plate count (M-63 count), moisture, microbial contamination, and heavy metals. M-63 type-C for use in infant formula undergoes additional microbial testing for *Cronobacter sakazakii* and *Bacillus cereus*.
- Thirteen GRAS Notices (GRNs) on *Bifidobacterium* species have received “no questions” letters from the FDA. This includes GRN 758, which allows for the use of a strain of *B. longum* subsp. *infantis* R0033 in infant formula at levels up to $5x10^7$ CFU/g.
- *B. longum* has been granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA).

- *B. infantis* M-63 has been tested for parameters outlined in the Food and Agriculture Organization of the United Nations/World Health Organization's (FAO/WHO) guidelines. Results from these tests provide evidence that *B. infantis* M-63 is safe for use in foods, namely:
  - *B. infantis* M-63 is resistant to streptomycin, however, the resistance does not reside on a transferable element.
  - *B. infantis* M-63 produces L-lactic acid but does not produce D-lactic acid.
  - *B. infantis* M-63 is shown to deconjugate bile salts, but no secondary bile acids are produced.
  - An *in vitro* study indicates that production of biogenic amines by *B. infantis* M-63 is not expected.
  - An *in vitro* study indicates that *B. infantis* M-63 is not expected to produce ammonia.
  - The use of 3 different methods indicates that *B. infantis* M-63 is not expected to degrade mucins.
  - Testing has confirmed the absence of plasmids in *B. infantis* M-63.
  - Genomic analysis of *B. infantis* M-63 did not reveal the presence of known toxin or virulence genes.
  - *B. infantis* M-63 was not observed to have hemolytic activity.
  - *B. infantis* M-63 is not expected to induce platelet aggregation.
- The safety of *B. infantis* M-63 is supported by a published acute toxicology study and a pivotal published 90-day repeated dose toxicology study, both in rats. In the single dose oral toxicity test using 3.2×10^{11} CFU/kg of *B. infantis* M-63, there were no deaths or M-63 related adverse findings. The no observed adverse effect level (NOAEL) from the 90-day study was determined to be at least 7.6×10^{10} CFU/kg bw/day (Abe et al., 2009).
- Three published studies of *B. infantis* M-63 in microbial mixtures in children, two published studies of *B. infantis* M-63 in mixtures or alone in adults, and three published studies of *B. infantis* M-63 in microbial mixtures with and without oligosaccharides in infants support the safety of *B. infantis* M-63. No adverse events were reported in any study. These studies support the safe use of *B. infantis* M-63 in

children at doses up to $1.0x10^9$ CFU/day for 8 weeks and adults at doses up to $1.25x10^{10}$ CFU/day for 12 weeks. Additionally, these studies support the safe use of *B. infantis* M-63 in infants up to $1.4x10^8$ CFU/100 mL for 6 months or $5x10^8$ CFU/day for up to 6 weeks.

- A literature search revealed six published studies of other *B. longum* subsp. *infantis* strains that corroborate the safety of *B. infantis* M-63. These studies administered doses of $1x10^7$-$2.8x10^{10}$ CFU/day to term and preterm infants ages for 1 day to 12 months. The study durations ranged from 3 to 12 weeks. No study reported adverse events related to *B. longum* subsp. *infantis* intake.
- *B. infantis* M-63 will be added to select general foods at levels sufficient to provide $1.25x10^{10}$ CFU/serving at the end of shelf life. This will result in a mean estimated daily intake (EDI) for consumers age 2+ of 1.45×10^{10} CFU/day (2.16×10^8 CFU/kg bw/day) and a 90th percentile intake of 2.68×10^{10} CFU/day (4.01×10^8 CFU/kg bw/day).
- *B. infantis* M-63 will also be added to powdered, term infant formula at levels sufficient to provide 1×10^8 CFU/g at the end of shelf life. This will result in an EDI of 9.9×10^9 CFU/day for a 1-month old infant and 1.35×10^{10} CFU/day for a 6-month old infant. These intakes are equivalent to 2.3×10^9 CFU/kg bw/day and 1.8×10^9 CFU/kg bw/day, respectively.

Determination of the GRAS status of *B. infantis* M-63 under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, FACN, FASN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, CNS, and Thomas E. Sox, PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of *B. infantis* M-63 and the potential human exposure to *B. infantis* M-63 resulting from its intended use as an ingredient in foods and have concluded:

> *There is no evidence in the available information on B. infantis M-63 that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when B. infantis M-63 is used at levels that might reasonably be expected from the proposed applications. B. infantis M-63 is GRAS for use in foods as proposed by Morinaga Milk Industry Co, Ltd.*

Therefore, *B. infantis* M-63 is safe and GRAS at the proposed levels of addition to foods and non-exempt infant formula. *B. infantis* M-63 is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

G. PREMARKET APPROVAL

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

H. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, PhD, DABT, Managing Partner, Spherix Consulting Group, Inc., at 751 Rockville Pike, Unit 30-B, Rockville, MD 20852. Telephone: 301-775-9476; Email: ckruger@spherixgroup.com, or be sent to FDA upon request.

I. FREEDOM OF INFORMATION ACT (FOIA)

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

J. INFORMATION INCLUDED IN THE GRAS NOTIFICATION

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

April 29, 2021
Date

Claire L. Kruger, PhD, DABT
Managing Partner, Spherix Consulting Group, Inc.
Signature of Authorized Representative of
Morinaga Milk Industry Co., Ltd.

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

A. COMMON OR USUAL NAME

Bifidobacterium longum subsp. *infantis* M-63*; Bifidobacterium infantis* M-63; *B. infantis* M-63; M-63

B. TRADE NAME

M-63, M-63 type-C

C. DESCRIPTION OF *BIFIDOBACTERIUM INFANTIS* M-63

1. Taxonomy and Origin

Bifidobacterium infantis was first isolated from a healthy infant and identified in Germany in 1963 (Reuter, 1963) and officially designated as *B. infantis* in 1971 (Reuter, 1971). In 2008, in response to controversy on the taxonomic classification of *B. longum*, *B. infantis*, and *B. suis*, as well as mounting evidence of their genetic similarity, the three strains were consolidated into a single species. *B. longum* and *B. infantis* was reclassified as *B. longum* subspecies *infantis* (Mattarelli, 2008).

The full taxonomic classification of *B. longum* subspecies *infantis* is as follows:

Kingdom: Bacteria
Subkingdom: Posibacteria
Phylum: Actinobacteria
Subclass: Actinobacteridae
Order: Bifidobacteriales
Family: Bifidobacteriaceae
Genus: Bifidobacterium
Species: *Bifidobacterium longum*
Subspecies: *Bifidobacterium longum infantis*

Bifidobacterium infantis M-63, a strain of *B. longum* subspecies *infantis* was originally isolated from a healthy infant in 1963. Morinaga Milk obtained a sample of *B. longum* subsp. *infantis* M-63 in the 1970s. Since that time, the original stock of this strain, deemed *B. infantis* M-63, has been stored at −80ºC. No selective pressures have been applied to this strain since it was obtained.

B. infantis M-63 has been deposited with the National Institute of Technology and Evaluation (NITE), Japan, under the designation NITE BP-02623.

2. Phenotypic Identification

B. infantis M-63 is a non-motile, non-spore forming, rod-shaped, anaerobic, Gram-positive bacterium (Figure 1).

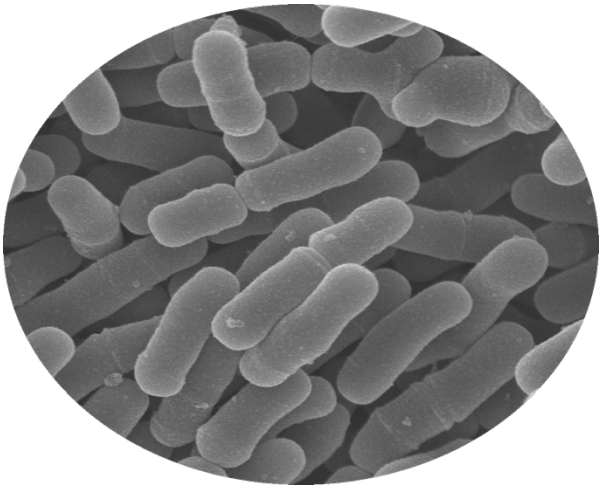

Figure 1. Scanning Election Microscope Image of *B. infantis* M-63

To confirm that *B. infantis* M-63 is phenotypically similar to other strains of *B. infantis*, Morinaga Milk compared the carbohydrate fermentation pattern (CFP) of *B. infantis* M-63 to that of the type strain *B. infantis* ATCC 15697^{T} using the method developed by Boyd et al. (2005). 1D-protein gel electrophoresis and cluster analysis were also performed by PROSAFE. The CFPs are qualitatively similar (Table 1), indicating that *B. infantis* M-63 is phenotypically similar to *B. infantis* ATCC 15697^{T}. Additionally, PROSAFE concluded that *B. longum* subsp. *infantis* M-63 is a strain of *B. longum* subsp. *infantis*.

Table 1. Carbohydrate Fermentation Patterns of *B. infantis*

Carbohydrate	*B. infantis*	
	ATCC 15697^T	M-63
Glycerol	–	–
Erythritol	–	–
D-Arabinose	(+)	±
L-Arabinose	–	–
Ribose	+	+
D-Xylose	–	–
L-Xylose	–	–
Adonitol	–	–
β-Methyl-xyloside	–	–
Galactose	+	+
D-Glucose	+	+
D-Fructose	+	+
D-Mannose	+	+
L-Sorbose	–	–
Rhamnose	–	–
Dulcitol	–	–
Inositol	–	–
Mannitol	–	–
Sorbitol	–	–
α-Methyl-D-Mannoside	–	–
α-Methyl-D-Glucoside	+	+
N-Acetyl-Glucosamine	+	+
Amygdalin	–	–
Arbutin	+	+
Esculin	–	–
Salicin	+S	+S
Cellobiose	–	–
Maltose	+	+
Lactose	+	+
Melibiose	+	+
Sucrose	+	+
Trehalose	–	–
Inulin	(+)	(+)
Melezitose	–	–
D-Raffinose	+	+
Starch	–	–
Glycogen	–	–
Xylitol	–	–

Table 1. Carbohydrate Fermentation Patterns of *B. infantis*

Carbohydrate	*B. infantis*	
	ATCC 15697^T	M-63
β-Gentiobiose	–	–
D-Turanose	+	+
D-Lyxose	–	–
D-Tagatose	–	–
D-Fucose	–	–
L-Fucose	+	+
D-Arabitol	–	–
L-Arabitol	–	–
Gluconate	–	–
2-Keto-Cluconate	–	–
5-Keto-Gluconate	–	–

*GRN 758 did not compare the notification strain to any type strain, and fermentation was only reported as positive or negative in this analysis.
+ positive; – negative; (+) weakly positive; ± very weakly positive; S delayed reaction.

3. Genotypic Identification

To confirm the genotypic identity of *B. infantis* M-63, Morinaga Milk compared the 16S rDNA sequences of *B. infantis* M-63 (obtained by direct sequencing) and type strain *B. longum* subsp. *infantis* ATCC 15697^T (obtained from the National Center for Biotechnology Information (NCBI) website (accession number KP326372)) using BLASTN (Version 2.6.1+) and conducted genome-wide homology analysis using colorimetric microplate hybridization Southern blotting as described by Yaeshima et al. (1996). Additionally, BOX-A1R-based repetitive extragenic palindromic-polymerase chain reaction (BOX-PCR) fingerprinting and cluster analysis were performed by PROSAFE. The 16S rDNA sequence of *B. infantis* M-63 was 100% identical to *B. longum* subsp. *infantis* ATCC 15697^T and Southern blotting showed 100% homology with *B. longum* subsp. *infantis* ATCC 15697^T, 87.8% homology with *B. longum* subsp. *longum* E194b, 73.0% homology with *B. breve* ATCC 15700, and 43.7% homology with *B. bifidum* IV-127 (Table 2). Together these data indicate that *B. longum* subsp. *infantis* M-63 is genetically similar to the *B. longum* subsp. *infantis* type strain. Additionally, PROSAFE concluded that *B. longum* subsp. *infantis* M-63 is a strain of *B. longum* subsp. *infantis*.

Table 2. Genome-Wide Homology of *B. infantis* M-63 Compared to Bifidobacteria Species

Strain	% similarity to DNA from:	
	***B. infantis* M-63**	***B. infantis* ATCC 15697^T**
B. infantis ATCC 15697^T	100.0	100.0
B. longum E194b	87.8	83.1
B. breve ATCC 15700	73.0	69.0
B. bifidum IV-127	43.7	40.3
Analysis conducted by Morinaga Milk using colorimetric microplate hybridization as described by Yaeshima et al. (1996).		

D. PRODUCTION PROCESS

The production of *B. infantis* M-63 consists of a culturing process and a non-culturing process (Figure 2), which are typical for the production of microbial ingredients used in food. The culturing process is a series of sequential expansions of working stocks, derived from the original stocks of *B. infantis* M-63, yielding a manufacturing culture. The manufacturing culture provides the material for the non-culturing process where *B. infantis* M-63 is refined and prepared for distribution.

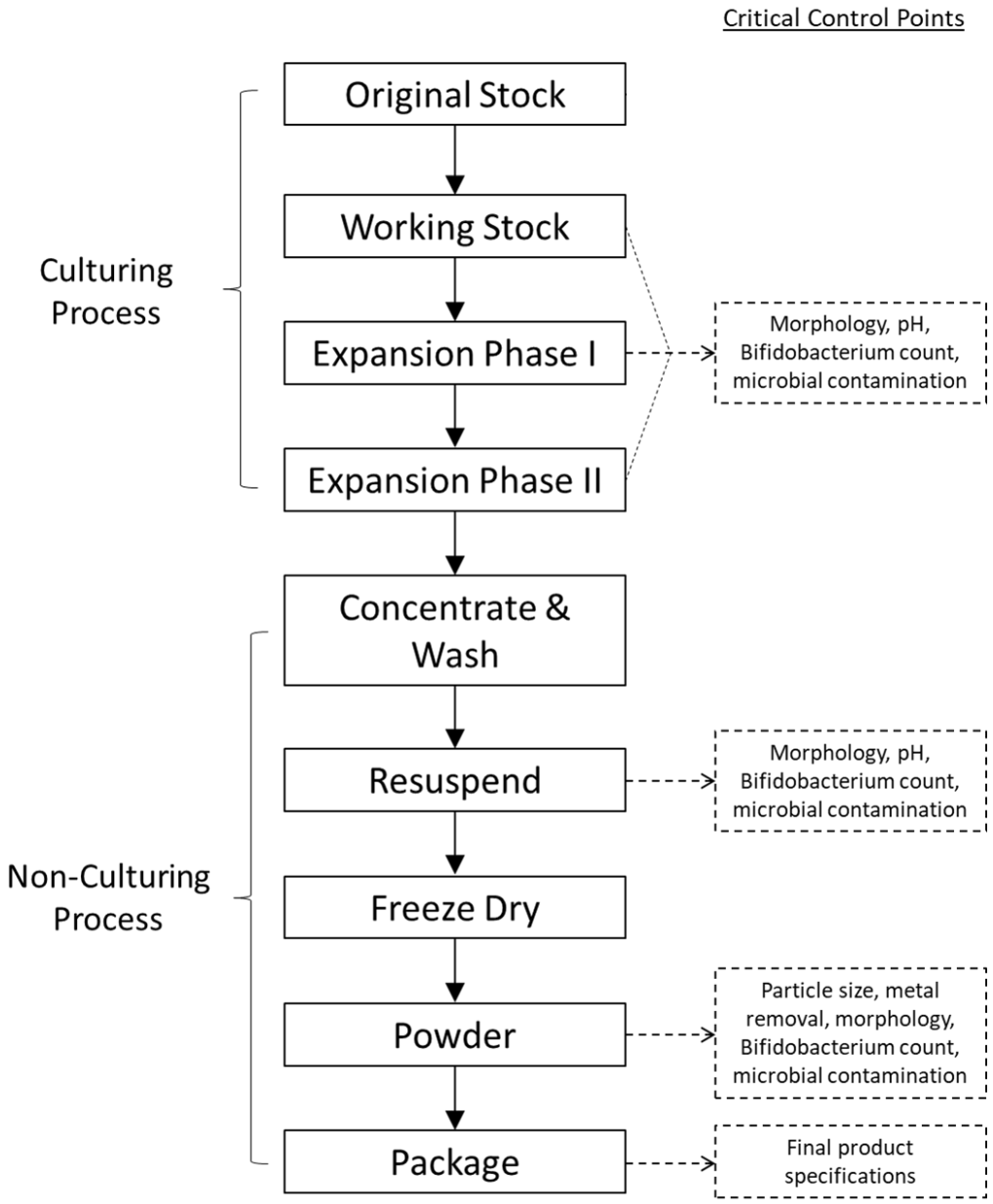

Figure 2. Production process for *B. infantis* M-63

This production process results in two different product formulations: M-63 (tapioca starch carrier) and M-63 type-C (cornstarch carrier). M-63 is used only in conventional foods, while M-63 type-C is used in either conventional foods or infant formula.

