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Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

5/9/2022

RE: GRAS Notification of ***Bifidobacterium infantis*** **CBT BT1**
II964.1-CBI.1.4

To Whom It Concerns,

In accordance with 21 CFR, Part 170, Subpart E, we as the agent [REJIMUS, INC., 600 W. Santa Ana Blvd. Ste 1100, Santa Ana, CA 92701], respectfully provides notice of a claim that the addition of the microorganism ***Bifidobacterium infantis*** **CBT BT1** to the foods identified in this notice at the specified levels is exempt from the premarket approval requirement of the Federal Food, Drug and Cosmetic Act because the notifier [**Cell Biotech Co. Ltd.**, 50, Agibong-ro, 409 Beon-gil, Wolgot-myeon, Gimpo, Republic of Korea] has determined that the intended uses are generally recognized as safe (GRAS). The attached documents contain the specific information and data that address the safety of the substance for use in human food applications.

Respectfully,

Jim Lassiter, COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

PART 1 – SIGNED STATEMENTS AND CERTIFICATION

Cell Biotech Co. Ltd. submits this notification of a conclusion of GRAS through its agent, REJIMUS, INC. in accordance with 21 CFR §170.30.

Name and Address of Notifier and Agent

Agent:

Jim Lassiter
President/COO
REJIMUS, INC.
600 W. Santa Ana Blvd., Suite 1100
Santa Ana, CA 92701
Tel: +1 (949) 485-2112
www.rejimus.com

Notifier:

Cell Biotech Co. Ltd.
50, Agibong-ro, 409 Beon-gil
Wolgot-myeon, Gimpo
Republic of Korea
Tel: +82 31 987 6205

Name and Address of Manufacturer:

Cell Biotech Co. Ltd.
397 Aegibong-rol
Wolgot-myeon, Gimpo-si, Gyeonggi-do 415-872
Republic of Korea
Tel: +82 31 987 8107

Name of the GRAS Substance

Cell Biotech Co. Ltd. (herein referred to as CBI) has undertaken an independent safety evaluation of the substance in this notification:

***Bifidobacterium infantis* CBT BT1**

Intended Conditions of Use and Levels of Inclusion

The intended use of *Bifidobacterium infantis* CBT BT1 is a food ingredient for inclusion in dairy products where standards of identity do not preclude such use. The intended addition level to these foods is up to 1×10^{11} CFU per serving.

Bifidobacterium infantis CBT BT1 will not be added to meat and poultry products (including soups and soup mixes containing meat or poultry), and will not be included in foods that are marketed towards infants and young children, inclusive of infant formula. *Bifidobacterium infantis* CBT BT1 is not intended for addition to standardized foods unless it is permitted by the applicable standard of identity.

Basis for GRAS Conclusion

The statutory basis for conclusion of GRAS status is through scientific procedures in accordance with 21 CFR §170.30(a) and (b).

Premarket Approval Exemption

We have concluded that the intended use of *Bifidobacterium infantis* CBT BT1 is GRAS for its intended conditions of use as stated in this notification and, such use of *Bifidobacterium infantis* CBT BT1 is not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act.*

Availability of Information

The data and information that serve as the basis of GRAS conclusion are available for review and copying at reasonable times at the offices of the Agent.

Should FDA have any questions of additional requests for information regarding this notification, the Agent shall provide further clarification and/or information at:

Attn: Jim Lassiter
REJIMUS, INC.
600 W. Santa Ana Blvd., Suite 1100
Santa Ana, CA 92701
Email: jim@rejimus.com

Trade Secrets

The notification does not contain trade secrets and the data are not exempt from disclosure under the Freedom of Information Act, 5 U.S.C. Part 552.

Authorization for FDA to share information with FSIS

As Agent for the Notifier, we authorize FDA to send any information deemed necessary to FSIS. The notice does not contain trade secrets and the data are not exempt from disclosure under the *Freedom of Information Act*, 5 U.S.C. 552.

Certification

Cell Biotech Co. Ltd. has concluded that *Bifidobacterium infantis* CBT BT1 is generally recognized as safe for use in dairy products based on scientific procedures and supported by a history of use in accordance with 21 CFR Part 170, Subpart E. As their Agent, REJIMUS, INC. takes responsibility for all communications on this matter. To the best of our knowledge, this GRAS Notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to

us and pertinent to the evaluation of the safety and GRAS status of the use of *Bifidobacterium infantis* CBT BT1.

Respectfully submitted,

Jim Lassiter, COO
REJIMUS, INC.
jim@rejimus.com

PART 2 – IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

Common Name: *Bifidobacterium infantis* CBT BT1 (KCTC 11859BP)

Taxonomic Lineage (Accessed from the Integrated Taxonomic Information System [http:www.itis.gov]):

Kingdom: Bacteria
Subkingdom: Posibacteria
Phylum: Actinobacteria
Class: Actinobacteridae
Order: Bifidobacteriales
Family: Bifidobacteriaceae
Genus: *Bifidobacterium*
Species: *infantis*
Strain: CBT BT1

Previously designated *Bifidobacterium longum* subsp. *infantis, Bifidobacterium infantis* is a species of the genus *Bifidobacterium* and the class Actinobacteridae that can be isolated from human feces (Ventura et al. 2007). *Bifidobacterium* spp. are gram-positive, non-motile, non-spore forming, anaerobic rods with variable appearance (Candela et al. 2007). The gram staining morphology of *Bifidobacterium* can vary as long, slender rods, in clusters, pairs or even independently. *Bifidobacterium* are studied as other Lactic Acid Bacteria (LAB) since they are found predominantly in the gastric and intestinal mucosa. Nursing newborns may have a bifidobacteria population of more than 95% with this population decreasing as humans age (Toure et al. 2003). It is estimated that, on average, approximately 4% of the bacterial population of the adult human colon are bifidobacteria (Turroni et al. 2014).

Identification

The organism that is the subject of notified substance, originally isolated from human feces or fermented food is identified as *Bifidobacterium infantis* and has been uniquely characterized as a distinct strain known as CBT BT1 by means of genomic typing. The strain was deposited in the Korean Collection for Type Cultures (KCTC), accession number KCTC 11859BP.

Carbohydrate Utilization

Fermentative characteristics of *Bifidobacterium infantis* CBT BT1 were analyzed using API 50 CHL kit. Results are shown in Table 1.

Table 1. Fermentative Characteristics of *Bifidobacterium infantis* CBT BT1 obtained with an API 50 CHL Kit. (Cellbiotech R&D Center (2018))

No	Carbohydrates	Utilized	No	Carbohydrates	Utilized
0	Control	-	25	Esculine	-
1	Glycerol	-	26	Salicine	-
2	Erythritol	-	27	Cellobiose	-
3	D-Arabinose	-	28	Maltose	+
4	L-Arabinose	-	29	Lactose	+
5	Ribose	+	30	Melibiose	+
6	D-Xylose	-	31	Saccharose	+
7	L-Xylose	-	32	Trehalose	-
8	Adonitol	-	33	Inuline	-
9	β-Methyl-xyloside	-	34	Melezitose	-
10	Galactose	-	35	D-Raffinose	+
11	D-Glucose	+	36	Amidon	-
12	D-Fructose	-	37	Glycogene	-
13	D-Mannose	-	38	Xylitol	-
14	L-Sorbose	-	39	β-Gentiobiose	+
15	Rhamnose	-	40	D-Turanose	-
16	Dulcitol	-	41	D-Lyxose	-
17	Inositol	-	42	D-Tagatose	-
18	Mannitol	+	43	D-Fucose	-
19	Sorbitol	-	44	L-Fucose	-
20	α-Methyl-D-mannoside	-	45	D-Arabitol	-
21	α-Methyl-D-glucoside	-	46	L-Arabitol	-
22	N-Acetyl glucosamine	-	47	Gluconate	-
23	Amygdaline	+	48	2-Ceto-gluconate	-
24	Arbutine	-	49	5-Ceto-gluconate	-

Genomic Classification, Sequence, and Profile

The 16S rRNA gene sequence were aligned and compared with different *Bifidobacterium* strains: *B. infantis* (KCTC 11859BP), *B. infantis* (ATCC 15697), *B. longum* (ATCC 15707), *B. bifidum* (DSM 20456), *B. breve* (ATCC 15700), *B. lactis* (DSM 10140), and *B. catenulatum* (KCTC 3221). Percent identity and divergence were compared between *Bifidobacterium* species and strains in Table 2. As presented in Table 2, distinctive sequences of 16S rRNA genes were used to generate the phylogenic tree shown in Figure 1 (Cellbiotech R&D Center 2018).