1. Regulatory Compliance

B. infantis M-63 is manufactured in Japan by Morinaga Milk Industry Co., Ltd., in FDA-registered facilities. Products are manufactured under food grade conditions and do not contain genetically modified organisms (GMOs) or ingredients derived from GMO-derived products. Morinaga Milk Industry Co., Ltd. operates under a Hazard Analysis Critical Control Point (HACCP) management system. Their facilities have been audited by a third party and determined to be compliant with the Food Safety System Certification (FSSC) 22000 and International Organization for Standardization (ISO) 22000:2005 standards.

The manufacturing process utilizes well water, treated with a reverse osmosis (RO) membrane, which is regularly tested and complies with the standards set forth in the Japan Water Supply Act. All food contact surfaces used in manufacturing *B. infantis* M-63 are either stainless steel, aluminum, or otherwise suitable for use in the production of food ingredients. Media ingredients are nutritional substances necessary for fermentation, do not contain major food allergens nor are they derived from major food allergens, and are safe and suitable for human consumption. *B. infantis* M-63 is thoroughly washed during the non-culturing process to minimize carry-over of the fermentation medium to the finished ingredient. Resuspension medium is not washed from the final product; all components comply with their respective sections within 21 CFR and/or Food Chemicals Codex (FCC). Carbohydrate carriers that are directly added to *B. infantis* M-63 to generate the final ingredient comply with Food Chemicals Codex (FCC). Packaging materials comply with 21 CFR (Table 3).

Table 3. Compliance with US Regulations

Role in Production	Processing Aid	Compliance
Carbohydrate carrier	Tapioca starch (unmodified)	FCC 11 3S
Carbohydrate carrier	Cornstarch (unmodified)	FCC 11 3S
Packaging	LLDPE/aluminum foil bag	21 CFR §177.1520

CFR: United States Code of Federal Regulations; FCC: Food Chemicals Codex; LLDPE: linear low-density polyethylene

2. Quality Control

Morinaga Milk Industry routinely evaluates the quality of the *B. infantis* M-63 products during the production process to ensure that the finished products are free of contaminants and the genotype and phenotype of the *B. infantis* M-63 in the finished product are consistent with that of the original stock. The timing and parameters measured during the culturing and non-culturing processes are provided in Tables 4 and 5. Although *B. infantis* M-63 is produced in the same facility and using the same line/equipment as other microbial strains, a clean in place (CIP) procedure is used, and manual cleaning is performed after each production run to prevent cross-contamination between the various strains. Additionally, the production line is sterilized by steam or dry heating before every production run. All cleaning is performed under approved SOPs and operators document the completion of each cleaning step.

Table 4. Quality Control Parameters Monitored During the Production of *Bifidobacterium infantis* M-63

Parameter*	Culturing Process			Non-Culturing Process		
	Seed Culture	Expansion Phase 1	Expansion Phase 2	Resuspension	Powdering	Finished Product
Culture pH	X	X	X	X		
Cell morphology	X	X	X	X	X	
Foreign body	X	X	X	X	X	X
Anaerobic CFU		X	X	X	X	X
Aerobic CFU		X	X	X	X	X
Mold		X	X	X	X	X
Yeast		X	X	X	X	X
Coliforms (including *E. coli*)				X	X	X
Staphylococcus aureus				X	X	X
Salmonella					X	X
Listeria monocytogenes						X
Moisture						X
Heavy Metals						X
Appearance						X
Odor and Taste						X
RAPD PCR						X

"X" denotes that the parameter is measured.
CFU: colony forming units; RAPD PCR: random amplification of polymorphic DNA polymerase chain reaction.
*Methods are validated and the same as those described in the product specifications.

Table 5. Quality Control Parameters Monitored During the Production of *Bifidobacterium infantis* M-63 type-C

Parameter*	Culturing Process			Non-Culturing Process		
	Seed Culture	Expansion Phase 1	Expansion Phase 2	Resuspension	Powdering	Finished Product
Culture pH	X	X	X	X		
Cell morphology	X	X	X	X	X	
Foreign body	X	X	X	X	X	X
Anaerobic CFU		X	X	X	X	X
Aerobic CFU		X	X	X	X	X
Mold		X	X	X	X	X
Yeast		X	X	X	X	X
Coliforms (including *E. coli*)				X	X	X
Staphylococcus aureus				X	X	X
Salmonella					X	X
Cronobacter sakazakii					X	X
Bacillus cereus						X
Listeria monocytogenes						X
Moisture						X
Heavy Metals						X
Appearance						X
Odor and Taste						X
RAPD PCR						X

"X" denotes that the parameter is measured.
CFU: colony forming units; RAPD PCR: random amplification of polymorphic DNA polymerase chain reaction.
*Methods are validated and the same as those described in the product specifications.

3. Manufacturing Process

a. Culturing Media

Three separate media, Base Medium, Manufacturing Medium, and Resuspension Medium, are used during different stages of the production process

Water used in the media is sterilized by passing through a reverse osmosis membrane, treatment with hypochlorous acid, and ultra-heat treatment (heating above 128ºC). Once prepared, all media are sterilized before use.

b. Culturing Process

i. Original Stock

Original stocks of *B. infantis* M-63 were established by suspending *B. infantis* M-63 in Base Medium in cryogenic vials. These stocks are stored at −80°C and maintained at the Morinaga Milk laboratory.

ii. Working Stocks

Working stocks are established by thawing and adding the original stock to Base Medium, expanding the stock, and freezing at −80°C. To initiate production of M-63, the frozen working stocks are thawed and verified by checking colony morphology, Bifidobacterium count, and microbial contamination. Working stocks are cultured in Base Medium to generate seed cultures, which provide the starting material for Expansion Phase 1.

iii. Expansion Phase 1

Base Medium is used for this phase. Seed cultures undergo a series of sequential expansions to yield the Main Starter Culture, which is approximately 1000 kg.

iv. Expansion Phase 2

The Main Starter Culture then undergoes final expansion using Manufacturing Medium. The resulting Manufacturing Culture is approximately 35,000 kg.

c. Non-Culturing Process

Once culturing is complete, the Manufacturing Culture is cooled and concentrated by centrifugation under refrigeration. The medium is discarded and the pellet washed 2-3 times using sterilized water. *B. infantis* M-63 is then resuspended in Resuspension Medium. If necessary, the

pH is adjusted using NaOH solution. This resuspension is freeze-dried, milled to a powder, and mixed with a powdered carbohydrate carrier. M-63 for use in general foods is mixed with tapioca starch, while M-63 type-C for use in either general foods or infant formula is mixed with non-GMO cornstarch.

This *B. infantis* M-63/carbohydrate mix is then sifted through a number 16 mesh sieve, passed through a magnetic tunnel to remove metal particles, weighed, packed into airtight bags, and stored at 10°C. Because the Resuspension Medium is not washed from the product prior to the final production steps, all processing aids present in the Resuspension Medium are present in the final product.

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Analytical Methods

All testing is performed using compendial and/or internal methods that have been validated.

2. Product Specifications

B. infantis M-63 is a white to light brown powder consisting of freeze-dried active *B. infantis* M-63 and a carbohydrate filler. To ensure a consistent food-grade product, each lot of *B. infantis* M-63 is evaluated against an established set of product specifications using validated methods. RAPD PCR fingerprinting is used to verify that *B. infantis* M-63 cells in the final products are identical to the original stocks (*i.e.*, no genetic drift has occurred during storage or culturing). Additional product specifications are in place for anaerobic plate count (which includes *B. infantis* M-63), microbial contamination, and heavy metals. Specifications for M-63 and M-63 type-C are generally the same, except that heavy metal limits for M-63 type-C are lower; when used for infant formula, M-63 type-C is also tested for *Cronobacter sakazakii* and *Bacillus cereus*. It should be noted that a single lot of M-63 type-C can be used both in general foods and infant formula, as long as it meets the indicated product specifications.

Data from three lots of each product type, shown in Tables 6, 7, and 8, demonstrate control of the production process and compliance with the product specifications.

Table 6. Final Product Specifications and Lot Data for M-63 for Use in General Foods

Parameter	Specification	Method	Lot Number		
			2019.06.19	2019.06.24	2019.07.01
Anaerobic plate count*	> 8.0×10^{10} CFU/g	Reinforced Clostridial Agar	1.1×10^{11}	1.3×10^{11}	1.2×10^{11}
B. infantis M-63	Banding pattern	RAPD PCR	Identified	Identified	Identified
Appearance	White to slightly brown powder	Visual	White to slightly brown powder	White to slightly brown powder	White to slightly brown powder
Foreign Body	Negative	Visual	Negative	Negative	Negative
Odor and Taste	No abnormal odor and taste	Sensory Evaluation	No abnormal odor and taste	No abnormal odor and taste	No abnormal odor and taste
Moisture	< 6g/100 g	Gravimetric method at 105°C for 4h	1.8	1.8	1.9
Microbial Contamination					
Aerobic plate count	< 300 CFU/g	ISO 4833-1	< 300	< 300	< 300
Molds	< 30 CFU/g	ISO 21527-2	< 30	< 30	< 30
Yeast	< 30 CFU/g	ISO 21527-2	< 30	< 30	< 30
Enterobacteriaceae	Negative/25 g	ISO 21528-1	Negative	Negative	Negative
Escherichia coli[1]	Negative/1 g	ISO 7251 : 2005	Negative	Negative	Negative
Staphylococcus aureus	Negative/0.01 g	ISO 6888-1	Negative	Negative	Negative
Salmonella	Negative/25 g	ISO 6579	Negative	Negative	Negative
Listeria monocytogenes	Negative/1 g	ISO 11290-1	Negative	Negative	Negative
Heavy Metals					
Arsenic	< 1 ppm	ICP-MS	< 1	< 1	< 1
Lead	< 0.5 ppm	ICP-MS	< 0.5	< 0.5	< 0.5
Mercury	< 1 ppm	ICP-MS	< 1	< 1	< 1
Cadmium	< 1 ppm	ICP-MS	< 1	< 1	< 1

**B. infantis* M-63 is included in anaerobic plate count. Differential selective medium is used occasionally to distinguish *B. infantis* M-63 from other anaerobes.
[1]Testing is not required when all samples for *Enterobacteriaceae* are negative.
CFU = colony forming units; ppm = parts per million; ICP-MS = inductively coupled plasma mass spectrometry.
Lot numbers indicate the date of production.
Limits of quantitation: heavy metals = 0.04 ppm.

Table 7. Final Product Specifications and Lot Data for M-63 type-C for Use in General Foods

Parameter	Specification	Method	Lot Number		
			2018.09.08	2019.06.19	2019.06.24
Anaerobic plate count*	> 8.0×10^{10} CFU/g	Reinforced Clostridial Agar	1.1×10^{11}	1.3×10^{11}	1.2×10^{11}
B. infantis M-63	Banding pattern	RAPD PCR	Identified	Identified	Identified
Appearance	White to slightly brown powder	Visual	White to slightly brown powder	White to slightly brown powder	White to slightly brown powder
Foreign Body	Negative	Visual	Negative	Negative	Negative
Odor and Taste	No abnormal odor and taste	Sensory Evaluation	No abnormal odor and taste	No abnormal odor and taste	No abnormal odor and taste
Moisture	< 6g/100 g	Gravimetric method at 105°C for 4h	1.9	0.6	0.8
Microbial Contamination					
Aerobic plate count	< 300 CFU/g	ISO 4833-1	< 300	< 300	< 300
Molds	< 30 CFU/g	ISO 21527-2	< 30	< 30	< 30
Yeast	< 30 CFU/g	ISO 21527-2	< 30	< 30	< 30
Enterobacteriaceae	Negative/25 g	ISO 21528-1	Negative	Negative	Negative
Escherichia coli[1]	Negative/1 g	ISO 7251 : 2005	Negative	Negative	Negative
Staphylococcus aureus	Negative/0.01 g	ISO 6888-1	Negative	Negative	Negative
Salmonella	Negative/25 g	ISO 6579	Negative	Negative	Negative
Listeria monocytogenes	Negative/1 g	ISO 11290-1	Negative	Negative	Negative
Heavy Metals					
Arsenic	< 1 ppm	ICP-MS	< 1	< 1	< 1
Cadmium	< 0.5 ppm	ICP-MS	< 0.5	< 0.5	< 0.5
Lead	< 0.5 ppm	ICP-MS	< 0.5	< 0.5	< 0.5
Mercury	< 0.1 ppm	ICP-MS	< 0.1	< 0.1	< 0.1

**B. infantis* M-63 is included in anaerobic plate count. Differential selective medium is used occasionally to distinguish *B. infantis* M-63 from other anaerobes.
[1]testing is not required when all samples for *Enterobacteriaceae* are negative.
CFU = colony forming units; ppm = parts per million; ICP-MS = inductively coupled plasma mass spectrometry.
Lot numbers indicate the date of production.
Limits of quantitation: heavy metals = 0.04 ppm.

Table 8. Final Product Specifications and Lot Data for M-63 type-C for Use in Infant Formula

Parameter	Specification	Method	Lot Number		
			2018.09.08	2019.06.19	2019.06.24
Anaerobic plate count*	> 8.0×10^{10} CFU/g	Reinforced Clostridial Agar	1.1×10^{11}	1.3×10^{11}	1.2×10^{11}
B. infantis M-63	Banding pattern	RAPD PCR	Identified	Identified	Identified
Appearance	White to slightly brown powder	Visual	White to slightly brown powder	White to slightly brown powder	White to slightly brown powder
Foreign Body	Negative	Visual	Negative	Negative	Negative
Odor and Taste	No abnormal odor and taste	Sensory Evaluation	No abnormal odor and taste	No abnormal odor and taste	No abnormal odor and taste
Moisture	< 6g/100 g	Gravimetric method at 105°C for 4h	1.9	0.6	0.8
Microbial Contamination					
Aerobic plate count	< 300 CFU/g	ISO 4833-1	< 300	< 300	< 300
Molds	< 30 CFU/g	ISO 21527-2	< 30	< 30	< 30
Yeast	< 30 CFU/g	ISO 21527-2	< 30	< 30	< 30
Enterobacteriaceae	Negative/25 g	ISO 21528-1	Negative	Negative	Negative
Escherichia coli[1]	Negative/1 g	ISO 7251 : 2005	Negative	Negative	Negative
Staphylococcus aureus	Negative/0.01 g	ISO 6888-1	Negative	Negative	Negative
Salmonella	Negative/25 g	ISO 6579	Negative	Negative	Negative
Listeria monocytogenes	Negative/1 g	ISO 11290-1	Negative	Negative	Negative
Cronobacter sakazakii	Negative/25 g	ISO 22964	Negative	Negative	Negative
Bacillus cereus	< 100 CFU/g	ISO 7932	< 100	< 100	< 100
Heavy Metals					
Arsenic	< 1 ppm	ICP-MS	< 1	< 1	< 1
Cadmium	< 0.5 ppm	ICP-MS	< 0.5	< 0.5	< 0.5
Lead	< 0.5 ppm	ICP-MS	< 0.5	< 0.5	< 0.5
Mercury	< 0.1 ppm	ICP-MS	< 0.1	< 0.1	< 0.1

**B. infantis* M-63 is included in anaerobic plate count
[1]testing is not required when all samples for *Enterobacteriaceae* are negative.
CFU = colony forming units; ppm = parts per million
Lot numbers indicate the date of production.
Limits of quantitation: heavy metals = 0.04 ppm.

F. STABILITY OF *BIFIDOBACTERIUM INFANTIS* M-63

The stability of powdered *B. infantis* M-63 and M-63 type-C was examined following its storage in aluminum bags. The storage temperature was maintained at less than 10°C, and humidity was not monitored.

For M-63, data on three non-consecutive lots demonstrate that the anaerobic plate count (representing *B. infantis*) continues to meet specifications through 36 months (Table 9). Measurements of moisture and microbial contamination are ongoing, but data on three lots demonstrate that these parameters continue to meet specifications at timepoints up to 45 months (Table 10).