Random Amplified Polymorphic DNA (RAPD) is a method used to obtain a molecular "fingerprint" from random DNA segments of genomic DNA that have been amplified using a single primer of an arbitrary nucleotide sequence. *Bifidobacterium infantis* CBT BT1 DNA was compared using RAPD with *Bifidobacterium infantis* ATCC 15697 strain. Both strains were amplified through PCR, ribotyping and pulsed-field gel electrophoresis (PFGE) in order to compare the RAPD patterns and genotypes between both species (Figure 2). Fragment yields presented difference between strains. DNA fragments were amplified with (GTG) primer (5' – GTGGTGGTGGTGGTG – 3') using genomic DNA as a template and analyzed in 0.8% agarose gel (Syngene, UK).

Pulse Field Gel Electrophoresis (PFGE) digests the genomic DNA with rare-cutting restriction enzymes. Separation of the macrofragments occurs via a continuously reorienting electric field. ***Bifidobacterium infantis* CBT BT1** (KCTC 11859BP) and *B. infantis* (ATCC 15697) strains were cultivated to OD_{600}=4 and treated with proteinase K and multiple restriction enzymes. DNA fragments from digestion were analyzed on agarose gel.

Table 2. Percent identity of *Bifidobacterium infantis* CBT BT1 with some closely related species and other closely related species based on 16S rRNA gene sequences. (Cellbiotech R&D Center 2018).

Percent Identity

Divergence

	1	2	3	4	5	6	7
1		99.5	99.2	93.9	96.4	90.3	93.2
2	0.2		98.9	93.4	96.8	90.8	93.0
3	0.8	0.9		93.4	95.8	90.6	93.7
4	4.5	4.6	5.1		92.9	89.3	94.1
5	2.4	2.3	3.1	4.6		91.4	93.1
6	7.2	6.9	7.0	7.6	6.3		90.9
7	5.2	5.2	5.0	4.9	5.1	6.7	

1 ***B. infantis* (KCTC 11859BP)**
2 *B. infantis* ATCC 15697
3 *B. longum* ATCC 15707
4 *B. bifidum* DSM 20456
5 *B. lactis* DSM 10140
6 *B. cantenulatum* KCTC 3221

Figure 1. Phylogenetic association between *Bifidobacterium infantis* CBT BT1 and closely related species based on 16S rRNA gene sequence. (Cellbiotech R&D Center 2018).

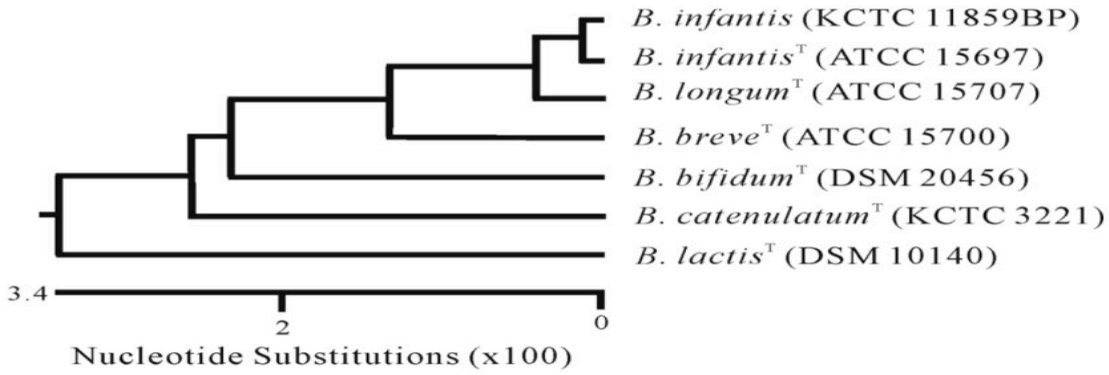
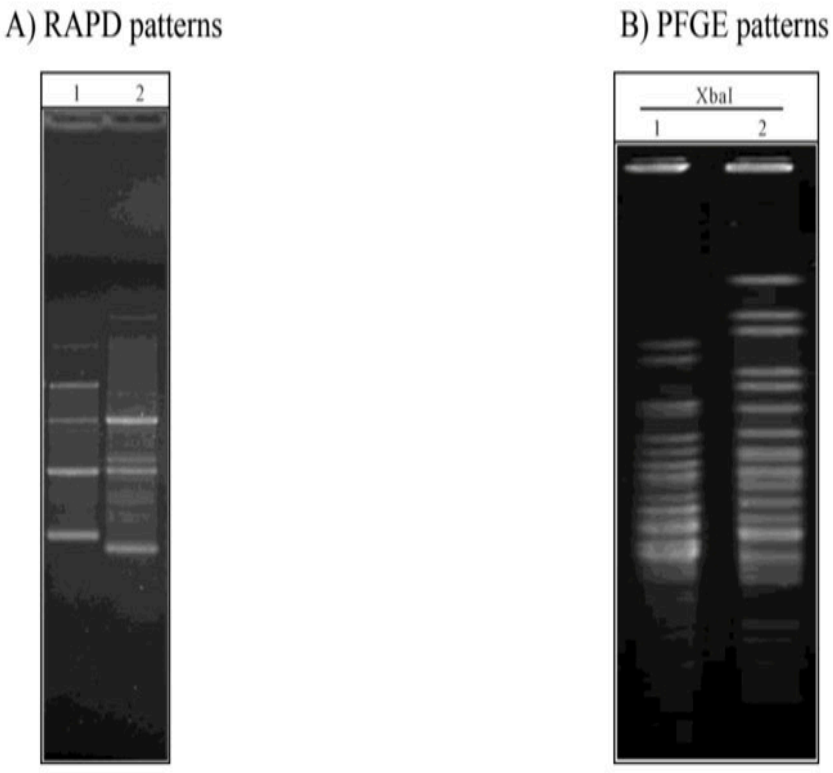


Figure 2. RAPD and PFGE results between *Bifidobacterium infantis* ATCC 15697- Lane 1 and *Bifidobacterium infantis* CBT BT1 (KCTC 11859BP).



Manufacturing

Components

All components employed in the manufacture of *Bifidobacterium infantis* CBT BT1 are suitably used for one or more effects described within FDA's Substances Added to Food Inventory as identified in Table 3.

Table 3. Identification of the ingredients used in the manufacturing process.

Fermentation Medium Ingredient	CAS No.	Reference
Dextrose Monohydrate	[77938-63-7]	21 CFR §168.111
Fructose	[57-48-7]	21 CFR §184.1866
Soy Peptone	[73049-73-7]	21 CFR §184.1553
Soy Protein Isolate	[977076-84-8]	21 CFR §184.1553
Yeast Extract Powder	[8013-01-1]	21 CFR §184.1983
Potassium Phosphate, Dibasic	[7758-11-4]	21 CFR §182.6285
Sodium acetate	[977127-84-6]	21 CFR §184.1721
Potassium Citrate	[6100-05-6]	21 CFR §184.1625
Calcium Chloride	[10043-52-4]	21 CFR §184.1193
Magnesium Sulfate	[10034-99-8]	21 CFR §184.1443
Manganese Sulfate	[15244-36-7]	21 CFR §182.5461
L-Cysteine Monohydrate	[7048-04-6]	21 CFR §184.1272
L-Ascorbic acid	[50-81-7]	21 CFR §182.8013
Monosodium L-Glutamate	[6106-04-3]	21 CFR §182.1
Polysorbate 80	[9005-65-6]	21 CFR §178.3400
Sodium Chloride	[7647-14-5]	21 CFR §182.1
Coating Ingredient	**CAS No.**	**Reference**
Trehalose	[6138-23-4]	FEMA No. 4600 (FEMA GRAS Publication No. 24)
Potassium Phosphate, Dibasic	[7758-11-4]	21 CFR §182.6285
Potassium Phosphate, Monobasic	[7778-7-0]	21 CFR §175.105
Xanthan Gum	[11138-66-2]	21 CFR §172.695
Cornstarch	[977050-21-3]	21 CFR §182.70 / 21 CFR §182.90
Sodium Carboxymethylcellulose	[9004-32-4]	21 CFR §182.1745

Fermentation Medium Ingredient	CAS No.	Reference
Sodium Chloride	[7647-14-5]	21 CFR §182.1
Excipient	**CAS No.**	**Reference**
Cornstarch	[977050-21-3]	21 CFR §182.70 / 21 CFR §182.90

Process Description and Flow Chart

The flowchart for the manufacturing process through packaging is shown at Figure 3.

Preparation of culture medium
All fermentation medium ingredients are blended together. The mixture is then sterilized with saturated steam.

Cultivation
Stock organism is prepared and tested for microbiological contaminants. The stock organism is then inoculated into the prepared medium where it is allowed to propagate. During fermentation, the process is monitored by testing for pH and for change in optical density approximately every two hours. Once the endpoint is reached, bacterial morphology is inspected by microscopy and the organisms are separated via filtration from the culture medium.

Preparation of coating materials
Coating ingredients are added to water, mixed, and sterilized with saturated steam.

Blending
The concentrated organisms, coating mixture, and cornstarch are blended together and then dispensed into trays for freezing.

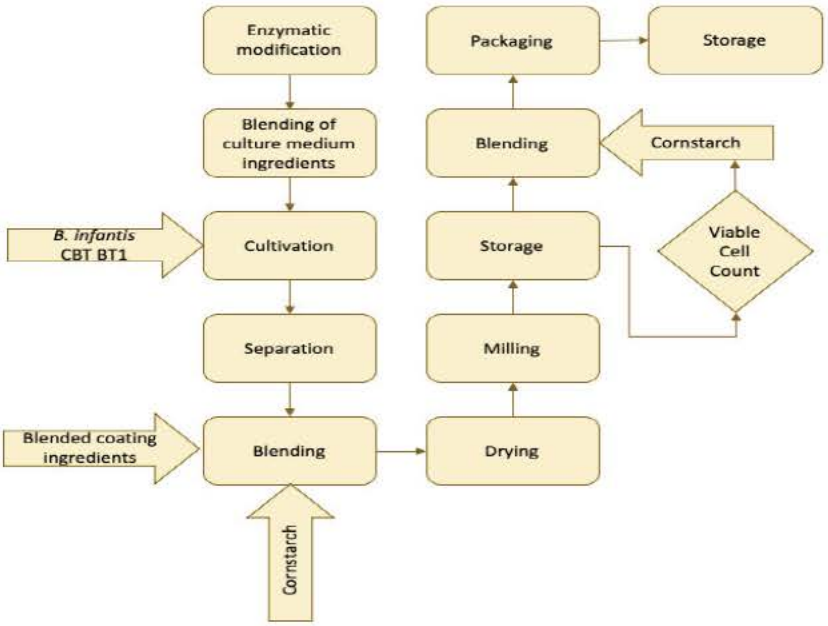
Drying
Trays containing the blended product are initially quick-frozen and then freeze dried.

Milling
Freeze-dried material is removed from the drying trays, milled, placed in polyethylene bags, passed through a metal detector, and stored as semi-finished product.

Standardization
The semi-finished product is tested for viable cell count and blended with a corresponding amount of cornstarch to ensure standardized potency.

Packaging
The standardized product is then packaged, passed through a metal detector again, sampled by QC for testing, and stored in a low -temperature warehouse.

Figure 3. Manufacturing process flow chart.



Specifications

Food grade specifications for *Bifidobacterium infantis* CBT BT1 have been established as shown in Table 4. Test results of three production batches are additionally presented in demonstration of the ability to consistently produce the notified substance in conformance with these specifications. Consistency of conformance to specifications is further evidenced by stability study results.

Table 4. *Bifidobacterium infantis* CBT BT1 food grade specifications and conforming test results.

Parameter	Limits	Method	Batch 08R	Batch 01R	Batch 44Q
Appearance	Light brown powder	Visual	Light brown powder	Light brown powder	Light brown powder
Viable Cell Count	$\geq 1.0 \times 10^{11}$ CFU/g	USP <2022> or equivalent	Conforms	Conforms	Conforms
Coliforms	Absent in 10g	USP <2023> or equivalent	Conforms	Conforms	Conforms

Stability Data

In order to determine the stability of *Bifidobacterium infantis* CBT BT1, the food ingredient was placed in a stability study by Cell Biotech Co. Ltd.

A 12-month stability study was conducted at 5 ± 3 °C using 3 different batches of *Bifidobacterium infantis* CBT BT1. At each time point, samples were analyzed in triplicate using 3 different analysts; the results of viable cell count assays are averaged and summarized in Table 5. Coliform testing was additionally performed by each analyst at all time points, the results of which are negative for all samples. Appearance test was performed by each analyst at all time points, the results of which were of a light brown powder.

Table 5. Viable cell count and percent survival rate of *Bifidobacterium infantis* CBT BT1 at 5 ± 3 °C.

Strain	Batch No.	Test	Time Point				
			Initial	3 Months	6 Months	9 Months	12 Months
***Bifidobacterium infantis* CBT BT1**	44Q	VCC (CFU/g)	2.64×10^{11}	2.39×10^{11}	2.05×10^{11}	1.84×10^{11}	1.69×10^{11}
		Survival Rate (%)	100.0	90.4	77.8	69.6	64.1
	01R	VCC (CFU/g)	3.35×10^{11}	3.04×10^{11}	2.72×10^{11}	2.33×10^{11}	2.04×10^{11}
		Survival Rate (%)	100.0	90.8	81.1	69.5	61.0
	08R	VCC (CFU/g)	4.48×10^{11}	4.19×10^{11}	3.49×10^{11}	3.15×10^{11}	2.85×10^{11}
		Survival Rate (%)	100.0	93.5	77.9	70.2	63.5
	Average Survival Rate (%)		100.0	91.6	78.9	69.8	62.9

Technical Effects

This substance will be used to provide as a dietary source of *Bifidobacterium infantis* CBT BT1 as a food ingredient to dairy products.

PART 3 – DIETARY EXPOSURE

Intended Use and All Sources in the Diet

The intended use of *Bifidobacterium infantis* CBT BT1 is as a food ingredient for inclusion in dairy products to provide at least 1×10^{11} CFU per serving.