Similarly, for M-63 type-C, data on three non-consecutive lots demonstrate that the anaerobic plate count (representing *B. infantis*) continues to meet specifications through 36 months (Table 11). Measurements of moisture and microbial contamination are ongoing, but data on three lots demonstrate that these parameters continue to meet specifications at timepoints up to 56 months (Table 12).

Overall, these data support a shelf life of M-63 and M-63 type-C of up to 36 months when stored at less than 10°C.

Table 9. Stability Analysis of M-63 less than 10°C

Parameter	Specification	Lot Number	Time (Months)						
			0	3	6	12	18	24	36
Anaerobic CFU/g	$>8.0x10^{10}$	2015.03.16	$1.4x10^{11}$	$1.2x10^{11}$	$1.0x10^{11}$	$1.1x10^{11}$	$1.1x10^{11}$	$1.2x10^{11}$	$1.1x10^{11}$
		2015.12.23	$1.4x10^{11}$	$1.4x10^{11}$	$1.3x10^{11}$	$1.4x10^{11}$	$1.2x10^{11}$	$1.3x10^{11}$	$1.4x10^{11}$
		2016.03.23	$1.4x10^{11}$	$1.3x10^{11}$	$1.4x10^{11}$	$1.3x10^{11}$	$1.3x10^{11}$	$1.1x10^{11}$	$1.1x10^{11}$
CFU: colony forming units.									

Table 10. Moisture and Microbial Contamination Stability Data for M-63 less than 10°C

Parameter	Method	Specification	Lot of M-63		
			2015.12.23	2016.03.23	2016.05.25
		Timepoint:	45 months	42 months	40 months
Moisture	Gravimetric 105°C, 4 hours	< 6 g/100 g	2.0	1.7	1.6
Total aerobic bacteria	ISO 4833-1	< 300 CFU/g	<300	<300	<300
Yeast	ISO 21527-2	< 30 CFU/g	<30	<30	<30
Mold	ISO 21527-2	< 30 CFU/g	<30	<30	<30
Enterobacteriacea	ISO 21528-1	Negative/25 g	Negative	Negative	Negative
Staphylococcus aureus	ISO 6888-1	Negative/0.01 g	Negative	Negative	Negative
Listeria monocytogenes	ISO 11290-1	Negative/1 g	Negative	Negative	Negative
Salmonella spp.	ISO 6579	Negative/25 g	Negative	Negative	Negative
CFU: colony forming units.					

Table 11. Stability Analysis of M-63 type-C less than 10°C

Parameter	Specification	Lot Number	Time (Months)						
			0	3	6	12	18	24	36
Anaerobic CFU/g	$>8.0x10^{10}$	2009.10.14	$1.1x10^{11}$	$1.1x10^{11}$	$1.1x10^{11}$	$1.2x10^{11}$	$1.1x10^{11}$	$9.8x10^{10}$	$9.4x10^{10}$
		2015.01.23	$1.4x10^{11}$	$1.3x10^{11}$	$1.1x10^{11}$	$9.4x10^{10}$	$1.0x10^{11}$	$1.2x10^{11}$	$1.2x10^{11}$
		2015.03.16	$1.3x10^{11}$	$1.1x10^{11}$	$1.0x10^{11}$	$1.1x10^{11}$	$9.3x10^{10}$	$1.0x10^{11}$	$1.0x10^{11}$
CFU: colony forming units.									

Table 12. Moisture and Microbial Contamination Stability Data for M-63 type-C less than 10°C

Parameter	Method	Specification	Lot of M-63		
			2015.01.23	2018.01.30	2018.09.08
		Timepoint:	56 months	20 months	12 months
Moisture	Gravimetric 105°C, 4 hours	< 6 g/100 g	2.8	1.4	1.5
Total aerobic bacteria	ISO 4833-1	< 300 CFU/g	<300	<300	<300
Yeast	ISO 21527-2	< 30 CFU/g	<30	<30	<30
Mold	ISO 21527-2	< 30 CFU/g	<30	<30	<30
Enterobacteriacea	ISO 21528-1	Negative/25 g	Negative	Negative	Negative
Staphylococcus aureus	ISO 6888-1	Negative/0.01 g	Negative	Negative	Negative
Listeria monocytogenes	ISO 11290-1	Negative/1 g	Negative	Negative	Negative
Salmonella spp.	ISO 6579	Negative/25 g	Negative	Negative	Negative
CFU: colony forming units.					

III. DIETARY EXPOSURE

A. INTENDED EFFECT

B. infantis M-63 will be used as an ingredient in general foods and cow's milk- and soy-based, non-exempt term infant formula.

B. HISTORY OF USE

Morinaga Milk Industry is the sole proprietor of *B. infantis* M-63 and has been selling its *B. infantis* M-63-containing ingredients for use in infant formulas in international markets since 2006. Through 2016, Spain has purchased 11,000 kg (2006-2016), France has purchased 2,000 kg (2012-2016), and Italy has purchased 180 kg (2009-2016).

M-63 is added to a variety of infant and follow-on formula products, including those described in Table 13. This product is also widely used in infant formula, and follow-on formula.

Table 13. *B. infantis* M-63 Use Levels in Infant and Follow-On Formula Worldwide

Country	Use level	Target Age
Indonesia	$9.6x10^6$ CFU/g	6-12 months
Indonesia	$4.5x10^7$ CFU/g	12-36 months
Indonesia	$6.4x10^7$ CFU/g	3-12 years
China	$1.0x10^6$ CFU/g	12-36 months
France	$1.4x10^8$ CFU/100 mL	6-12 months
France	$1.0x10^7$ CFU/100 mL	0-6 months
CFU: colony forming units.		

Other *B. infantis* strains are available in the United States and around the world. As described in GRN 758 (pg. 69), *B. longum* subsp. *infantis* R0033 has been sold worldwide for many years in foods for infants, toddlers, and children, providing 3×10^8 CFU/serving. These foods were first launched in China in October 2002 and have since been sold in more than ten countries, including Australia, Canada, France, South Africa, Ukraine, and the United Kingdom. Additionally, *B. longum* subsp. *infantis* R0033 has been marketed in more than 40 other formulas with no reports of adverse effects. *B. longum* subsp. *infantis* R0033 is also GRAS for use in powdered infant formula at levels up to 5×10^7 CFU/g (GRN 758).

C. INTENDED USE

Morinaga Milk Industry Co., Ltd., intends to add *B. infantis* M-63 to selected food products (Table 14) containing up to 1.25 x 10^{10} CFU/serving at the end of shelf life. Also, they intend to add it to cow milk- and soy-based term infant formulas containing up to 1 x 10^{8} CFU/g of *B. infantis* M-63 at the end of shelf life.

Table 14. Proposed Conventional Food Categories for the Addition of *B. infantis* M-63

Food Category	Specific Food	CFU/serving
Breads/baked goods	• Bars; includes meal replacement, high protein, snack bars • Biscuits • Breads/roll (yeast), including bagels, croissants, English muffins, pizza crust • Breakfast pastries; includes Danish • Cakes, includes coffee cakes • Cobblers, turnovers, strudels, crisps • Cookie bars • Crackers • Doughnuts • Pies • Quick breads; includes breads, muffins, popovers, cornbread	1.25 x 10^{10}
Cereals	• Breakfast cereals, cooked; includes grits, oatmeal, cream of wheat, and wheat cereal • Breakfast cereals, ready-to-eat	1.25 x 10^{10}
Fruits	• Juices and nectars, including citrus, non-citrus, vegetable and blends, frozen fruit, frozen juice bars, ices	1.25 x 10^{10}
Dairy products/dairy-based foods and dairy substitutes	• Skim milk • Cheese spreads • Cheese, imitation • Cheese, processed • Cream substitutes • Cream, heavy • Fermented milk (flavored, heat treated), including buttermilk, kefir, and flavored milk beverage mixes • Frozen desserts, including ice cream, ice milk, frozen yogurt, frozen novelties, and imitation milk • Meal replacements, liquids and dry mixes • Milk shakes • Milk (plain and flavored), including cocoa, chocolate milk, fruit milks, coffee drinks (fluid/dry) • Puddings and custards • Smoothies • Whipped toppings • Yogurt • Butter and dried milk products	1.25 x 10^{10}

Table 14. Proposed Conventional Food Categories for the Addition of *B. infantis* M-63		
Food Category	**Specific Food**	**CFU/serving**
	• Milk powder for pregnant women, plain and flavored • Milk powder for adult people, plain and flavored • Milk powder for elderly people, plain and flavored	
Miscellaneous	• Candies, including hard candies, mints, chocolate and all other types of confections (i.e., chewing gum), cocoa powder, condiment sauces, (i.e., catsup, BBQ, taco, steak, cocktail, Worcestershire, teriyaki, cheese-based, hollandaise, tartar, béarnaise) • Gelatin desserts, plain or with fruit gravies • Peanut and other nut butters/spreads • Snack foods, including chips, popcorn mixtures • Weaning foods, including meals, desserts, fruits, cereal, vegetables, snacks, juices	1.25×10^{10}

D. ESTIMATED DAILY INTAKE

1. Assessment of *B. infantis* M-63 Use in General Foods

The estimated daily intake (EDI) of *B. infantis* M-63 (including both M-63 and M-63 type-C) in general foods is calculated based on the food uses and maximum use levels listed in Table 14, in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2015-2016 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2018; USDA, 2018). Food codes representative of each use were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS) for the corresponding biennial NHANES survey. Calculations from NHANES for the mean and 90th percentile intakes were performed for representative food uses of *B. infantis* M-63.

a. Food Consumption Survey Data

i. Survey Description

The most recent NHANES data for the years 2015-2016 are available for public use. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. In each cycle, approximately 10,000 people across the U.S. completed the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Hayes et al., 2014). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and

Day 2) are available from the NHANES 2015-2016 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provide the most appropriate data for evaluating food-use and food-consumption patterns in the United States, containing 2 years of data on individuals selected via stratified multistage probability sample of civilian non-institutionalized population of the U.S. NHANES survey data were collected from individuals and households via 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person in the Mobile Examination Center (MEC), and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For example, in the 2009-2010 NHANES, there were 13,272 persons selected; of these 10,253 were considered respondents to the MEC examination and data collection. 9754 of the MEC respondents provided complete dietary intakes for Day 1 and of those providing the Day 1 data, 8,405 provided complete dietary intakes for Day 2. The release data do not necessarily include all the questions asked in a section. Data items may have been removed due to confidentiality, quality, or other considerations. For this reason, it is possible that a dataset does not completely match all the questions asked in a questionnaire section. Each data file has been edited to include only those sample persons eligible for that particular section or component, so the numbers vary.

In addition to collecting information on the types and quantities of foods being consumed, the NHANES surveys collect socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population.

Sample weights are incorporated with NHANES surveys to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2012).

ii. Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer in Octave and used to generate estimates for the intake of *B. infantis* M-63 by the U.S. population. Estimates for the daily intake of *B. infantis* M-63 represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating sample weights in order to provide representative intakes for the entire U.S. population. "All-user" intake refers to the estimated intake of *B. infantis* M-63 by those individuals consuming food products containing *B. infantis* M-63. Individuals were considered users if they consumed 1 or more food products containing *B. infantis* M-63 on either Day 1 or Day 2 of the survey.

b. Food Usage

The estimated "all-user" total intakes of *B. infantis* M-63 from the proposed food uses listed in NHANES in the U.S. by population group is described in Table 15. The mean intake by all *B. infantis* M-63 consumers age 2+ from the selected food uses was estimated to be 1.45×10^{10} CFU/person/day or 2.16×10^{8} CFU/kg bw/day. The heavy consumer (90^{th} percentile) intake was estimated to be 2.68×10^{10} CFU/person/day or 4.01×10^{8} CFU/kg bw/day.

Table 15. Estimated "All-user" Daily Intake of *B. infantis* M-63 in Targeted Foods by Population Group (2015-2016 NHANES Data)

Population Group	N users	N population	% Users	Mean mass (kg)	Mean EDI (CFU)	90th % EDI (CFU)	Mean EDI (CFU/kg)	90th % EDI (CFU/kg)
ages 0-1	223	293	76.11	11.22	9.25E+09	1.55E+10	8.25E+08	1.38E+09
ages 1-2	223	291	76.63	13.59	9.95E+09	1.73E+10	7.30E+08	1.27E+09
ages 2-5	665	915	72.68	16.92	1.04E+10	1.81E+10	6.15E+08	1.07E+09
ages 6-12	1186	1505	78.80	36.59	1.28E+10	2.29E+10	3.49E+08	6.25E+08
ages 13-19	994	1143	86.96	67.35	1.60E+10	2.83E+10	2.37E+08	4.20E+08
ages 20 and up	4880	5748	84.90	79.95	1.51E+10	2.87E+10	1.89E+08	3.59E+08
ages 2 and up	7725	9311	82.97	66.96	1.45E+10	2.68E+10	2.16E+08	4.01E+08

CFU: colony forming units; N = number; EDI = estimated daily intake.

2. Assessment of *B. infantis* M-63 type-C Use in Infant Formula

Powdered non-exempt term infant formula will contain 1×10^{8} CFU/g *B. infantis* M-63. Infant formula in the US market provides approximately 670 kcal/L (20 kcal/fl oz) (Martinez and Ballew, 2011). Since powdered formula are typically reconstituted at a caloric density of 670 kcal/L (141 g/L), this will result in an intake of *B. infantis* M-63 type-C of 1.41×10^{10} CFU/L.

Assuming powdered infant formula is the sole source of nutrition and the caloric requirements of a one month-old and six month-old infants are 472 kcal/day and 645 kcal/day, respectively (Institute of Medicine (US) Panel on Macronutrients and Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005), 1 and 6 month-old infants consume approximately 0.704 and 0.963 L formula/day, respectively. The addition of 1 x 10^8 CFU/g (equivalent to 1.41 x 10^{10} CFU/L) *B. infantis* M-63 to infant formula will therefore result in an intake of approximately 9.9 x 10^9 CFU/day of *B. infantis* M-63 for a 1 month-old infant and 1.35 x 10^{10} CFU/day of *B. infantis* M-63 for a 6 month-old infant. Assuming an average weight of 4.35 kg for a 1 month old and 7.60 kg for a 6 month-old (based on the 50th percentile data for boys and girls as reported by the World Health Organization (https://www.who.int/childgrowth/standards/WFA_boys_0_5_percentiles.pdf?ua=1. Accessed 30 January 2020; https://www.who.int/childgrowth/standards/WFA_girls_0_5_percentiles.pdf?ua=1. Accessed 30 January 2020), these intakes are equivalent to 2.3 x 10^9 CFU/kg bw/day and 1.8 x 10^9 CFU/kg bw/day, respectively. These levels are consistent with intake levels reported in other GRAS notifications where *B. lactis* Bb12, *Streptococcus thermophilus* Th4, *L. reuteri* DSM 17938, *B. breve* M-16V, and *B. longum* BB536 are used in infant formulas (GRN 49; GRN 410; GRN 454, GRN 455; GRN 877).

As indicated in Table 15, some general foods containing *B. infantis* M-63 may be consumed by infants (ages 0-1 year) who also consume formula. It is assumed that consumption of solid foods displaces consumption of infant formula; as such, infants consuming both solid food and formula will not be consuming the entire EDI for either category. Thus, it is expected that infants will not exceed the EDI for *B. infantis* M-63 (Table 15).

IV. SELF-LIMITING LEVELS OF USE

This part does not apply.

V. COMMON USE IN FOOD BEFORE 1958

This part does not apply.

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

The general recognition of safety of *B. infantis* M-63 under the specified conditions of use in general foods and non-exempt term infant formula is based on the established safety of Bifidobacteria and *Bifdobacterium longum* subsp. *infantis*, as well as published and unpublished studies of *B. infantis* M-63. Bifidobacteria are ubiquitous, are generally recognized to be non-pathogenic to humans, lack invasive properties, and have been the subject of numerous GRNs. *B. longum* strains, which include *B. longum* subsp. *infantis*, have been granted Qualified Presumption of Safety (QPS) status by EFSA. *In vitro* studies of *B. infantis* M-63, including antibiotic resistance, bile salt conjugation, biogenic amine production, ammonia production, mucin degradation, absence of plasmids, genomic analysis for toxins and pathogenic markers, and measurement of hemolytic potential provide supportive evidence that *B. infantis* M-63 does not pose safety risks.

The pivotal studies that directly support the safety of *B. infantis* M-63 include a published acute oral toxicity study and a published 90-day repeated oral dose toxicity study, both conducted in rats (Abe et al., 2009). No adverse test article related effects were observed in either study and the NOAEL from the 90-day study was determined to be greater than 7.6×10^{10} CFU/kg bw/day.