The consensus of an international scientific expert panel categorized live microorganisms for human use as defined in Table 6. The panel suggested a minimum level of 1×10^{9} CFU of LAB per serving to be the minimum criteria in support a claim of "contains live and active cultures." (Hill 2014)

Table 6. Categories of live microorganisms for human use (Hill et al. 2014).

Description	Claim	Criteria*	Minimum level of evidence required to make claim	Comments
Not probiotic				
Live or active cultures	"Contains live and active cultures"	Any food fermentation microbe(s) Proof of viability at a minimum level reflective of typical levels seen in fermented foods, suggested to be 1×10^{9} CFU per serving[73]	No product-specific efficacy studies needed	The terms 'live' or 'active' do not imply probiotic activity Fermented foods containing live cultures might also qualify as a 'probiotic' if they meet the criteria for that category (e.g. evidence that yogurt can improve lactose digestion in lactose maldigesters would qualify it as a 'probiotic'[74,75])
Probiotic				
Probiotic in food or supplement without health claim	"Contains probiotics"	A member(s) of a safe[76,77] species, which is supported by sufficient evidence of a general beneficial effect in humans OR a safe microbe(s) with a property (e.g. a structure, activity or end product) for which there is sufficient evidence for a general beneficial effect in humans Proof of viability at the appropriate level used in supporting human studies[73]	Well-conducted human studies (e.g. these could involve RCT(s), observational studies, systematic reviews or meta-analyses supporting the observed general beneficial effect for the taxonomical category concerned) The evidence does not have to be generated for the specific strain included in the product	Extrapolation of evidence must be based on reasonable expectations that the strain(s) incorporated in the product would have similar general beneficial effects in humans This evidence could be based on taxonomical or functional comparisons
Probiotic in food or supplement with a specific health claim	Specific health claim, such as "helps to reinforce the body's natural defences in children" or "helps reduce the risk of antibiotic-associated diarrhoea"	Defined probiotic strain(s) Proof of delivery of viable strain(s) at efficacious dose at end of shelf-life[73]	Convincing evidence needed for specific strain(s) or strain combination in the specified health indication Such evidence includes well-conducted studies in humans, including: positive meta-analyses on specific strain(s) or strain combinations, as per principles outlined by Cochrane,[78] PASSCLAIM,[79] or GRADE;[80] well-conducted RCT(s) OR strong evidence from large observational studies[81]	Well-designed observational studies are useful to detect the effect of foods on health in 'real life', that is, outside the controlled environment of an RCT (e.g. data on health benefits by dietary fibre are mostly observational) Sample sizes must be large enough to manage confounding factors
Probiotic drug	Specific indication for treatment or prevention of disease, such as "useful for the prevention of relapse of ulcerative colitis"	A defined strain(s) of live microbe Proof of delivery of viable probiotic at efficacious dose at end of shelf-life Risk–benefit assessment justifies use	Appropriate trials to meet regulatory standards for drugs	What constitutes a drug claim varies among countries

*Unless otherwise indicated, all criteria indicated must be met. Abbreviations: CFU, colony forming unit; GRADE, Grades of Recommendation Assessment, Development and Evaluation; PASSCLAIM, Process for the Assessment of Scientific Support for Claims on Food; RCT, randomized controlled trial.

Consumption Data

Based on the food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES 2017-2018) dataset compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics, and the Nutrition Coordinating Center, the EDIs of dairy products were determined by several age groups.

The intended use of at least 1.0 x 10^{11} CFU per serving in dairy products would result in intakes in all users of 8.94 x 10^{10} CFU and 1.85 x 10^{11} CFU per person per day in the mean and 90th percentile, respectively (Table 7). A maximum exposure would occur in male adults with a 90th percentile EDI of 2.05 x 10^{11} per person per day.

Table 7. EDIs of *Bifidobacterium infantis* CBT BT1 from proposed uses in dairy products across all users based on 2017-2018 NHANES.

Group	% (n)	Dairy intake g/day		Dairy, serving/day		*Bifidobacterium infantis* CBT BT1, cfu/day	
		Mean	90th percentile	Mean	90th percentile	Mean	90th percentile
Children, 3-11	74.04 (739)	360.44	456.85	0.97	1.87	9.74×10^{10}	1.87×10^{11}
Females, 12-19	42.44 (191)	186.02	362.90	0.76	1.49	7.62×10^{10}	1.49×10^{11}
Males, 12-19	54.73 (243)	265.10	477.28	1.09	1.96	1.09×10^{11}	1.96×10^{11}
Females, 20 and up	38.21(826)	179.05	360.87	0.73	1.48	7.34×10^{10}	1.48×10^{11}
Males, 20 and up	44.06(871)	222.93	499.63	0.91	2.05	9.13×10^{10}	2.05×10^{11}
All users	47.61(3161)	218.16	452.44	0.89	1.85	8.94×10^{10}	1.85×10^{11}

Assuming all servings of the intended dairy products consumed contain *Bifidobacterium infantis* CBT BT1, the suggested three daily servings would result in a cumulative exposure of 2.68 × 10^{11} CFU per day ($8.94\times10^{10}\times3$). The estimated 90th percentile of consumers of dairy products at this level of recommended consumption adjusted for the findings of the per capita data would potentially be exposed to up to 5.55 × 10^{11} CFU per day *Bifidobacterium infantis* CBT BT1. The LD_{50} identified is the uppermost safety point that has been studied to date. The study presented by CBI R&D Center (2018) demonstrated that > 10^{11} CFU/kg was still safe for the rats at that dosage. In point of fact, no true LD_{50} nor NOAEL has ever been determined for this organism. This is due to the fact that an amount of organism greater than this cannot feasibly be administered to the rats.

The LD_{50} of greater than 10^{11} CFU/kg from the animal studies from the Cell Biotech R&D Center corresponds to the human equivalent dose of 9.6 × 10^{11} CFU in a 60 kg human (using the animal-specific body surface area-based conversion factor presented in the Center for Drug Evaluation and Research's Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers 2005). Therefore, even if the general population consumers of dairy products were to meet these guidelines, the recommended levels of the cumulative exposure of 2.68 × 10^{11} CFU

per day and the cumulative exposure at an estimated 90th percentile of 5.55 × 10^{11} CFU per day is less than the LD_{50} levels of greater than 10^{11} CFU/kg (or 9.6 × 10^{11}) of *Bifidobacterium infantis* CBT BT1**.**

Substances Expected to Be Formed in Food

Under the intended conditions of use, there are no substances expected to be formed in the foods in which *Bifidobacterium infantis* CBT BT1 is included. The metabolic by-products from *Bifidobacterium infantis* CBT BT1 do not go beyond the expected fermentation products from any of the other LAB microorganisms. These include lactic acid, carbon dioxide and the ATP necessary for the cell. *Bifidobacterium infantis* CBT BT1 is not known to secrete any exotoxins or any other substances that are classified as harmful to humans. Additionally, the number of viable organisms will decline during a product's shelf life to further minimize the exposure to any of the metabolic by-products.

Substances Naturally Present or Due to Manufacturing

Any remaining ingredients used to produce the fermentation media should have little to no presence in the overall finished output and therefore, the EDIs for these ingredients were not determined or calculated.

The coating ingredients and excipients used in the manufacturing process are listed in FDA's Substances Added to Food Inventory for various uses:

- Trehalose is listed as a flavoring agent or adjuvant.
- Potassium phosphate, dibasic is listed as an emulsifier or emulsifier salt, nutrient supplement, pH control agent, sequestrant, or stabilizer or thickener.
- Potassium phosphate, monobasic is listed as malting or fermenting aid, nutrient supplement, pH control agent, or stabilizer or thickener.
- Xanthan gum is listed as an anticaking agent or free-flow agent, color or coloring adjunct, drying agent, emulsifier or emulsifier salt, formulation aid, processing aid, solvent or vehicle, stabilizer or thickener, surface-finishing agent, or texturizer.
- Cornstarch is listed as an anticaking agent or free-flow agent, drying agent, flavoring agent or adjuvant, formulation aid, humectant, non-nutritive sweetener, nutritive sweetener, solvent or vehicle, stabilizer or thickener, or texturizer.
- Sodium carboxymethylcellulose is listed as an anticaking agent or free-flow agent, drying agent, emulsifier or emulsifier salt, formulation aid, processing aid, humectant, stabilizer or thickener, or texturizer.
- Sodium chloride is listed as an anticaking agent or free-flow agent, antimicrobial agent, color or coloring adjunct, emulsifier or emulsifier salt, firming agent, flavoring agent or adjuvant, formulation aid, nutrient supplement, solvent or vehicle, stabilizer or thickener.

PART 4 – SELF-LIMITING LEVELS OF USE

There is no recognized self-limiting level of use for this organism. Issues of palatability of the substance are not present at the levels of inclusion identified.

PART 5 – EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

As the conclusion of general recognition of safety is through scientific procedures, this Part is not applicable. Information about the current international marketplace availability of products containing *Bifidobacterium infantis* CBT BT1 as an ingredient is discussed as part of the scientific procedures upon which the general recognition of safety is based. Nevertheless, the historical use of foods with *Bifidobacterium infantis* is discussed in Part 6.

PART 6 – NARRATIVE

Introduction

Fermented foods have a long history of consumption in the human population, with some of the earliest records of such in Southeast Asia and Africa (Nout 1992). Prevalence of fermented foods is much higher in some parts of the world outside the U.S., such as in Sudan where it seems the majority of foods are prepared and preserved by fermentation (Dirar 1992).

Used as an inexpensive means throughout the world, lactic acid-producing bacteria (LAB) are one major group of microorganisms used to process milk, meat, and various plant material like vegetables, cereals, and legumes into fermented foods that undergo flavor and nutritive profile changes from their original forms as well as gain the benefit of improved stability (Steinkraus 1992). By preventing the formation of pathogenic and spoilage organisms, fermented foods have an increased shelf life and decreased potential for causing food poisoning (Hesseltine 1981).

In the United States, LAB in general are permitted for use in several standardized foods. A variety of cheeses, whose requirements are found within 21 CFR Part 133—Cheeses and Related Cheese Products, include the use of these and other types of bacterial cultures. LAB are also used in the production of Sour Cream [§131.160], are optional ingredients for use in Bread, Rolls, and Buns [§136.110(c)(10)], and may be used as characterizing microbial organisms or as microbial cultures to produce aroma and flavor in the production of Acidified Milk [§131.111] and Cultured Milk [§131.112].

History of GRAS Notices

There is a history of successfully notified GRAS substances intended for inclusion in foods dating back to 2002 (GRAS No. 49).

GRAS notices of food ingredient substances containing the same species as *Bifidobacterium infantis* CBT BT1 to which FDA has no questions are presented below in Table 8. These GRAS notices reference and address a large body of established scientific procedures evidencing the safe and common use of various strains of *Bifidobacterium infantis* and its subspecies. GRAS notices of *Bifidobacterium* organisms of species other than *infantis* which FDA has no questions are presented below in Table 9.

Table 7. GRAS notices containing *Bifidobacterium infantis* receiving reply from FDA that it had no questions (GRAS Notices Inventory Database).

GRAS No.	Date of Closure	Substance
758	20-Aug-2018	*Lactobacillus helveticus* strain R0052, *Bifidobacterium longum* subsp. *infantis* strain R0033, and *Bifidobacterium bifidum* strain R0071
268	08-Jul-2009	*Bifidobacterium longum* strain BB536

Table 9. GRAS notices of *Bifidobacterium* organisms of species other than *infantis* receiving reply from FDA of no questions (GRAS Notices Inventory Database)

GRAS No.	Date of Closure	Substance
877	26-Dec-2019	*Bifidobacterium longum* BB536
872	9-Dec-2019	*Bifidobacterium animalis* subsp. *lactis* UABla-12
856	09-Dec-2019	*Bifidobacterium animalis* subsp. *lactis* strain BB012
855	05-Feb-2020	*Bifidobacterium animalis* subsp. *lactis* strain R0421
814	25-Jun-2019	*Bifidobacterium bifidum* BGN4
813	21-Jun-2019	*Bifidobacterium bifidum* BORI
455	30-Sep-2013	*Bifidobacterium breve* M-16V
454	27-Sep-2013	*Bifidobacterium breve* M-16V
453	27-Sep-2013	*Bifidobacterium breve* M-16V
445	10-Apr-2013	*Bifidobacterium animalis* subsp. *lactis* strains HN019, Bi-07, BI-04 and B420
377	29-Sep-2011	*Bifidobacterium animalis* subsp. *lactis* strain Bf-6

Approved Use

The status of *Bifidobacterium infantis* in Canada involves the accepted use of the microorganism in food products. Specific claims may be made about these products when the level of use is a minimum of 1×10^9 CFU per serving.

In a December 12th, 2019 update to their Qualified Presumption of Safety list, the European Food Safety Authority confirmed *Bifidobacterium* spp. (including *Bifidobacterium longum*) presence in an inventory of recommended biological agents intentionally added to food or feed based on review of latest applicable literature. *Bifidobacterium infantis* is a subspecies of *Bifidobacterium longum.*

Antibiotic Resistance

Determination of the minimal inhibitory concentration (MIC) of select antibiotics [ampicillin (AMP), gentamycin (GEN), kanamycin (KAN), streptomycin (STM), erythromycin (ERM), clindamycin (CLM), tetracycline (TET), and chloramphenicol (CP)] was performed in accordance with ISO 10932:2010 using *Bifidobacterium infantis* CBT BT1 as the test strain. Observed MIC values for *Bifidobacterium infantis* CBT BT1 were determined to be lower than the cut-off values prescribed by 2012 Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance published by the European Food Safety Authority (EFSA), as shown in Table 10 and therefore the strain is susceptible to AMP, GEN, KAN, STM, ERM, CLM, TET, and CP. Most *Bifidobacterium* species are reported to be resistant to aminoglycosides, because of the lack of a cytochrome-mediated drug transport system and the particular resistance to kanamycin is well known and testing for such in *Bifidobacterium infantis* is not required by EFSA guidance (EFSA 2012).

Table 10. Antibiotic susceptibility of *Bifidobacterium infantis* CBT BT1 (Cellbiotech R&D Center (2018)).

Strain	**Minimum Inhibitory Concentrations (μg/mL) of Antibiotics**								
	AMP	VAN	GEN	KAN	STM	ERM	CLM	TET	CP
***B. infantis* CBT BT1**	<2	<0.5	<8	<128	<16	<0.5	<0.06	<8	<2
EFSA Cut-off Value	2	2	64	NR	128	1	1	8	4

Current Marketplace Availability of *Bifidobacterium infantis* CBT BT1

While the conclusion of general recognition of safety (GRAS) is based upon scientific procedures, there is a history of use of *Bifidobacterium infantis* CBT BT1 in foreign countries and in multiple food products.

In vitro Toxicity Studies

Hemolysis Assay

The Cell Biotech R&D Center tested *Bifidobacterium infantis* CBT BT1 for its hemolytic activity by inoculating microorganism in MRS agar supplemented with 5% horse blood and incubated under anaerobic conditions. The test showed no hemolytic activity.