The safety of *B. infantis* M-63 is corroborated by four published clinical studies in children and adults, as well as four published clinical studies in infants. Additionally, other *B. longum* subsp. *infantis* strains have been tested in four published studies in animals and seven clinical studies. No test article related adverse effects were reported in any study.

Thus, based on the weight of the evidence there is reasonable certainty that the use *B. infantis* M-63 in conventional foods and infant formulas is expected to be safe under its intended uses.

A. REVIEW OF BIFIDOBACTERIA

The safety of Bifidobacteria is reviewed in GRN 268, pg. 42-46, which is incorporated by reference. Briefly, Bifidobacteria are generally held to be non-pathogenic to humans (Carr et al., 2002). *Bifidobacterium* spp. lack invasive properties, *i.e.*, they will not pass the epithelial boundary of the intestine to reach deep tissue, and they are not mucinolytic (Zhou et al., 2000a; 2000b; 2001). *Bifidobacterium* spp. have been used in a variety of food products and are regularly consumed by humans on a daily basis. Bifidobacteria are components of the normal flora of the human gastrointestinal tract (Ahrné et al., 1998; Germond et al., 2002; Picard et al., 2005; Reuter, 2001; Yang et al., 2019). The lack of pathogenicity has been demonstrated across all age groups and in immunocompromised individuals (Borriello et al., 2003).

As of March 31, 2020, 15 GRAS Notices have been submitted for various *Bifidobacterium* species and all have received “no questions” letters from the FDA (GRNs 49, 268, 377, 445, 453, 454, 455, 758, 813, 814, 855, 856, 872, 875, and 877).

B. REVIEW OF *BIFIDOBACTERIA LONGUM* SUBSP. *INFANTIS*

Bifidobacterium longum has been granted QPS status by EFSA (EFSA BIOHAZ Panel, 2019). Additionally, *Bifidobacterium longum* strains, including *B. longum* subspecies *infantis*, are included in an inventory, assembled by the International Dairy Foundation (IDF) in collaboration with the European Food & Feed Cultures Association (EFFCA), of microorganisms that have a documented history of safe use in food (Bourdichon et al., 2012).

Recently, Yang et al. (2019) showed that *B. longum* subsp. *infantis* is a major component of the cultivable infant gastrointestinal microbiota. Although some significant differences were seen between delivery (c section versus vaginal) and feeding methods (breast fed, milk-powder fed, and mixed fed), *B. longum* subsp. *infantis* was a major gastrointestinal component in all tested groups (≈10-20% of total).

Additionally, the subject of GRN 758 is a mixture of *B. longum* subsp. *infantis* R0033, *Lactobacillus helveticus* R0052, and *Bifidobacterium bifidum* R0071 for use in infant formula, which received a "no questions" from the FDA. The safety of *B. longum* subsp. *infantis* R0033 provides corroborative evidence for the safety of *B. infantis* M-63.

C. *IN VITRO* SAFETY STUDIES OF *BIFIDOBACTERIA LONGUM* SUBSP. *INFANTIS* M-63

1. Antibiotic Resistance

The antibiotic resistance of *B. infantis* M-63 has been evaluated in three studies (Xiao et al., 2010; Toscano et al., 2015). Two of the studies were published by Xiao et al. (2010) and Toscano et al. (2015). The third was conducted by PROSAFE, a European Union funded research project whose goal was to investigate the biosafety of lactic acid bacteria used for human consumption. Although there was some variability in the results across the studies, which may be due to methodological differences, *B. infantis* M-63 was reproducibly found to be resistant to streptomycin, which is likely due to a chromosomally-located mutation or recombination.

PROSAFE evaluated the antibiotic susceptibility of *B. infantis* M-63 to penicillin, ampicillin, ampicillin/sulbactam, gentamicin and streptomycin, vancomycin, teicoplanin, quinupristin/dalfopristin, erythromycin, clindamycin, oxytetracycline, chloramphenicol, fusidic acid, trimethoprim, sulfamethoxazole/trimethoprim using the method described by Klare et al. (2005) and compared the resulting minimum inhibitory concentrations (MICs) to their internal cut-offs for *Bifidobacterium longum*. Except for streptomycin, *B. infantis* M-63 had MICs below the cut-offs for *Bifidobacterium longum* for all antibiotics tested, including gentamicin. The MIC to vancomycin was 0.5 mg/L, which was four-fold lower than the cut-off value. PROSAFE concluded that *B. infantis* M-63 had "acquired resistance" to streptomycin, which is defined as a type of resistance present in strains with MICs that are higher than the normal range of the MIC

distribution of the wild type population of a given taxonomic group and that the resistance usually originates from gene mutations or recombinations. PROSAFE also concluded that no resistance genes were detected.

Xiao et al. (2010) evaluated the antimicrobial susceptibility of a variety of Bifidobacteria species, including *B. infantis* M-63 and the *B. infantis* type strain ATCC 15697^T, to ampicillin, chloramphenicol, ciproflaxacin, clindamycin, erythromycin, gentamicin, kanamycin, linezolid, neomycin, rifampicin, streptomycin, tetracycline, trimethoprim, virginamycin, and vancomycin using a broth microdilution method outlined in a Draft International Standard (ISO/IDF, 2010). *Bifidobacterium infantis* M-63 and *B. infantis* type strain ATCC 15697^T had similar minimum inhibitory concentrations (MICs; less than 2-fold) to all of the antibiotics tested, and both strains exhibited a MIC to streptomycin of greater than 1024 mg/L, which is approximately 10-fold greater than the EFSA cut off value for Bifidobacteria (Table 16; EFSA Panel on Additives Products or Substances used in Animal Feed, 2012). *Bifidobacterium infantis* M-63 also had an MIC for vancomycin that was two-fold greater than the EFSA cut off value for Bifidobacteria. A subsequent survey of the genomic sequence of *B. infantis* M-63 and *B. infantis* type strain ATCC 15697^T revealed that the streptomycin resistance of the two strains was likely due to chromosomally located alanine to guanine substitution at nucleotide position 128 of the rpsl gene for ribosomal protein S12, rather than a streptomycin resistance gene located on a mobile element such as a plasmid. An explanation of the increase resistance to *B. infantis* M-63 of vancomycin was not provided, however, since the MIC was only two-fold greater and this was not found in other study (Prosafe), this may arise from methodological experimental variability.

Toscano et al. (2015) evaluated the antimicrobial susceptibility of *B. infantis* M-63 to erythromycin, gentamicin, tetracycline, and penicillin using an E-test and compared the MICs for erythromycin, gentamicin, tetracycline to the EFSA cut off value for Bifidobacteria and the MIC for penicillin to the National Committee for Clinical Laboratory Standards. Toscano et al. concluded that *B. infantis* M-63 was sensitive to erythromycin, tetracycline, and penicillin, and resistant to gentamicin; however, the MICs for *B. infantis* M-63 to each of the antimicrobials and an explanation for why *B. infantis* M-63 was resistant to gentamicin were not provided.

Taken together, the results from these two studies indicate that *B. infantis* M-63 is resistant to streptomycin, which is similar to the type strain *B. infantis* type strain ATCC 15697^T and likely due to a gene mutation or recombination event rather than due to the presence of an actual streptomycin resistance gene. Additionally, although Toscano et al. concluded that *B. infantis* M-63 is resistant to gentamicin and Xiao et al. (2010) reported a vancomycin MIC that was greater than the EFSA cut-off, there are methodological differences between the studies that may have led to MIC variability and the actual gentamicin and vancomycin MICs for *B. infantis* M-63 reported Xiao et al. (2010) and PROSAFE were either below or approximate the EFSA cut-off values. Additionally, *Bifidobacteria* are also not known to be particularly resistant to

vancomycin, although there appears to be variability across the genus (Charteris et al., 1998; D'Aimmo et al., 2007; Zhou et al., 2005). Thus, there is reasonable certainty that *B. infantis* M-63 is resistant to streptomycin and that the resistance does not pose risks to *B. infantis* M-63 consumers because it is likely due to a gene mutation or recombination event and because no plasmids have been found (section C. 3.), the risk of transfer is negligible.

Table 16. Antibiotic Resistance of *B. infantis* M-63

Antibiotic	Reported by Xiao et al., 2010		Toscano et al., 2015	PROSAFE		EFSA cut-off values for Bifidobacteria * (mg/L)
	B. infantis M-63	*B. infantis* ATCC 15697^T		*B. infantis* M-63	*Bifidobacterium longum* cut-off values (mg/L)	
Ampicillin	0.25	0.25	NT	0.125	Inconclusive**	≤ 2
Ampicillin/sulbactam	NT	NT	NT	0.125	Inconclusive	NP
Chloramphenicol	2	2	NT	2	≤ 8	≤ 4
Ciproflaxacin	8	8	NT	NT	NP	NP
Clindamycin	0.125	0.125	NT	0.25	Inconclusive	≤ 1
Erythromycin	0.5	0.25	S	0.125	≤ 25	≤ 1
Fusidic acid	NT	NT	NT	4	≤ 32	NP
Gentamicin	32	16	R	4	≤ 128	≤ 64
Kanamycin	128	64	NT	NT	NP	NR
Linezolid	1	1	NT	NT	NP	NP
Neomycin	128	32	NT	NT	NP	NP
Penicillin	NT	NT	S	0.125	Inconclusive	NP
Quinupristin/Dalfopristin	NT	NT	NT	0.063	≤ 0.032	NP
Rifampicin	≤ 0.125	≤ 0.125	NT	NT	NP	NP
Streptomycin	>1024	>1024	NT	> 4096	≤ 128	≤ 128
Sufamethoxazole/trimethoprim	NT	NT	NT	16	Inconclusive	NP
Tetracycline	8	8	S	4	≤ 4	≤ 8
Teicoplanin	NT	NT	NT	≤ 0.125	Inconclusive	NP
Trimethoprim	16	16	NT	8	Inconclusive	NP
Virginamycin	1	2	NT	NT	NP	NP
Vancomycin	4	2	NT	0.5	≤ 0.5	≤ 2

NT = Not tested; NR = Not Required; NP = Not Provided; MIC = Minimum inhibitory concentration; S= sensitive; R= resistant.
*EFSA Panel on Additives Products or Substances used in Animal Feed, 2012.
**Inconclusive = no clear distinction between intrinsic and acquired resistance can be made due to insufficient information.

2. Metabolic Activity

a. Lactic acid production

Lactic acid isomer production by *B. infantis* M-63 was compared to that of *B. infantis* ATCC 15697^{T} (type strain) and *Lactobacillus plantarum* MCC-00621, a known D-lactic acid producer. While *L. plantarum* MCC-00621 produced both D- and L-lactic acids, *B. infantis* M-63 produced L-lactic acid, but not D-lactic acid, similar to that of the *B. infantis* type strain (Figure 3). Thus, there is no concern of toxicity due to D-lactic acid production by *B. infantis* M-63.

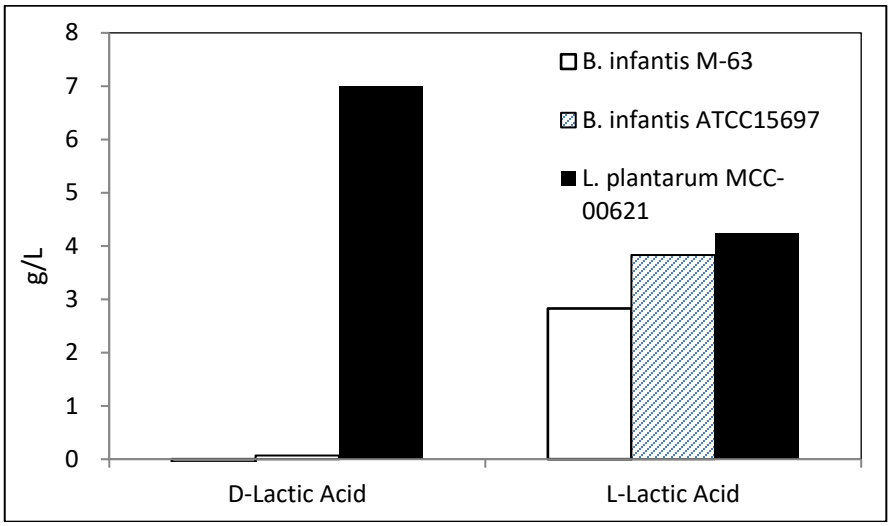

Figure 3. D- and L-lactic Acid Production by *Bifidobacterium infantis* M-63
D- and L-lactic acid production by *B. infantis* M-63, *B. infantis* ATCC 15697 (type strain) and *L. plantarum* MCC-00621 was determined using enzymatic assays involving D- and L-lactate dehydrogenase.

b. Bile salt conjugation

Although many *Bifidobacterium* strains are capable of deconjugating bile acids (Ridlon et al., 2006), production of secondary bile acids by *B. longum* subsp. *infantis* strains has not been reported. To confirm this finding, *B. infantis* M-63 was incubated with broth containing 0.1 mM of various bile acids (taurocholic acid sodium salt hydrate, glycocholic acid hydrate, taurochenodeoxycholic acid sodium salt, and sodium glycochenodeoxycholate) anaerobically at 37°C overnight. *B. infantis* M-63 converted all tested conjugated bile acids into the corresponding unconjugated forms to some extent (Table 17). No secondary bile acids were detected after incubation. Thus, although *B. infantis* M-63 can deconjugate bile salts, it does not produce secondary bile acids.

Table 17. Bile Salt Deconjugation Activity of *B. infantis* M-63

Bile Acid Substrate	Product After Culturing	Residual Substrate (µM)	Product Concentration (µM)	Ratio of Product to Total Bile Acid
Taurocholic acid	Cholic acid	0.1 ± 0.1	76.6 ± 1.9	100%
Glycocholic acid	Cholic acid	ND	86.1 ± 2.3	100%
Taurochenodeoxy cholic acid	Chenodeoxy cholic acid	53.3 ± 4.4	46.0 ± 1.9	43.9 ± 3.1 %
Glycochenodeoxy cholic acid	Chenodeoxy cholic acid	3.0 ± 1.3	88.1 ± 1.9	96.7 ± 1.4 %
Cholic acid	Deoxycholic acid	87.8 ± 1.2	ND	0%
Chenodeoxy cholic acid	Lithocholic acid	81.9 ± 1.8	ND	0%

Data are shown as mean ± SD, n=3.
ND = not detected.

c. Biogenic amine production

The ability of *B. infantis* M-63 to produce biogenic amines was examined. *Enterococcus faecalis* ATCC 19433 and *Clostridium perfringens* ATCC 12124 were used as positive controls for tyramine and histamine production, respectively. Strains were cultured overnight in the appropriate medium at 37°C under aerobic or anaerobic conditions, as required. The culture supernatant was collected for analysis, and the bacteria were pelleted by centrifugation and resuspended in test buffer containing no additives or supplemented with tyrosine or histidine. The pH of the buffer was tested before and after a 6 hour incubation with the bacteria. As expected, incubation of *E. faecalis* ATCC 19433 and *C. perfringens* ATCC 12124 with tyrosine and histidine, respectively, resulted in significant increased in the buffer pH (Figure 4). Incubation with *B. infantis* M-63 did not cause changes in buffer pH following incubation with either tyrosine or histidine which indicates no biogenic amine production.

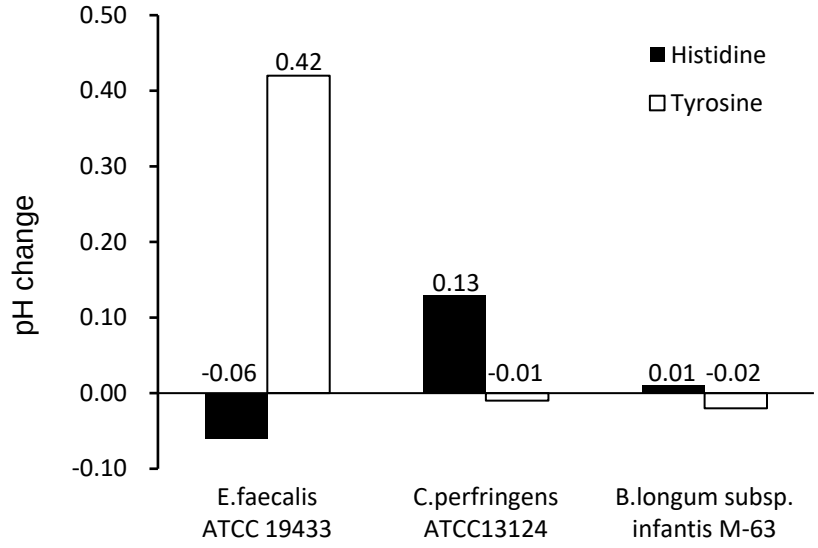

Figure 4. Change in pH of histidine- or Tyrosine-Containing Test Buffer Incubated with *E. faecalis, C. perfringens,* or *B. infantis* M-63 for 6 Hours at 37˚C

Following the incubation, unsupplemented test buffer was also analyzed for tyrosine and histidine content by HPLC-MS. As expected, buffer from *E. faecalis* and *C. perfringens* contained significant amounts of tyramine and histamine, respectively (Figure 5). However, buffer from *B. infantis* M-63 cultures contained trace or undetectable amounts of the amines. Therefore, *B. infantis* M-63 is not expected to produce biogenic amines.