Animal Studies

The pathogenicity and acute toxicity of ***Bifidobacterium infantis* CBT BT1** were investigated using male and female Sprague-Dawley rats (5 of each sex in each group). The animals were intragastrically

administered either 0.85% saline solution or 1×10^{11} CFU/kg ***Bifidobacterium infantis*** **CBT BT1** and observed for the ensuing 14 days. The net body weight gain, gross pathological findings, feed and water consumption, organ weight, and body temperature were monitored and recorded for two (2) weeks.

This investigation revealed no mortalities or obvious adverse clinical signs in rats administered with the live bacterial cells at the investigated dose level as shown on Table 11. In addition, results indicate no significant differences in net body weight gain (Figure 4), gross pathological findings (Table 12), feed and water consumption (Figure 5), organ weight (Table 13), and body temperature (Table 14) among the different treatment groups and between the treated and control rats.

Table 11. Mortality of male and female rats orally administered with 1×10^{11} CFU/kg *Bifidobacterium infantis* CBT BT1 (Cellbiotech R&D Center (2018))

Sex	Group	Days After Administration														Final Mortality (%)	LD_{50}
		1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Male	CBT BT1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	> 1 x 10^{11} CFU/kg
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Female	CBT BT1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	> 1 x 10^{11} CFU/kg
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Figure 4. Body weight curves for male and female rats given 10^{11} CFU/kg *Bifidobacterium infantis* CBT BT1 and control for 14 days. Values are mean ± SE. (Cellbiotech R&D Center (2018))

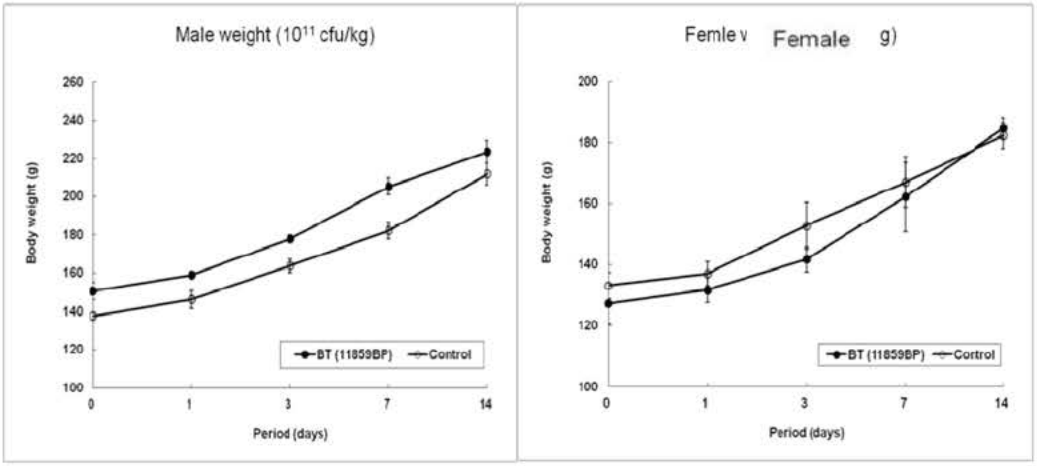


Table 12. Clinical findings of male and female rats orally administered with 10^{11} CFU/kg *Bifidobacterium infantis* CBT BT1 (Cellbiotech R&D Center (2018))

Sex	LAB Strain	Clinical Signs	Hours after treatment				Days after treatment				
			1	2	5	6	1	3	5	7	14
Male	CBT BT1	NAD	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Control	NAD	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Female	CBT BT1	NAD	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Control	NAD	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

NAD: No abnormality detected

Figure 5. Food and water consumption of male and female rats given 10^{11} CFU/kg *Bifidobacterium infantis* CBT BT1 and control for 14 days. (Cellbiotech R&D Center (2018))

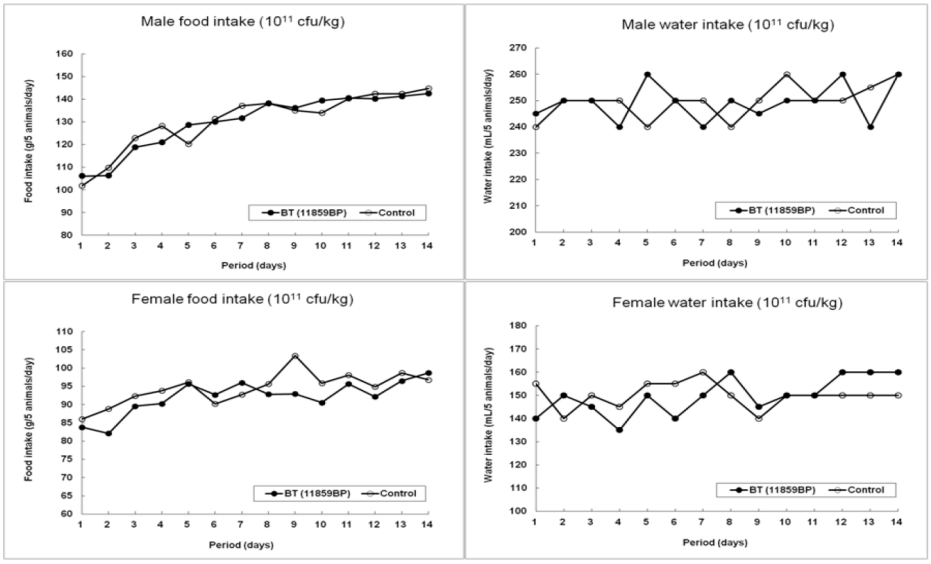


Table 13. Absolute organ weights (g) of male and female orally administered with 10^{11} CFU/kg *Bifidobacterium infantis* CBT BT1 (Cellbiotech R&D Center (2018))

Sex	Parameters	Lab	CBT BT1	Control
		No. of Animals	5	5
Male	Body weight (g)		223.56 ± 5.84	211.90 ± 5.66
	Liver (g)		7.45 ± 0.52	7.20 ± 0.70
	Spleen (g)		0.87 ± 0.05	0.79 ± 0.05
	Kidney (g)	Right	0.88 ± 0.04	0.81 ± 0.09
		Left	0.45 ± 0.07	0.30 ± 0.06
Female	Body weight (g)		184.67 ± 3.65	182.32 ± 4.33
	Liver (g)		5.52 ± 0.92	5.32 ± 0.53
	Spleen (g)		0.62 ± 0.07	0.63 ± 0.05
	Kidney (g)	Right	0.63 ± 0.11	0.66 ± 0.05
		Left	0.37 ± 0.06	0.32 ± 0.04

Table 14. Body temperature changes in male and female orally treated with 10^{11} CFU/kg *Bifidobacterium infantis* CBT BT1 (Cellbiotech R&D Center (2018))

Day	No.	Male body temperature		Female body temperature	
		CBT BT1 (˚C)	Control (˚C)	CBT BT1 (˚C)	Control (˚C)
Pre-treatment	Ave	35.52	34.40	32.20	35.16
	SEM	0.96	0.24	0.81	0.70
Day 1	Ave	35.18	34.70	34.64	35.08
	SEM	0.83	0.92	0.43	0.66
Day 2	Ave	35.08	34.90	35.42	35.12
	SEM	0.46	0.56	0.39	0.83
Day 3	Ave	35.40	35.10	35.20	35.36
	SEM	0.62	0.69	0.25	0.32

Day 4	Ave	35.82	34.10	35.12	35.30
	SEM	0.39	0.60	0.13	0.30

Human Studies

Study 1

Del Giudice et al. (2017) conducted a placebo-controlled, double-blind, randomized clinical trial that investigated the effects of *Bifidobacterium* on allergic symptoms due to pollen allergy. Forty children were treated with either bacterial (oral supplementation containing 1×10^9 CFU of *Bifidobacterium infantis* with *Bifidobacterium longum* and *Bifidobacterium breve*) or placebo daily for 8 weeks. The treatment was well tolerated, significantly reduced nasal symptoms, and improved quality of life. No clinically relevant side effects were noted in either group.

Study 2

Groeger et al. (2013) studied the effects of *Bifidobacterium infantis* on inflammatory biomarker and plasma cytokine levels in 96 patients suffering from ulcerative colitis, chronic fatigue syndrome or psoriasis in three separate randomized, double-blind, placebo-controlled interventions performed over 6-8 weeks. Effects of treatment on immunological biomarkers in 22 healthy subjects were also assessed. Each participant received either 1×10^{10} CFU viable *Bifidobacterium infantis* cells or placebo daily. The results showed an overall reduction in systemic pro-inflammatory biomarkers in all three conditions as well as in the healthy subjects administered the microbial treatment. No adverse events were reported.

Study 3

Smilowitz et al. (2017) conducted a Phase I clinical trial with 80 mother-breast fed infants to determine safety and tolerability of supplementing breast milk with *Bifidobacterium infantis*. Infants were fed breastmilk supplemented with $1.8 - 10^8 – 2.8 \times 10^8$ CFU *Bifidobacterium infantis* daily or breast milk alone for 21 days. The *Bifidobacterium infantis* treated group passed fewer, better formed stools than the control group. There were no differences in the safety and tolerability endpoints between supplemented and non-supplemented infants.

Study 4

Hoyos (1999) *Bifidobacterium infantis* and *Lactobacillus acidophilus* (2.5×10^8 viable cells each) were given as daily doses, until discharge from the hospital, to all 1237 newborns admitted to an intensive care unit over the course of a year. Information from 1282 infants hospitalized during the previous year were used as the study control. The incidence of necrotizing enterocolitis in patients treated with specific microorganisms was reduced to one third compared to the control group. No complications were attributed to the microbial therapy treatment (Hoyos 1999).

Study 5

Whorwell et al. (2006) conducted a randomized, double-blind, placebo-controlled, multicenter, dose-ranging study with 362 female patients suffering from irritable bowel syndrome to confirm the efficacy of treatment with an encapsulated *Bifidobacterium infantis* strain and to determine the optimal dosage. Subjects received once daily treatment with a capsule containing either 1×10^6, 1×10^8, or 1×10^{10} CFU of *B. infantis*, or placebo, for 4 weeks. The median microbial dose was significantly superior to placebo and other treatments in symptom reduction (the high dose suffered from formulation coagulation problems). The authors reported that the treatment was remarkably well tolerated.

Study 6

Bazanella et al. (2017) conducted a randomized, double-blind, placebo-controlled study to determine the first year of life effects of a formula containing *Bifidobacterium* spp. on the healthy infant intestinal microbiome. The treatment group consisted of 48 newborn infants provided with a supplemented formula containing a total of 1×10^8 CFU/g of *Bifidobacterium*, including *Bifidobacterium infantis* CBT BT1 with 3 other *Bifidobacterium* in equal amounts, from birth to 12 months. The supplemented formula was shown to impact the early stage of microbiome development with no detectable long-term consequences.

Study 7

Escribano et al. (2017) conducted a multicenter, double-blind, randomized, placebo-controlled clinical trial. Infants were fed an infant formula supplemented with 1×10^7 CFU/g of *Bifidobacterium infantis* over twelve weeks. Evaluation of the 73 treated infants and 78 controls that completed the follow up led researchers to conclude that the supplemented infant formula was well tolerated, safe, and may reduce the occurrence of diarrhea while lowering the prevalence of constipation.

Study 8

Hod et al. (2017 and 2018) investigated the effects of a microorganism mixture in 107 adult women diagnosed with diarrhea-dominant-IBS (IBS-D). The study was designed as a randomized double-blind, placebo-controlled, parallel-group trial with a 2-week run-in period prior to treatment and a treatment period for 8 weeks. Those subjects in the BIO-25 group were given a BIO-25 capsule containing 2.5×10^{10} CFU microorganism mixture of 11 bacteria twice daily that contained 2×10^9 CFU *Bifidobacterium infantis* CBT BT1. A total of 54 subjects were used in the BIO-25 group and 53 subjects were used in the placebo group. Nine subjects in the placebo group and five subjects in the BIO-25 group did not complete the study. No serious adverse events were reported in either group. The studies concluded improved symptoms in women with IBS-D but did not demonstrate superiority of symptoms and microbial diversity of the microorganism mixture over the placebo group.

Conclusion

The scientific data, information, methods, and principles described in this notification provide the basis for conclusion that *Bifidobacterium infantis* CBT BT1 is generally recognized among qualified experts to be safe for inclusion in the food types described in the amounts noted. The historic safe use of *Bifidobacterium infantis* in the food supply along with the evaluation of the consumption data serve as the foundation on which the safety of this uniquely identified strain is established.

Inclusion of *Bifidobacterium infantis* and other lactic acid-producing bacteria is identified and sometimes mandated in FDA regulations surrounding standards of identity for select food types. FDA has also responded with no questions to numerous GRAS notices submitted for other strains of *Bifidobacterium infantis*, other species of *Bifidobacterium*, as well as members of other genera of lactic acid-producing bacteria, intended for inclusion as food ingredients. The applicable GRAS notices, referenced in Table 8 and Table 9 within Part 6 of this notice, incorporate myriad studies demonstrating the safety of ingestion of substances closely related to *Bifidobacterium infantis* CBT BT1**.**

Bifidobacterium infantis CBT BT1 is well characterized genetically, taxonomically known as an organism lacking potential for harm, and supported by analyses conducted by Cell Biotech R&D Center (2018) in demonstration of its safety and elucidation of its genotypic and phenotypic traits. The substance's potential for pathogenicity and acute toxicity tested negative. *Bifidobacterium infantis* CBT BT1's potential for antibiotic resistance was tested in accordance with EFSA guidelines where *Bifidobacterium* strains are intrinsically resistant to kanamycin.

Additional efficacy studies in humans and animals have been performed without the occurrence of observation of adverse events. An LD_{50} of greater than 10^{11} CFU/kg was established in rats which corresponds to a human equivalent amount of 9.6×10^{11} CFU in a 60kg human (using the animal-specific body surface area-based conversion factor presented in the Center for Drug Evaluation and Research's Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers [2005]). The estimated level of cumulative daily intake of *Bifidobacterium infantis* CBT BT1 at the 90th percentile of high-level consumers of products of the intended inclusion food is 5.55×10^{11} CFU per day of *Bifidobacterium infantis* CBT BT1. The 90th percentile for actual consumption of 5.55×10^{11} CFU/day is below the maximum safe starting dose of 9.6×10^{11} CFU/serving.

All data and information pertaining to the studies performed on the material, in-house documentation, and additional information were made available to the Expert Panel, and their findings reflect review of the totality of the information used in the preparation of this notice as shown on the Expert Panel Endorsement pages.

PART 7 – SUPPORTING DATA AND INFORMATION

Generally Unavailable

Cellbiotech R&D Center (2018) Identification. Molecular Typing and Safety Assessment of *Bifidobacterium infantis* CBT BT1 (KCTC 11859BP).

Generally Available

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Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of Cell Biotech Co. Ltd. *Bifidobacterium infantis* CBT BT1

February 25, 2021

Cell Biotech Co. Ltd. intends to market ***Bifidobacterium infantis* CBT BT1** as an ingredient in dairy products. ***Bifidobacterium infantis* CBT BT1** is produced by growth of a certified source strain of the organism in an appropriate medium. The strain is verified prior to inoculation of the medium. The resultant microorganism is freeze-dried for use in dairy products.