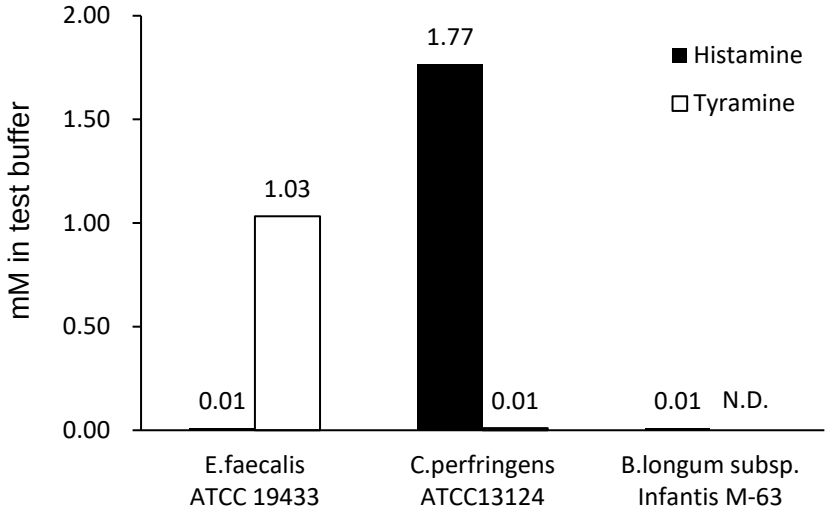

Figure 5. Histamine and Tyramine Content in Buffer Incubated with *E. faecalis, C. perfringens,* or *B. infantis* M-63 for 6 Hours at 37˚C

d. Ammonia production

The ability of *B. infantis* M-63 to produce ammonia was examined. *Enterococcus faecium* JCM5804 and *Lactobacillus rhamnosus* JCM1136 were used as positive and negative controls, respectively. Bacteria were cultured at 37°C overnight under anaerobic conditions as precultures. An aliquot of each precultured was inoculated into Gifu Anaerobic Medium (GAM broth), then incubated anaerobically at 37˚C for 48 hours. Culture medium was collected and adjusted to pH 7 with sodium hydroxide. Samples were diluted appropriately, and ammonia concentration determined using an enzymatic photometric assay kit (Roche Diagnostics GmbH, Germany). Uncultured GAM broth was used for the baseline value. As expected, media from *E. faecium* JCM5804 contained a substantial ammonia concentration, but media from *L. rhamnosus* JCM1136 contained very little (Table 18). The ammonia content of media from *B. infantis* M-63 was below the baseline level. Thus, *B. infantis* M-63 does not produce ammonia *in vitro* and is not expected to increase blood ammonia levels *in vivo*.

Table 18. Ammonia Concentration in GAM Broth Following 48 Hour Culture

Bacteria	Ammonia (mM)
E. faecium JCM5804	6.42
L. rhamnosus JCM1136	0.14
B. infantis M-63	-0.67

e. Mucin degradation

B. infantis M-63 was also evaluated for mucin degradation, using three different methods: 1) *B. infantis* M-63 growth in liquid medium providing mucin as a carbon source; 2) sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] analysis of mucin residues obtained from the growth medium containing mucin; and 3) degradation of mucins during *B. infantis* M-63 growth on a Petri dish (Abe et al. 2010). None of the methods indicated that *B. infantis* M-63 is capable of mucin degradation.

3. Presence of Plasmids

DNA was extracted from *B. infantis* M-63 using a QIAGEN Plasmid kit according to the manufacturer's instructions. Extracted DNA was analyzed by gel electrophoresis on a 1.0% agarose gel together with wide-range DNA Ladder (50-10,000 bp, Takara Bio Inc.) (Figure 6). Although the *B. infantis* M-63 DNA contained contaminating DNA and degraded RNA, no discrete bands, which would be indicative of plasmids were detected between 500 and 10,000 bp, confirming that *B. infantis* M-63 does not harbor plasmids.

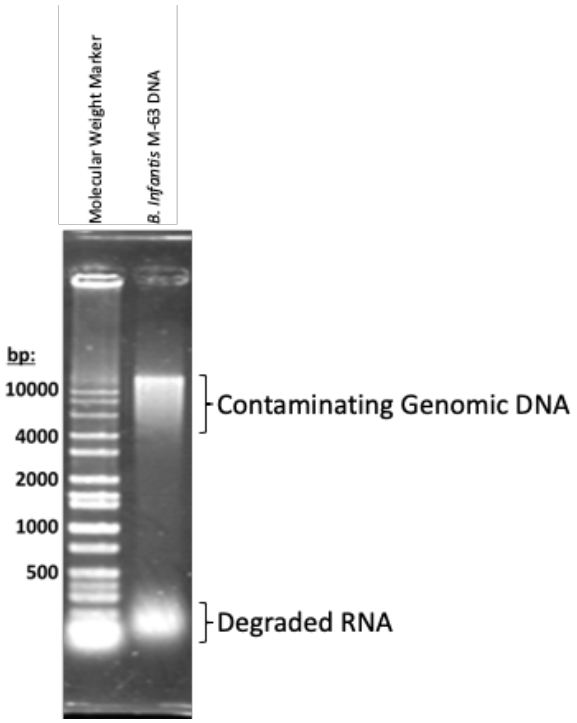

Figure 6. Evaluation of *B. infantis* M-63 for the Presence of Plasmids by Gel Electrophoresis

The high molecular weight material (~10,000 bp) is contaminating genomic DNA. The low molecular weight material <500 bp is degraded RNA. Plasmids, which are not present, migrate as discrete bands between 10,000 bp and 500 bp.

4. Genomic Analysis for Toxins and Pathogenic Markers

To confirm the absence of toxin-producing genes and pathogenic markers, the genomic sequence of *B. infantis* M-63 was determined using PacBio RS II sequencer. The entire sequence was assembled using SMRT Analysis ver.2.3 with a program of RS_HGAP_Assembly.3. Open Reading Frame prediction was performed using PRODIGAL prediction software version 2.6. Automatic annotation was performed on the basis of BLASTP v2.2.26 analysis using the non-redundant protein database curated by the National Centre for Biotechnology (e-value < 10^{-5}). Where necessary, manual editing was performed using Artemis v.15 which was employed for output visualization. Except for hemolysin, hemolysin III, and Hly-III family protein, which are related to hemolysin-like proteins. No known toxin or virulence genes were identified aside from the hemolytic genes.

5. Hemolytic Potential

To evaluate the hemolytic potential of M-63, *B. infantis* M-63, *Listeria ivanovii* subsp. *ivanovii* ATCC 19119^T (positive control) and *Bifidobacterium breve* M-16V (negative control) were plated on horse blood-supplemented agar and incubated at 37°C for up to 72 hours (24 for *Listeria*). Compared to *L. ivanovii* subsp. *ivanovii* ATCC 19119^T, which is known to cause hemolysis, no hemolysis was detected when *B. infantis* M-63 and *B. breve* M-16V, a strain of Bifidobacteria that has already been deemed GRAS (GRN 453; GRN 454; GRN 455) (Figure 7) were cultured on the horse blood-supplemented agar. Thus, *B. infantis* M-63 does not induce hemolysis *in vitro*.

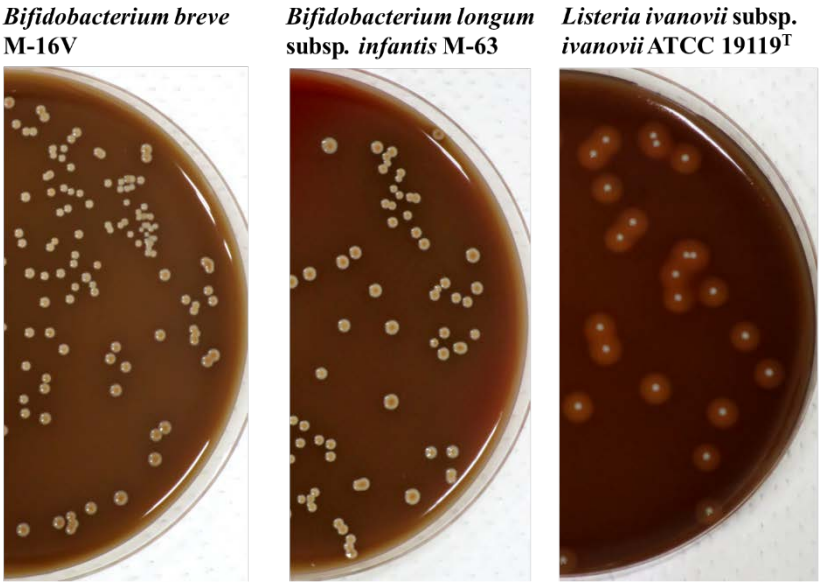

Figure 7. Hemolysis Induced by *B. breve* M-16V, *B. longum* subsp. *infantis* M-63 and *L. ivanovii* subsp. *ivanovii* ATCC 19119^T Plated on Blood Agar

6. Platelet Aggregation

The potential for *B. infantis* M-63 to induce platelet aggregation was evaluated. Blood was collected from informed, consenting donors and processed to isolate the platelets. All platelet samples were verified for proper aggregation prior to use by initial testing with adenosine diphosphate (ADP) as a stimulus. Medium alone (negative control) or bacterial suspensions of *B. infantis* M-63, positive bacterial control *S. aureus*, and negative bacterial control *B. breve* were incubated with platelets with gentle mixing. Aggregation was measured after 1 and 3 hours of incubation using light transmission platelet aggregometry. *S. aureus* at a concentration of 0.05-0.35x10^9/mL induced aggregation, while medium alone or incubation with *B. breve* did not. *B. infantis* M-63 at concentrations of 0.67x10^9/mL and 0.17x10^9/mL did not induce aggregation. Thus, *B. infantis* M-63 does not induce platelet aggregation.

D. TOXICOLOGY STUDIES

The toxicity of *B. infantis* M-63 has been evaluated in published acute and subchronic oral toxicity studies (Abe et al., 2009). *B. infantis* M-63 is not acutely toxic at levels up to 3.2×10^{11} CFU/kg and has a no observed adverse effect level (NOAEL) of at least 7.6×10^{10} CFU/kg bw/day, based on the results from a 90-day oral toxicity study. Additional studies in animal models have also been published and, although the studies were designed to evaluate the beneficial effects of ingesting *B. infantis* M-63, they reported that the ingestion of *B. infantis* M-63 is well-tolerated.

1. Acute Toxicity

In a single dose oral toxicity study, 5 male and 5 female Crj:CD (SD)IGS(SPF) rats received a single oral dose of *B. infantis* M-63 at 4000 mg/kg (3.2×10^{11} CFU/kg suspended in saline) using a metal gastric tube (Abe et al., 2009). All animals were housed five to a cage in a climate-controlled room (20-24°C, 40-70% humidity) with a 12 hour light/dark cycle and pelleted feed and water were consumed *ad libitum*. Clinical signs and body weight were monitored continuously over the following 14 days and at the end of the study period, animals were euthanized and necropsied.

No deaths or clinical signs of toxicity were reported in any animal during the study. Body weights increased normally. At necropsy, no abnormalities were reported in any animal. The LD_{50} for *B. infantis* M-63 was determined to be higher than the tested dose of 4000 mg/kg (3.2×10^{11} CFU/kg).

2. Subchronic Toxicity

B. infantis M-63 was evaluated in a 90-day repeated dose oral toxicity study conducted in compliance with Japanese guidelines: Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives (1996). The results of this study were published by Abe et al. (2009).

Crj:CD(SD)IGS rats (10/sex/group; Charles River Japan Inc. (Tokyo, Japan)) were administered a cornstarch suspension (control) or 7.6×10^{10} CFU/kg bw *B. infantis* M-63 suspended in saline via gavage for 90 days. All animals were housed individually in bracket-type metallic wire-mesh cages in a room with temperature controlled at 21–24°C and humidity at 25–58% with a 12 hour light/dark cycle, allowed free access to a pelleted feed and water, and acclimatized for 8 days before the study.

Over the course of the 90-day treatment period, all animals were observed for general conditions such as external appearance, nutritional condition, posture, behavior, and abnormalities in excreta three times a day. Body weight and food consumption were measured continuously. Ophthalmological examinations were conducted before and at the end of the experiments. Urinalysis was performed using urine collected during week 13 (day 85 to day 87). At the end of the test period, all animals were subjected to laparotomy under ether anesthesia. Blood and serum were collected for measurement of hematological, biochemical, and coagulation parameters. All animals that survived until termination were killed by exsanguination and subjected to necropsy, organ weight measurements, and histopathological examination.

No mortalities or abnormalities in clinical signs were observed in either group. Animals receiving *B. infantis* M-63 showed no difference in body weight, water, or feed consumption compared to the control group. No abnormalities in ophthalmological examinations, urinalysis, hematology, blood chemistries, or gross pathology were detected. The weight of the seminal vesicle in male rats administered *B. infantis* M-63 was significantly lower ($p<0.05$) compared to the control animals, but all other organ weights showed no difference. Histopathological examination showed no abnormalities in any organ, including the seminal vesicle.

Due to the lack of observed toxicity, the NOAEL for *B. infantis* M-63 was determined to be at least 7.6×10^{10} CFU/kg bw/day under the tested conditions.

3. Corroborative Studies of *B. longum* subsp. *infantis*

Five corroborative studies of *B. longum* subsp. *infantis* in various animal models were found in the literature (Jena et al., 2018; Liu et al., 2017; Mi et al., 2017; Moreno Muñoz et al., 2011; Musilova et al., 2017). These studies are summarized in Table 19. In these studies, *B. longum* subsp. *infantis* was administered at doses ranging from 1×10^9 CFU/week to 1×10^{10} CFU/day for durations of 6 days to 7 months. No adverse effects or signs of intolerance were reported in any study. Overall, these studies provide corroborative evidence for the safety of *B. longum* subsp. *infantis*.

Table 19. Corroborative Animal Studies of *B. longum* subspecies *infantis*

Reference	Study Design and Animals	Duration	Groups	Results Safety Endpoints
Moreno Muñoz et al., 2011	BALB/c mice, normal or immunosuppressed with cyclophosphamide	6 days	1. Placebo 2. *B. infantis* $1x10^9$ CFU 3. Immunosuppressed + placebo 4. Immunosuppressed + *B. infantis* $1x10^9$ CFU N=9/group	• No morbidity or mortality was observed in any group.
Liu et al., 2017	Male BALB/c mice with ovalbumin-induced allergic asthma (OVA) or β-lactoglobulin and cholera toxin-induced food allergy (BLG)	7 days (pre-treatment) OR 21 days (prevention)	<u>Allergic asthma:</u> 1. Normal control 2. OVA control 3. OVA + *B. infantis* $1x10^{10}$ CFU/day during sensitization (prevention group) 4. OVA + *B. infantis* $1x10^{10}$ CFU/day during challenge (pre-treatment group) <u>Food allergy:</u> 5. Normal control 6. BLG control 7. BLG + *B. infantis* $1x10^{10}$ CFU/day during sensitization (prevention group) 8. BLG + *B. infantis* $1x10^{10}$ CFU/day during challenge (pre-treatment group) N=10/group	• No adverse effects were reported. • No worsening of the allergic model with *B. infantis* administration was reported.

Table 19. Corroborative Animal Studies of *B. longum* subspecies *infantis*

Reference	Study Design and Animals	Duration	Groups	Results Safety Endpoints
Mi et al., 2017	Male Sprague-Dawley rats using a model of colorectal cancer (CRC)	11 days	1. CRC control 2. CRC + chemotherapy 3. CRC + chemotherapy + *B. infantis* $1x10^9$ CFU/day N=10/group	• No mortalities occurred, and all treatments were well tolerated. • Rats in Group 2 had significantly lower final body weights ($p<0.01$) compared to Group 1, but this weight loss was partially reduced in Group 3 ($p<0.05$ vs. Group 2). • Both Groups 2 and 3 developed diarrhea following chemotherapy; the severity of diarrhea was attenuated in Group 3 vs. Group 2 ($p<0.05$)
Musilova et al., 2017	Germ-free immunocompetent BALB/c mice humanized through colonization of the gut by oral gavage of a fecal mixture from Bifidobacteria-free human infants	14 days	1. Control 2. Human milk (instead of water) + *B. infantis* $4x10^5$ CFU every 3-4 days 3. Water supplemented with HMO 7 g/L + *B. infantis* $4x10^5$ CFU every 3-4 days N=2/sex/group.	• No adverse effects of *B. infantis* were reported.
Jena et al., 2018	C57BL/6 wild-type (WT) and age- and sex-matched FXR (farnesoid × receptor) knockout (KO) mice fed a Western diet to induce non-alcoholic steatohepatitis (NASH)	2 months OR 7 months	1. WT control 2. KO control 3. KO + *B. infantis* $1x10^9$ CFU once a week 4. KO + BMO 7% in diet 5. KO + *B. infantis* $1x10^9$ CFU once a week + BMO 7% in diet N=6-10/group	• No adverse effects were reported. • *B. infantis* administration did not worsen the NASH model.

ALP: alkaline phosphatase; ALT: alanine aminotransferase; *B. infantis*: *Bifidobacterium longum* subspecies *infantis;* BLG: β-lactoglobulin; BMO: bovine milk oligosaccharides; CFU: colony forming units; IBS: Irritable Bowel Syndrome; KO: knock out; NASH: non-alcoholic steatohepatitis; OVA: ovalbumin; PEG: polyethylene glycol; MM: microbial mixture including *B. longum* BB536, *B. infantis* M-63, and *B. breve* M-16 V; WT: wild-type; TC: total cholesterol; TG: triglycerides.

E. CLINICAL STUDIES

Pivotal clinical studies assessing the safety of *B. infantis* M-63 have been conducted in infants, children, and adults, while corroborative studies of *B. longum* subsp. *infantis* have been conducted infants. The following discussion provides the safety-related parameters reported from each study.

1. Studies of *B. infantis* M-63 in Children and Adults

There are three published studies of *B. infantis* M-63 in microbial mixtures in children (Del Giudice et al., 2017; Giannetti et al., 2017; Russo et al., 2017) and two studies of *B.* infantis M-63 in mixtures or alone in adults (Inoue et al., 2018; Ma et al., 2019). These studies are summarized in Table 20.

Del Giudice et al. (2017) conducted a randomized, prospective, double-blind, placebo-controlled study in children ages 4-17 years suffering from seasonal allergic rhinitis and well-controlled asthma. Subjects were administered placebo or microbial mixture containing 1 x 10^9 CFU/day *B. infantis* M-63 (n=20/group) for 8 weeks. All subjects completed the study, and no clinically relevant adverse effects were reported.

Giannetti et al. (2017) conducted a multi-center, randomized, double-blind, placebo-controlled, crossover study in children ages 8-17 years with IBS (n=50) or functional dyspepsia (FD, n=28). Subjects received placebo and microbial mixture including 1 x 10^9 CFU/day *B. infantis* M-63, in random order, for 6 weeks. Study periods were separated by a 2-week washout period. Four subjects were lost to follow-up, and one was withdrawn due to the use of antibiotics. No adverse events were reported.

Russo et al. (2017) conducted a randomized, double-blind, controlled study in children aged 4-12 years with constipation. Subjects received polyethylene glycol (PEG; control, n=28) or PEG plus a microbial mixture including 2.5 x 10^9 CFU/day *B. infantis* M-63 (n=27) for 8 weeks. Five subjects withdrew from the study due to “bad taste” of the test article or loss to follow-up. No clinically significant adverse effects were reported except for transient diarrhea, which disappeared with dose reduction. There were no complaints of abdominal distention, increased flatus, or new onset of abdominal pain. Children in both groups continued to grow in weight and height, along their growth curves.

Inoue et al. (2018) conducted a randomized, double-blind, placebo-controlled trial in adults aged > 65 years who had undergone stretch training for the previous 12 months. Subjects completed moderate resistance training and received placebo (n=19) or a microbial mixture

including $1.25x10^{10}$ CFU/day *B. infantis* M-63 (n=20) for 12 weeks. One subject in the *B. infantis* M-63 group dropped out due to non-compliance. No adverse events were reported.

Finally, Ma et al. (2019) conducted a prospective, non-randomized, open-label intervention and controlled before-and-after study in adults aged ≥ 18 years with irritable bowel syndrome (IBS). Subjects received no intervention (control, n=33) or $2.5x10^{9}$ CFU/day *B. infantis* M-63 (n=20) for 3 months. No additional symptoms (beyond established IBS) or adverse events were reported in either group.

Overall, no adverse events or other safety concerns were observed with *B. infantis* M-63 administration in any study. These studies support the safe use of *B. infantis* M-63 in children at doses up to 1.0×10^{9} CFU/day for 8 weeks and adults at doses up to 1.25×10^{10} CFU/day for 12 weeks.

Table 20. Clinical Studies using *B. infantis* M-63 in Children and Adults

Reference	Design and Population	Duration	Groups	Results Safety Endpoints
Del Giudice et al., 2017	Randomized, prospective, double-blind, placebo-controlled study in children ages 4-17 years suffering from seasonal allergic rhinitis and well-controlled asthma	8 weeks	1. Placebo 2. Microbial mixture (PM) including $1x10^9$ CFU/day *B. infantis* M-63 N=20/group	• All subjects completed the study. • PM was well-tolerated and there were no clinically relevant side effects observed.
Giannetti et al., 2017	Multi-center, randomized, double-blind, placebo-controlled, crossover study in children ages 8-17 years with IBS (n=50) or FD (n=28)	12 weeks (total): 6 weeks each placebo and PM	1. Placebo 2. PM including $1x10^9$ CFU/day *B. infantis* M-63 The order of test article administration was randomized for each subject. Study periods were separated by a wash-out period of 2 weeks. 3. N=78 (IBS: n=50; FD: n=28)	• Five subjects dropped out of the study, including 2 IBS subjects and 3 FD subjects. o Four lost during washout o One lost due to need for antibiotic therapy • No adverse events were recorded.
Russo et al., 2017	Randomized, double-blind, controlled study in children ages 4-12 years with constipation	8 weeks	1. PEG (control), n=28 2. PEG plus PM including an unknown number of *B. infantis* M-63, n=27	• Three subjects withdrew from the Group 1 and 2 from the Group 2. Withdrawals were due to "bad taste" of the test article or subjects were lost to follow-up. • No clinically significant adverse effects were reported except for transient diarrhea, which disappeared with dose reduction. • There were no complaints of abdominal distention, increased flatus, or new onset of abdominal pain. • Children in both groups continued to grow in weight and height, along their growth curves. • There were no new abnormal physical findings on examination.

Table 20. Clinical Studies using *B. infantis* M-63 in Children and Adults

Reference	Design and Population	Duration	Groups	Results Safety Endpoints
Inoue et al., 2018	Randomized, double blind, placebo-controlled trial in adults aged > 65 years who had undergone stretch training for the previous 12 months	12 weeks	12-week moderate resistance training program plus: 1. Placebo, n=19 2. PM including 1.25×10^{10} CFU/day *B. infantis* M-63, n=20	• One subject in the PM group dropped out due to challenges in complying with the study protocol. • No adverse events were reported by either group during the study.
Ma et al., 2019	Prospective, non-randomized, open-label intervention and controlled before-and-after study in adults aged ≥ 18 years; flood victims who fulfilled the Rome III criteria for IBS after flood	3 months	1. Control, n=33 2. *B. infantis* M-63 $2.5x10^9$ CFU/day, n=20	• Thirteen subjects dropped out in the control group and 9 dropped out in the M-63 group. All withdrawals were all due to inability complete the full course and/or provide stool samples. • No additional symptoms (beyond established IBS) or adverse events were reported in either group.

IBS: Irritable Bowel Syndrome; PEG: polyethylene glycol; MM: microbial mixture including *B. longum* BB536, *B. infantis* M-63, and *B. breve* M-16 V.

2. Studies of *B. infantis* M-63 in Infants

There are three published studies of *B. infantis* M-63 in microbial mixtures with and without oligosaccharides in infants (Dupont et al., 2010; Ishizeki et al., 2013; Rozé et al., 2012). These studies are summarized in Table 21.

Dupont et al. (2010) conducted a double-blind, placebo-controlled study in healthy infants with colic. Subjects received control formula (n=32) or the formula containing 1 x 10^7 CFU/100 mL *B. infantis* M-63 (n=30) for 1 month. The experimental formula also contained *Lactobacillus rhamnosus*, galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), and bovine α-lactalbumin. Withdrawals (n=16) were similar between groups and occurred due to side effects, therapeutic failure, consent withdrawal, or loss to follow-up. No differences were observed in weight or height gain between groups. Overall gastrointestinal (GI) side effects were comparable, but "feeding-related" GI side effects were significantly lower (p=0.011) in the group receiving *B. infantis* M-63 versus control.

Rozé et al. (2012) conducted a double-blind, multicenter, randomized, controlled trial in non-breastfed term neonates. Subjects received control formula (n=49) or the formula containing 1.4 x 10^8 CFU/100 mL *B. infantis* M-63 (n=48) for 6 months. The experimental formula also contained *Lactobacillus rhamnosus*, galacto-oligosaccharides, fructo-oligosaccharides, and bovine α-lactalbumin. Compliance was not different between groups. Reasons for non-compliance were not different between groups and included poor digestive tolerance and poor parental compliance. At 6 months, weight, height, and head circumference were not significantly different between groups.

Ishizeki et al. (2013) conducted a study in low birth-weight babies. Subjects consumed unsupplemented formula or breastmilk (control, n=17) or formula/breastmilk supplemented with a microbial mixture including 5 x 10^8 CFU/day *B. infantis* M-63 (n=14) for 6 weeks. One subject withdrew from each group due to infection unrelated to test article administration. Adverse events were not reported.

Overall, *B. infantis* M-63 was well-tolerated in infants and resulted in no adverse events. These studies support the safe use of *B. infantis* M-63 in infants up to 1.4 x 10^8 CFU/100 mL for 6 months or 5 x 10^8 CFU/day for up to 6 weeks.

Table 21. Clinical Studies using *B. infantis* M-63 in Infants

Reference	Design	Duration	Groups	Results Safety Endpoints
Dupont et al., 2010	Double-blind, placebo-controlled study in healthy infants with colic	1 month	1. Control formula, n=32 2. Experimental formula containing $1x10^7$ CFU/100 mL *B. infantis* M-63, n=30 Experimental formula also contained *Lactobacillus rhamnosus* LCS-742, GOS, FOS, and bovine α-lactalbumin	• Sixteen subjects (6 in Group 1, 10 in Group 2) dropped out, due to side effects, therapeutic failure, consent withdrawal, or loss to follow-up. • No difference in weight or height gain was observed between groups. • Overall GI side effects were comparable between groups. • "Feeding-related" GI side effects were significantly reduced (p=0.011) in Group 2 versus Group 1.
Rozé et al., 2012	Double-blind, multicenter, randomized, controlled trial in non-breastfed term neonates	6 months	1. Control formula, n=49 2. Experimental formula containing $1.4x10^8$ CFU/100 mL *B. infantis* M-63, n=48 Experimental formula also contained *Lactobacillus rhamnosus* LCS-742, GOS, FOS, and bovine α-lactalbumin	• Three subjects (1 from Group 1, 2 from Group 2) were lost to follow-up at one month. • Three subjects (2 in Group 1, 1 in Group 2) received antibiotics during month 1 of the study. • Compliance was not different between groups. Reasons for non-compliance were not different between groups and included poor digestive tolerance and poor parental compliance. • At 6 months, weight, height, and head circumference were not significantly different between groups.
Ishizeki et al., 2013	Study in low birth-weight babies	6 weeks	Subjects consumed formula and/or breast milk supplemented with: 1. Nothing (control), n=17 2. MM including $5x10^8$ CFU/day *B. infantis* M-63, n=14	• One subject withdrew from each group due to infection. • No adverse events were reported.

FD: Functional Dyspepsia; FOS: short chain fructo-oligosaccharides; GI: gastrointestinal; GOS: galacto-oligosaccharides; IBS: Irritable Bowel Syndrome; PEG: polyethylene glycol; MM: microbial mixture including *B. longum* BB536, *B. infantis* M-63, and *B. breve* M-16 V.

3. Corroborative Studies of *B. longum* subsp. *infantis*

A search of PubMed for "*Bifidobacterium longum* subspecies *infantis*" revealed 6 clinical studies corroborating the safety of *B. infantis* M-63 (De Andrés et al., 2018; Escribano et al., 2018; Hoy-Schulz et al., 2016; Manzano et al., 2017; Robertson et al., 2019; Smilowitz et al., 2017). These studies are summarized in Table 22. All studies were conducted in infants.

In these studies, *B. longum* subsp. *infantis* was administered at doses of 1×10^7-2.8×10^{10} CFU/day to term and preterm infants ages 1 day to 12 months. The duration of *B. longum* subspecies *infantis* administration ranged from 21 days to 12 weeks. No study reported adverse events related to *B. longum* subsp. *infantis* administration. Symptoms of gastrointestinal intolerance were infrequently reported.

Overall, these studies provide corroborative evidence for the safe use of *B. infantis* M-63 at dose of up to 2.8×10^{10} CFU/g in infants.

Table 22. Corroborative Clinical Studies using *B. longum* subsp. *infantis* in Infants

Reference	Design	Duration	Groups	Results Safety Endpoints
Hoy-Schulz et al., 2016	Controlled, parallel, randomized, Phase I trial in healthy infants ages 4-12 weeks	1 month	1. Control 2. *B. infantis* $1x10^9$ CFU, daily 3. *B. infantis* $1x10^9$ CFU, weekly 4. *B. infantis* $1x10^9$ CFU, biweekly *B. infantis* groups also received *L. reuteri* N=40/group	• A total of 23 infants withdrew or were lost to follow-up after baseline data collection but before the intervention began (n=8, 5, 5, and 5 in Groups 1-4, respectively). • Twenty-four subjects withdrew after initiation of the intervention (n=8, 6, 6, and 4 in Groups 1-4, respectively), most often due to moving away from the study area (58%), the family being too busy (13%), or the family perceiving no benefit from study (13%). • Cough and congestion were the most commonly reported symptoms. GI symptoms were rare. No differences among groups were seen in the follow up time with these symptoms. • No sudden adverse and allergic reactions were observed. • Ten hospitalizations occurred but were unrelated to microbial use.

Table 22. Corroborative Clinical Studies using *B. longum* subsp. *infantis* in Infants

Reference	Design	Duration	Groups	Results Safety Endpoints
Manzano et al., 2017	Multi-center, randomized, double-blind, placebo-controlled intervention study in healthy infants ages 3-12 months	8 weeks	1. Placebo, n=52 2. *B. infantis* $3x10^9$ CFU/day, n=53	• Four subjects (1 in Group 1, 3 in Group 2) did not comply with the product intake and were therefore eliminated from the per-protocol analysis. • No serious AEs were reported. • In the per-protocol analysis, the total number of AEs were not significantly different between groups. • In the intention to treat population, the total number of AEs was not equivalent between groups (p=0.085). This was due to the high number of AEs (n=9: 2 gastrointestinal; 4 respiratory, thoracic and mediastinal; 1 eye and 1 skin and subcutaneous tissue disorders; and 1 episode of fever) in one subject in group 2 who was non-compliant with the product intake. All AEs in this subject were deemed not related to product intake • No differences in growth (height, weight, head circumference) were observed between groups. • The incidence of gastrointestinal symptoms, fever, rashes, and unscheduled visits to the doctor was low and not different between groups.
Smilowitz et al., 2017	Parallel, partially-randomized, controlled Phase I trial in healthy mother-infant dyads (mothers ages 21-45 years; infants started study at postnatal day 7)	21 days	1. Lactation support alone, n=39 2. Lactation support + *B. infantis* $1.8x10^{10}$-$2.8x10^{10}$ CFU/day, n=41	• Twelve subjects withdrew from the study due to using formula within 24 hours of the initial visit (n=4) or unexpectedly discontinuing breastfeeding (n=8). • No difference in infant weight or mean number of feeds per day were observed between groups. • None of the safety and tolerability endpoints, including flatulence, bloody stool, body temperature, ratings of gastrointestinal symptoms, use of antibiotics or gas-relieving medications, infant colic, jaundice, number of illnesses, sick doctor visits, or diagnoses of eczema were different between groups at any point.

Table 22. Corroborative Clinical Studies using *B. longum* subsp. *infantis* in Infants

Reference	Design	Duration	Groups	Results Safety Endpoints
De Andrés et al., 2018	Randomized, double-blind, placebo-controlled intervention study in healthy infants ages 3-12 months	8 weeks	1. Placebo 2. *B. infantis* $3x10^9$ CFU/day N=23/group	• No safety endpoints were discussed.
Escribano et al., 2018	Double-blind, randomized, multicenter, placebo-controlled trial in healthy infants < 3 months of age	12 weeks	1. Standard formula, n=97 2. Standard formula + *B. infantis* $1x10^7$ CFU/g, n=93	• In the control group, 13 subjects dropped out before 4 weeks, 2 subjects dropped out between weeks 5-8, and 4 subjects dropped out between weeks 9-12. • In the *B. infantis* group, 11 subjects dropped out before 4 weeks, 6 subjects dropped out between weeks 5-8, and 3 subjects dropped out between weeks 9-12. • No differences between groups were observed in growth or formula intake.
Robertson et al., 2019	Single-center retrospective observational study in preterm neonates at high risk of NEC	Until ~34 weeks postmenstrual age (≈3-6 weeks)	1. No microbials (January 2008 - December 2012), n=496 2. *L. acidophilus* and *B. bifidum* (January 2013 – March 2016) 3. MM including $0.5x10^9$ CFU/day *B. infantis* (April 2016 – December 2017) Results are reported for the combined "microbials" group (Groups 2 and 3), n=513	• No safety issues occurred related to microbial administration.

B. infantis: *Bifidobacterium longum* subspecies *infantis*; NEC: necrotizing enterocolitis; MM: microbial mixture containing *L. acidophilus*, *B. bifidum*, and *B. infantis*.

F. ALLERGENICITY

There are no published reports of allergic reactions resulting from the ingestion of *B. longum* subsp. *infantis* generally or *B. infantis* M-63 specifically.

G. REGULATORY APPROVALS ACROSS THE WORLD

1. *B. infantis* M-63

B. infantis M-63 is currently approved for use in infant and follow-on formula in countries around the world as previously described in Table 13. *B. infantis* M-63 is also used in Japan in foods and infant and follow-on formula.

2. *B. longum* subsp. *infantis*

In the United States, *Bifidobacterium longum* subsp. *infantis* strain R0033 is GRAS for use in powdered, milk-based, term infant formula alone or in combination with other microbial strains at levels up to $5x10^7$ CFU/g powdered formula or $3x10^9$ CFU/day (GRN 758). Notably by 16S rDNA homology, *B. longum* subsp. *infantis* R0033, like M-63, was shown to have the closest similarity to the type strain ATCC 15697.

Bifidobacterium longum species (including *infantis*) have been granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA BIOHAZ Panel, 2019). A strain belonging to a species listed on QPS and meeting the established criteria can freely be added to foods in Europe. Additionally, the International Dairy Federation (IDF) in collaboration with the European Food and Feed Cultures Association (EFFCA) has included *Bifidobacterium longum* on its list of microorganisms with a documented history of safe use in food (Bourdichon et al., 2012).

VII. SUPPORTING DATA AND INFORMATION

A. REFERENCES

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

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B. EXPERT PANEL STATEMENT

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of *B. infantis* M-63 as an ingredient in conventional foods and non-exempt infant formula. *B. infantis* M-63 has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b).

This GRAS determination for the use of *B. infantis* M-63 as an ingredient in conventional foods at a maximum level of 1.25×10^{10} CFU per serving and powdered, cow's milk- and soy-based, non-exempt, term infant formula at a maximum level of 1×10^{8} CFU/g is based upon scientific procedures as described under 21 CFR §170.30(b). The intake of *B. infantis* M-63 from the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). To demonstrate that *B. infantis* M-63 is safe, and GRAS, under the intended conditions of use, the safety of the intake of *B. infantis* M-63 has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of *B. infantis* M-63 as an ingredient in foods and non-exempt infant formula has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

- Bifidobacteria are naturally occurring bacteria that contribute to the composition of the gut microflora of humans. *Bifidobacterium longum* species have been detected in feces from infants and adults.

- *Bifidobacterium infantis* M-63, a strain of *B. longum* subspecies *infantis*, is a Gram-positive anaerobic bacterium that was originally isolated from a healthy infant in 1963. The bacterium has been deposited with the National Institute of Technology and Evaluation (NITE, Japan) and is designated NITE BP-02623.

- *B. infantis* M-63 was first commercially available in 2006 and has since been sold in a variety of markets including China, France, Japan, Indonesia, Italy, and Spain.

- The original stock culture of *B. infantis* M-63 has been maintained at −80°C since it was obtained by Morinaga Milk in the 1970s, and no selective pressures have been applied.
- *B. infantis* M-63 cultures are used to produce two product formulations: 1) M-63, using tapioca starch, for use in general foods, and 2) M-63 type-C, using cornstarch, for use in general foods and infant formula.
- Finished products made with *B. infantis* M-63 consistently comply with established, food-grade product specifications. Specifications are in place to control anaerobic plate count (M-63 count), moisture, microbial contamination, and heavy metals. M-63 type-C for use in infant formula undergoes additional microbial testing for *Cronobacter sakazakii* and *Bacillus cereus*.
- Thirteen GRAS Notices (GRNs) on *Bifidobacterium* species have received "no questions" letters from the FDA. This includes GRN 758, which allows for the use of a strain of *B. longum* subsp. *infantis* R0033 in infant formula at levels up to $5x10^7$ CFU/g.
- *B. longum* has been granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA).
- *B. infantis* M-63 has been tested for parameters outlined in the Food and Agriculture Organization of the United Nations/World Health Organization's (FAO/WHO) guidelines. Results from these tests provide evidence that *B. infantis* M-63 is safe for use in foods, namely:
    - *B. infantis* M-63 is resistant to streptomycin, however, the resistance does not reside on a transferable element.
    - *B. infantis* M-63 produces L-lactic acid but does not produce D-lactic acid.
    - *B. infantis* M-63 is shown to deconjugate bile salts, but no secondary bile acids are produced.
    - An *in vitro* study indicates that production of biogenic amines by *B. infantis* M-63 is not expected.
    - An *in vitro* study indicates that *B. infantis* M-63 is not expected to produce ammonia.

- The use of 3 different methods indicates that *B. infantis* M-63 is not expected to degrade mucins.
- Testing has confirmed the absence of plasmids in *B. infantis* M-63.
- Genomic analysis of *B. infantis* M-63 did not reveal the presence of known toxin or virulence genes.
- *B. infantis* M-63 was not observed to have hemolytic activity.
- *B. infantis* M-63 is not expected to induce platelet aggregation.

- The safety of *B. infantis* M-63 is supported by a published acute toxicology study and a pivotal published 90-day repeated dose toxicology study, both in rats. In the single dose oral toxicity test using 3.2×10^{11} CFU/kg of *B. infantis* M-63, there were no deaths or M-63 related adverse findings. The no observed adverse effect level (NOAEL) from the 90-day study was determined to be at least 7.6×10^{10} CFU/kg bw/day (Abe et al., 2009).

- Three published studies of *B. infantis* M-63 in microbial mixtures in children, two published studies of *B. infantis* M-63 in mixtures or alone in adults, and three published studies of *B. infantis* M-63 in microbial mixtures with and without oligosaccharides in infants support the safety of *B. infantis* M-63. No adverse events were reported in any study. These studies support the safe use of *B. infantis* M-63 in children at doses up to 1.0×10^9 CFU/day for 8 weeks and adults at doses up to 1.25×10^{10} CFU/day for 12 weeks. Additionally, these studies support the safe use of *B. infantis* M-63 in infants up to 1.4×10^8 CFU/100 mL for 6 months or 5×10^8 CFU/day for up to 6 weeks.

- A literature search revealed six published studies of other *B. longum* subsp. *infantis* strains that corroborate the safety of *B. infantis* M-63. These studies administered doses of 1×10^7-2.8×10^{10} CFU/day to term and preterm infants ages for 1 day to 12 months. The study durations ranged from 3 to 12 weeks. No study reported adverse events related to *B. longum* subsp. *infantis* intake.

- *B. infantis* M-63 will be added to select general foods at levels sufficient to provide 1.25×10^{10} CFU/serving at the end of shelf life. This will result in a mean estimated daily intake (EDI) for consumers age 2+ of 1.45×10^{10} CFU/day (2.16×10^8 CFU/kg bw/day) and a 90th percentile intake of 2.68×10^{10} CFU/day (4.01×10^8 CFU/kg bw/day).

- *B. infantis* M-63 will also be added to powdered, term infant formula at levels sufficient to provide 1 x 10^8 CFU/g at the end of shelf life. This will result in an EDI of 9.9 x 10^9 CFU/day for a 1-month old infant and 1.35 x 10^{10} CFU/day for a 6-month old infant. These intakes are equivalent to 2.3 x 10^9 CFU/kg bw/day and 1.8 x 10^9 CFU/kg bw/day, respectively.

Determination of the GRAS status of *B. infantis* M-63 under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, FACN, FASN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, CNS, and Thomas E. Sox, PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of *B. infantis* M-63 and the potential human exposure to *B. infantis* M-63 resulting from its intended use as an ingredient in foods and non-exempt infant formula and have concluded:

> *There is no evidence in the available information on B. infantis M-63 that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when B. infantis M-63 is used at levels that might reasonably be expected from the proposed applications. B. infantis M-63 is GRAS for use in foods and non-exempt infant formula as proposed by Morinaga Milk Industry Co, Ltd.*

Therefore, *B. infantis* M-63 is GRAS at the proposed levels of use. It is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

Roger Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
School of Pharmacy
University of Southern California

Signature: [redacted]

Date: April 29, 2021

A. Wallace Hayes PhD, DATS, FACT, FACN
GRAS Expert Panel Member
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Thomas E. Sox, PhD, JD
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Signature: [redacted]

Date: April 29, 2021

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January 14, 2022

Ellen Anderson, Ph.D.
Regulatory Review Scientist
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RE: Questions Regarding GRN 001003

Dear Dr. Anderson:

Below are our responses to your requests for additional information regarding GRN 001003 as stated in your email on December 9, 2021. Your requests are in italicized text and our responses are below in plain text:

1. *For the administrative record, please describe whether B. infantis M-63 produces antibiotics.*

   A literature search of Pubmed was conducted on December 22, 2021 with the search terms "Bifidobacterium infantis AND antibiotic" and did not reveal any reports of antibiotic production by *B. infantis*. Moreover, whole genome sequencing of *B. infantis* M-63 did not revealed any open-reading frames that encode genes for antibiotics. Therefore, *B. infantis* M-63 does not produce antibiotics.

2. *For the administrative record, please state if B. infantis M-63 is non-pathogenic and non-toxigenic.*

   B. infantis M-63 is non-pathogenic and non-toxigenic.

3. *Please state whether all analytical methods used to analyze the batches for conformance with the stated specifications have been validated for that particular purpose.*

   All analytical methods used to qualify each batch with the product specifications have been validated for the particular purpose.

4. *The notifier provides a specification for Staphylococcus aureus, listed as negative by test in 0.01 grams. The method referenced is ISP 6888-1. We note that this method is based on the analysis of a 0.1 mL test portion. Please clarify that the analytical method used to detect Staphylococcus aureus has been validated for the stated sample size.*

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Morinaga uses ISO 6888-1 for the quantitation of *S. aureus* as it is intended to be used and has set a specification based on the dilutions of the product and volumes specified in ISO 6887-1 and ISO 6888-1. Specifically, Section 8 of ISO 6888-1 states, "Prepare the test sample from the laboratory sample in accordance with the specific International Standard dealing with the product concerned: follow the procedures specified in the ISO 6887 series." According to section 3.6 of ISO 6887-1, an initial suspension is "obtained after a weighed or measured quantity of the product under examination (or of a test sample prepared from the product) has been mixed with, normally, a nine-fold quantity of diluent, allowing large particles, if present, to settle." Section 9.2 of ISO 6888-1 then states, "Transfer, by means of a sterile pipette (6.7), 0,1 ml of the test sample if liquid, or 0,1 ml of the initial suspension (10^{-1} dilution) in the case of other products, to a Baird-Parker agar (BPA) plate (see B.2)." Therefore, the 0.1 ml of the initial suspension that is added to the BPA plate represents 0.01 gram of the product and the method used and specification established by Morinaga are fit-for use and comply with ISO 6888-1.

5. *The notifier provides a specification for Cronobacter sakazakii, listed as negative by test in 25 grams. The method referenced is ISO 22964. We note that this method is based on the analysis of a 10-gram test portion. Please clarify the analytical method used to detect Cronobacter sakazakii has been validated for that particular purpose.*

   The analytical method used to detect *Cronobacter sakazakii* has been validated and is fit-for-purpose.

6. *We understand that the unmodified corn starch is food grade and used in accordance with current good manufacturing practices as a carrier. We are also aware that corn starch can be used as a thickener. Please clarify that the technical effect of cornstarch is not as a thickener.*

   The unmodified corn starch is being used as a carrier, not as a thickener.

7. *The notifier presents "final product specifications" for use in general foods and infant formula in Tables 6-8. For heavy metals, the limit of quantitation is given as 0.04 ppm, while the results of batch analyses mirror the specifications (e.g., specification <1 ppm for arsenic, batch analyses <1 ppm for arsenic). We request that, for both forms of the ingredient, the notifier provides results of batch analyses for heavy metals rather than a "< (insert specification)" value that is above the limit of quantification. Additionally, we would expect the specifications for heavy metals to be as low as possible. Please consider revising the specifications for heavy metals to be consistent with the results of the batch analyses.*

   The measured values for the arsenic, lead, mercury, and cadmium levels in *Bifidobacterium infantis* M-63 and M-63 type-C are provided in Tables 1 and 2, respectively. The limits of detection for arsenic, lead, mercury, and cadmium are 0.04 ppm. In *B. infantis* M-63, the arsenic levels ranged from the limit of detection to 0.04 ppm whereas lead, mercury, and cadmium were not detected. In *B. infantis* M-63 type-C, arsenic, lead, mercury, and cadmium were not detected.

Table 1. Heavy Metal Residues in *Bifidobacterium infantis* M-63					
Parameter	**Specification**	**Method**	**Lot Number**		
			2019.06.19	**2019.06.24**	**2019.07.01**
Arsenic	< 1 ppm	ICP-MS	0.04	< 0.04*	< 0.04
Lead	< 0.5 ppm	ICP-MS	< 0.04*	< 0.04	< 0.04
Mercury	< 1 ppm	ICP-MS	< 0.04*	< 0.04	< 0.04
Cadmium	< 1 ppm	ICP-MS	< 0.04*	< 0.04	< 0.04
*Limit of quantitation.					

Table 2. Heavy Metal Residues in *Bifidobacterium infantis* M-63 type-C					
Parameter	**Specification**	**Method**	**Lot Number**		
			2018.09.08	**2019.06.19**	**2019.06.24**
Arsenic	< 1 ppm	ICP-MS	< 0.04*	< 0.04	< 0.04
Lead	< 0.5 ppm	ICP-MS	< 0.04*	< 0.04	< 0.04
Mercury	< 0.5 ppm	ICP-MS	< 0.04*	< 0.04	< 0.04
Cadmium	< 0.1 ppm	ICP-MS	< 0.04*	< 0.04	< 0.04
*Limit of quantitation.					

8. *On p. 25 in Table 14, the notifier provides a list of the intended use levels for B. infantis M-63 in a variety of foods, including "weaning foods", in per serving amounts. Please clarify the serving amounts used in the dietary exposure estimates. Please confirm that the Reference Amounts Customarily Consumed (RACC) that are established in 21 CFR 101.12(b) were used. In addition, please clarify for the exposure estimates when converting use levels in colony forming units (CFU) per serving to CFU per 100 g, which foods were assigned serving sizes based on the RACCs for young children ages 1-3 years of age and which foods were assigned the RACCs for ages 4 years and above.*

The serving sizes used to calculate the Estimated Daily Intakes (EDIs) of *B. infantis* M-63 correspond to the reference amounts customarily consumed (RACC) per eating occasion specified in 21 CFR 101.12. The food categories and their corresponding RACCs are shown below in a revised version of Table 14 (see below). When RACCs are listed in imperial measurement units in 21 CFR 101.12 (e.g. tablespoons and cups), a conversion factor of 15 g/1 tablespoon was assumed for the exposure estimates. Any RACC that was provided in milliliters was converted to grams, assuming 1 mL is equal to 1 g. The EDIs were calculated by converting colony forming units (CFU)/serving to CFU/g using the above assumptions and RACCs.

The EDIs provided in the GRAS Notice were calculated using only the RACCs for ages 4 years and above for all food categories and did not use the RACCs specific for the foods intended for infants and young children 1 through 3 years of age, as described in 21 CFR 101.12 (a)(2), for the "weaning foods" described in Table 14 of the GRAS Notice. The

EDIs provided in the GRAS Notice also did not include non-exempt infant formula, because those estimates were calculated using a different method. The EDIs have now been recalculated using the RACCs for infants and young children 1 through 3 years of age for the weaning products, the RACCs for ages 4 years and above for all other food products, and infant formula (Table 14). The EDIs for users 0-1 years of age were also stratified into 0-0.5 years-old (0-6 months) and 0.5-1 years-old (7-12 months of age) to address Question 9. The mean and 90th percentile EDIs for all users ages 2 and up are now estimated to be 1.43 x 10^{10}CFU/day and 2.65 x 10^{10} CFU/day, respectively (Table 3). Importantly, the new EDIs are comparable to those provided in the GRAS Notice and do not change Morinaga's conclusion regarding the GRAS status of the use of *B. infantis* M-63 in infant formula and conventional food products.

9. *While the notifier presents a dietary exposure for infants 0-6 months consuming infant formula and, separately, a dietary exposure for infants 0-12 months consuming weaning foods and conventional foods, a dietary exposure for infants 6-12 months consuming both foods and infant formula is not provided (although it is noted that the exposure would not be strictly additive). Please provide a dietary exposure estimate for infants 6-12 months that includes both infant formula and conventional food uses.*

To provide a dietary exposure estimate for infants 6-12 months that includes both infant formula and conventional food uses, the EDIs *B. infantis* M-63 were recalculated using non-exempt infant formula with an inclusion rate of 1.41 x 10^{10} CFU/L (as described in Chapter III, Section D. Estimated Daily Intake), as well as the use levels for the intended conventional foods uses and the corresponding RACCs provided in the revised Table 14. The EDIs for users 0-1 years of age were also stratified into 0-0.5 years-old (0-6 months of age) and 0.5 - 1 year-old (6-12 months of age) to illustrate the dietary exposure of infants consuming both food and infant formula (Table 3). The mean and 90th percentile EDIs for infants 0-0.5 years-old (0-6 months of age) are 4.42 x 10^{9} CFU/day and 1.14 x 10^{10} CFU/day, respectively, whereas the mean and 90th percentile EDIs for infants 0.5 - 1 year-old (6-12 months of age) are 9.49 x 10^{9} CFU/day CFU/day and 1.55 x 10^{10} CFU/day, respectively.

Table 14. Proposed Conventional Food Categories for the Addition of *B. infantis* M63

Food Category	Specific Food	CFU/serving	RACCs* used
Breads/baked goods	Bars; includes meal replacement, high protein, snack bars[1]	1.25×10^{10}	40 g
	Biscuits, croissants, English Muffins, pizza crusts	1.25×10^{10}	55 g
	Bagels	1.25×10^{10}	110 g
	Breads/roll (yeast)	1.25×10^{10}	50 g
	Breakfast pastries; includes Danish	1.25×10^{10}	55 g
	coffee cakes	1.25×10^{10}	55 g
	Cakes, heavyweight (as defined in 21 CFR 101.12)	1.25×10^{10}	125 g
	Cakes, mediumweight (as defined in 21 CFR 101.12)	1.25×10^{10}	80 g
	Cakes, lightweight (as defined in 21 CFR 101.12)	1.25×10^{10}	55 g
	Cobblers, turnovers, strudels, crisps	1.25×10^{10}	125 g
	Cookie bars	1.25×10^{10}	30 g
	Crackers that are usually used as snacks	1.25×10^{10}	30 g
	Crackers that are not usually used as snacks (as defined in 21 CFR 101.12)	1.25×10^{10}	15 g
	Doughnuts	1.25×10^{10}	55 g
	Pies	1.25×10^{10}	125 g
	Quick breads; includes breads, muffins, popovers, cornbread	1.25×10^{10}	55 g
Cereals	Breakfast cereals, cooked; includes grits, oatmeal, cream of wheat, and wheat cereal	1.25×10^{10}	40 g
	Breakfast cereals, ready-to-eat, weighing less than 20 g per cup, e.g., plain puffed cereal grains	1.25×10^{10}	15 g
	Breakfast cereals, ready-to-eat, weighing 20 g or more but less than 43 g per cup; high fiber cereals containing 28 g or more of fiber per 100 g	1.25×10^{10}	40 g
	Breakfast cereals, ready-to-eat, weighing 43 g or more pre cup; biscuit type	1.25×10^{10}	60 g
Fruits	Juices and nectars, including citrus, non-citrus, vegetable and blends, frozen fruit, frozen juice bars, ices	1.25×10^{10}	240 mL (g)[2]
Dairy products/dairy-based foods and dairy substitutes	Skim milk	1.25×10^{10}	240 mL (g)[2]
	Cheese spreads	1.25×10^{10}	30 g
	Cheese, imitation	1.25×10^{10}	30 g
	Cheese, processed	1.25×10^{10}	30 g
	Cream substitutes	1.25×10^{10}	15 g
	Cream, heavy	1.25×10^{10}	15 g
	Fermented milk (flavored, heat treated), including buttermilk, kefir, and flavored milk beverage mixes	1.25×10^{10}	240 mL (g)[2]
	Frozen desserts, including ice cream, ice milk, frozen yogurt, frozen novelties, and imitation milk	1.25×10^{10}	2/3 c (160 g)[3]
	Meal replacements, liquids and dry mixes	1.25×10^{10}	240 mL (g)[2]
	Milk shakes	1.25×10^{10}	240 mL (g)[2]
	Milk (plain and flavored), including cocoa, chocolate milk, fruit milks, coffee drinks (fluid/dry) (coffee 360)	1.25×10^{10}	240 mL (g)[2]
	Puddings and custards	1.25×10^{10}	½ c (120 g)[3]
	Smoothies[4]	1.25×10^{10}	240 mL (g)[2]
	Whipped toppings	1.25×10^{10}	30 g
	Yogurt	1.25×10^{10}	170 g
	Butter and dried milk products[5]	1.25×10^{10}	15 g
	Milk powder for pregnant women, plain and flavored	1.25×10^{10}	240 g

Table 14. Proposed Conventional Food Categories for the Addition of *B. infantis* M63

Food Category	Specific Food	CFU/serving	RACCs* used
	Milk powder for adult people, plain and flavored	1.25×10^{10}	240 g
	Milk powder for elderly people, plain and flavored	1.25×10^{10}	240 g
Miscellaneous	Hard candies[6]	1.25×10^{10}	15 g
	All other candies	1.25×10^{10}	30 g
	Chewing gum	1.25×10^{10}	3 g
	Cocoa powder	1.25×10^{10}	1 Tbsp (15 g)[3]
	All sauces for dipping	1.25×10^{10}	30 g
	Catsup, steak sauce, soy sauce, vinegar, teriyaki marinades	1.25×10^{10}	15 g
	Minor condiments: horseradish, horseradish, hot sauces, mustards, Worcestershire sauce	1.25×10^{10}	5 g
	Gelatin desserts, plain or with fruit gravies	1.25×10^{10}	1/2 c (120 g)[3]
	Peanut and other nut butters/spreads	1.25×10^{10}	2 Tbsp (30 g)[3]
	Snack foods, including chips, popcorn mixtures	1.25×10^{10}	30 g
Weaning foods	Cereals, dry instant	1.25×10^{10}	15 g
	Cereals, prepared, ready-to-serve	1.25×10^{10}	110 g
	Other cereal and grain products, dry ready-to-eat, e.g., ready-to-eat cereals, cookies, teething biscuits, and toasts	1.25×10^{10}	7 g
	Dinners, desserts, fruits, vegetables or soups, ready-to-serve, junior type	1.25×10^{10}	110 g
	Dinners, desserts, fruits, vegetables or soups, ready-to-serve, strained type	1.25×10^{10}	110 g
	Dinners, stews or soups for young children, ready-to-serve	1.25×10^{10}	170 g
	Fruits for young children, ready-to-serve	1.25×10^{10}	125 g
	Vegetables for young children, ready-to-serve	1.25×10^{10}	70 g
	Juices all varieties	1.25×10^{10}	120 mL (g)[2]

*RACC: Reference amounts customarily consumed, as described in 21 CFR 101.12.
[1]"Grain-based bars with or without filling or coating, e.g., breakfast bars, granola bars, rice cereal bars" was chosen as a surrogate category to estimate the RACC for "Bars, includes meal replacement, high protein, snack bars".
[2]When RACC is described in mL, the RACC was converted to grams, assuming 1 mL is equal to 1 gram.
[3]When the RACC is provided in imperial measurements (cups and tablespoons), the value was converted to metric grams assuming 1 tablespoon is equal to 14.79 mL, or approximately 15 g (As described in the National Institute of Standards and Technology (NIST) Guide for the use of the International System of Units https://www.nist.gov/pml/special-publication-811).
[4]"Shakes or shake substitutes, e.g. dairy shake mixes, fruit frost mixes" was chosen as a surrogate category to estimate the RACC for "Smoothies".
[5]"Butter, margarine, oil, shortening" was chosen as a surrogate category to estimate the RACC for "butter and dried milk products".
[6]"Hard candies, others" was chosen as a surrogate category to estimate the RACC for "Hard candies." This RACC is for all hard candies except breath mints, or roll-type, mini-size hard candies.

Table 3. Estimated "All-user" Daily Intake (EDI) of *B. infantis* M63 in Targeted Foods by Population Group (2015-2016 NHANES Data) Calculated with Corrected RACCs and Non-Exempt Infant Formula Uses Added

Population Group	N users	N population	% Users	Mean mass (kg)	Mean EDI (CFU)	90th % EDI (CFU)	Mean EDI (CFU/kg)	90th % EDI (CFU/kg)
ages 0-0.5 years	304	396	76.77	7.76	4.42E+09	1.14E+10	5.70E+08	1.47E+09
ages 0.5-1 year	223	293	76.11	11.22	9.49E+09	1.55E+10	8.46E+08	1.38E+09
ages 1-2 years	368	487	75.56	14.49	9.89E+09	1.71E+10	6.82E+08	1.18E+09
ages 2-5 years	467	643	72.63	21.50	1.07E+10	1.79E+10	4.97E+08	8.34E+08
ages 6-12 years	1170	1473	79.43	40.94	1.31E+10	2.35E+10	3.19E+08	5.75E+08
ages 13-19 years	840	960	87.50	69.01	1.57E+10	2.71E+10	2.27E+08	3.93E+08
ages 20 years and up	4814	5665	84.98	79.97	1.48E+10	2.77E+10	1.85E+08	3.46E+08
ages 2 years and up	7291	9020	80.83	66.96	1.42E+10	2.62E+10	2.12E+08	3.91E+08

Should you need any additional information, please feel free to contact me at 240-367-6089 or dconze@spherixgroup.com.

Sincerely,

Dietrich B. Conze, Ph.D.
Managing Partner

From: Dietrich Conze
To: Anderson, Ellen
Cc: Kathy Brailer; Claire Kruger
Subject: [EXTERNAL] Re: GRN 001003 - clarification request
Date: Monday, February 28, 2022 10:15:29 AM

Hi Ellen.

Kathy just alerted me to a typo in my email. To clarify, the resuspension medium does not contain major food allergens and its ingredients are not derived from major food allergens. If you have any additional questions, please let us know.

Regards.
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

> On Feb 28, 2022, at 9:57 AM, Dietrich Conze <dconze@spherixgroup.com> wrote:
>
> Hi Ellen,
>
> Thanks for your request. The resuspension medium does not major food allergens and its ingredients are not derived from major food allergens.
>
> Regards.
> Dietz
>
> Dietrich Conze, PhD
> Managing Partner
> Spherix Consulting Group
> 751 Rockville Pike, Unit 30-B
> Rockville, MD 20852
>
> Tel: 240-367-6089
> Fax: 301-230-2188
> dconze@spherixgroup.com

From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Friday, February 25, 2022 1:35 PM
To: ckruger@spherixgroup.com
Subject: GRN 001003 - clarification request

Dear Dr. Kruger,

We are finishing up our review of GRN 001003 and have an additional request for information.

We note that on page 12 the notice states, "Media ingredients are nutritional substances necessary for fermentation, do not contain major food allergens nor are they derived from major food allergens, and are safe and suitable for human consumption. *B. infantis* M-63 is thoroughly washed during the non-culturing process to minimize carry-over of the fermentation medium to the finished ingredient. Resuspension medium is not washed from the final product; all components comply with their respective sections within 21 CFR and/or Food Chemicals Codex (FCC)."

Please clarify if the resuspension medium contains any major food allergens or is derived from major food allergens.

Thank you in advance for the clarification.

Sincerely,
Ellen

Ellen Anderson
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

Pronouns: she/her/hers

<image001.png>

From: Dietrich Conze
To: Anderson, Ellen
Cc: Claire Kruger; Kathy Brailer
Subject: [EXTERNAL] Re: request for clarification - GRN 001003
Date: Tuesday, March 22, 2022 10:13:03 AM

Hi Ellen,
Sorry for the delay. Your questions are in italicized text and our responses are in plain text below.

1. Does the resuspension medium contain buffer and cryoprotectant or any other ingredients?

The resuspension medium, which has no technical function other than to ensure the viability of the bifidobacterium, is composed of carbohydrates, amino acids, phosphate, and a vitamin, all of which comply with the specifications listed in the FCC monographs for each ingredient.

2. We sent several questions to the notifier in a letter dated December 9, 2021. It recently came to our attention that question #9 contained a typographical error. In question #9, we noted that the notifier presented a dietary exposure estimate for infants aged 0-6 months consuming infant formula, and we asked the notifier to provide a dietary exposure estimate for infants aged 6-12 months that included infant formula and conventional food uses. The age group "6-12 months" was intended to be stated as "7-12 months" in question #9. The notifier responded to question #9 in a letter dated January 14, 2022 (attached for your reference) by providing dietary exposure estimates for "0-0.5 years-old (0-6 months of age) and 0.5 - 1 year-old (6-12 months of age)." We believe the notifier intended to state the older age group as "0.5 – 1 year-old (7-12 months of age)" rather than "0.5 – 1 year-old (6-12 months of age)." Please state whether you concur that the older age group is intended to be "0.5 – 1 year-old (7-12 months of age)."

We concur that the older age group is intended to be 0.5 - 1 year-old (7-12 months of age).

If you have an additional questions, please let us know.
Regards.
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Monday, March 21, 2022 5:20 PM
To: Claire Kruger <ckruger@spherixgroup.com>
Subject: FW: request for clarification - GRN 001003

Hello Dr. Kruger,

I just wanted to touch base with you again about our 3/10/2022 request for clarification (see below) in case the email was inadvertently overlooked. We would appreciate a response at your earliest convenience.

Sincerely,
Ellen

Ellen Anderson
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

Pronouns: she/her/hers

From: Anderson, Ellen
Sent: Thursday, March 10, 2022 10:13 AM
To: Claire Kruger <ckruger@spherixgroup.com>
Subject: request for clarification - GRN 001003

Good morning Dr. Kruger,

We have just about completed our review of GRN 001003, but request two additional pieces of information for clarification.

1. Does the resuspension medium contain buffer and cryoprotectant or any other ingredients?

2. We sent several questions to the notifier in a letter dated December 9, 2021. It recently came to our attention that question #9 contained a typographical error. In question #9, we noted that the notifier presented a dietary exposure estimate for infants aged 0-6 months consuming infant formula, and we asked the notifier to provide a dietary exposure estimate for infants aged 6-12 months that included infant formula and conventional food uses. The age group "6-12 months" was intended to be stated as "7-12 months" in question #9. The notifier responded to question #9 in a letter dated January 14, 2022 (attached for your reference) by providing

dietary exposure estimates for "0-0.5 years-old (0-6 months of age) and 0.5 - 1 year-old (6-12 months of age)." We believe the notifier intended to state the older age group as "0.5 – 1 year-old (7-12 months of age)" rather than "0.5 – 1 year-old (6-12 months of age)." Please state whether you concur that the older age group is intended to be "0.5 – 1 year-old (7-12 months of age)."

Thank you for providing this additional information.

Sincerely,
Ellen

Ellen Anderson
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

Pronouns: she/her/hers

<Morinaga Response to FDA on GRN1003 1-14-22.pdf>