The use of this microorganism in the production of food products is historic. The application of the specific strain ***Bifidobacterium infantis* CBT BT1** identified in this dossier is further demonstrated in this submission as Generally Recognized as Safe through support from the application of scientific procedures evaluating the safety of the item.

At the request of Cell Biotech Co. Ltd., a panel of independent scientists (the "Expert Panel"), qualified by their relevant national experience, education and training, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of ***Bifidobacterium infantis* CBT BT1** as an ingredient in dairy products is safe, suitable, and would be Generally Recognized as Safe (GRAS) based on a combination of historic use and scientific procedures. The Expert Panel consisted of following experts: Steven Dentali, Ph.D. (Dentali Botanical Sciences), Mary C. Mulry, Ph.D. (Foodwise), and Ms. Jeanne Moldenhauer, M.Sc. (Excellent Pharma Consulting).

The Expert Panel, independently and collectively, evaluated the dossier inclusive of the following:

Basis for GRAS Determination	Narrative Summary
Claim Regarding GRAS Status	Determination of the Expert Panel
Manufacturing Process	Summary and Diagrams
Stability Data	Data and Presentation
Dietary Exposure	Summary of intended exposure
Basis for Determination	Discussion of studies
Public and Private Studies	Supporting studies included

In addition, the Expert Panel evaluated all other information deemed necessary and/or sufficient in order to arrive at its independent, critical evaluation of these data and information. The Expert Panel has attained a unanimous conclusion that the intended uses described herein for Cell Biotech Co. Ltd. ***Bifidobacterium infantis* CBT BT1**, meeting appropriate food-grade specifications as described in the supporting dossier, as a dairy ingredient is identified as Generally Recognized as Safe (GRAS) by Self-determination for use as a food ingredient across a range of food categories identified in the dossier. Such dairy products that include Cell Biotech Co. Ltd. ***Bifidobacterium infantis* CBT BT1** in accordance with the described applications and levels specified in the dossier, manufactured according to current Good

Manufacturing Practice (cGMP), are safe for human consumption. These determinations are made based on a combination of historic use of the microorganism in food products with support from scientific procedures.

The individual endorsement pages follow hereunder.

ENDORSEMENT BY STEVEN DENTALI, PH.D.

I, Steven Dentali, hereby affirm that ***Bifidobacterium infantis* CBT BT1** is Generally Recognized as Safe by Self-determination based upon my review and participation in the appointed Expert Panel.

Signature:__  Date:17 March 2021

Steven Dentali, Ph.D.
Dentali Botanical Sciences

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The use of this microorganism in the production of food products is historic. The application of the specific strain ***Bifidobacterium infantis* CBT BT1** identified in this dossier is further demonstrated in this submission as Generally Recognized as Safe through support from the application of scientific procedures evaluating the safety of the item.

At the request of Cell Biotech Co. Ltd., a panel of independent scientists (the "Expert Panel"), qualified by their relevant national experience, education and training, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of ***Bifidobacterium infantis* CBT BT1** as an ingredient in dairy products is safe, suitable, and would be Generally Recognized as Safe (GRAS) based on a combination of historic use and scientific procedures. The Expert Panel consisted of following experts: Steven Dentali, Ph.D. (Dentali Botanical Sciences), Mary C. Mulry, Ph.D. (Foodwise), and Ms. Jeanne Moldenhauer, M.Sc. (Excellent Pharma Consulting).

The Expert Panel, independently and collectively, evaluated the dossier inclusive of the following:

Basis for GRAS Determination	Narrative Summary
Claim Regarding GRAS Status	Determination of the Expert Panel
Manufacturing Process	Summary and Diagrams
Stability Data	Data and Presentation
Dietary Exposure	Summary of intended exposure
Basis for Determination	Discussion of studies
Public and Private Studies	Supporting studies included

In addition, the Expert Panel evaluated all other information deemed necessary and/or sufficient in order to arrive at its independent, critical evaluation of these data and information. The Expert Panel has attained a unanimous conclusion that the intended uses described herein for Cell Biotech Co. Ltd. ***Bifidobacterium infantis* CBT BT1**, meeting appropriate food-grade specifications as described in the supporting dossier, as a dairy ingredient is identified as Generally Recognized as Safe (GRAS) by Self-determination for use as a food ingredient across a range of food categories identified in the dossier. Such dairy products that include Cell Biotech Co. Ltd. ***Bifidobacterium infantis* CBT BT1** in accordance with the described applications and levels specified in the dossier, manufactured according to current Good

Manufacturing Practice (cGMP), are safe for human consumption. These determinations are made based on a combination of historic use of the microorganism in food products with support from scientific procedures.

The individual endorsement pages follow hereunder.

ENDORSEMENT BY JEANNE MOLDENHAUER, M. SC.

I, Jeanne Moldenhauer, hereby affirm that ***Bifidobacterium infantis* CBT BT1** is Generally Recognized as Safe by Self-determination based upon my review and participation in the appointed Expert Panel.

Signature: [redacted] Date: 6APR21

Jeanne Moldenhauer, M. Sc.
Excellent Pharma Consulting

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Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of Cell Biotech Co. Ltd. *Bifidobacterium infantis* CBT BT1

February 25, 2021

Cell Biotech Co. Ltd. intends to market ***Bifidobacterium infantis* CBT BT1** as an ingredient in dairy products. ***Bifidobacterium infantis* CBT BT1** is produced by growth of a certified source strain of the organism in an appropriate medium. The strain is verified prior to inoculation of the medium. The resultant microorganism is freeze-dried for use in dairy products.

The use of this microorganism in the production of food products is historic. The application of the specific strain ***Bifidobacterium infantis* CBT BT1** identified in this dossier is further demonstrated in this submission as Generally Recognized as Safe through support from the application of scientific procedures evaluating the safety of the item.

At the request of Cell Biotech Co. Ltd., a panel of independent scientists (the "Expert Panel"), qualified by their relevant national experience, education and training, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of ***Bifidobacterium infantis* CBT BT1** as an ingredient in dairy products is safe, suitable, and would be Generally Recognized as Safe (GRAS) based on a combination of historic use and scientific procedures. The Expert Panel consisted of following experts: Steven Dentali, Ph.D. (Dentali Botanical Sciences), Mary C. Mulry, Ph.D. CFS (FoodWise One LLC), and Ms. Jeanne Moldenhauer, M.Sc. (Excellent Pharma Consulting).

The Expert Panel, independently and collectively, evaluated the dossier inclusive of the following:

Basis for GRAS Determination	Narrative Summary
Claim Regarding GRAS Status	Determination of the Expert Panel
Manufacturing Process	Summary and Diagrams
Stability Data	Data and Presentation
Dietary Exposure	Summary of intended exposure
Basis for Determination	Discussion of studies
Public and Private Studies	Supporting studies included

In addition, the Expert Panel evaluated all other information deemed necessary and/or sufficient in order to arrive at its independent, critical evaluation of these data and information. The Expert Panel has attained a unanimous conclusion that the intended uses described herein for Cell Biotech Co. Ltd. ***Bifidobacterium infantis* CBT BT1**, meeting appropriate food-grade specifications as described in the supporting dossier, as a dairy ingredient is identified as Generally Recognized as Safe (GRAS) by Self-determination for use as a food ingredient across a range of food categories identified in the dossier. Such dairy products that include Cell Biotech Co. Ltd. ***Bifidobacterium infantis* CBT BT1** in accordance with the described applications and levels specified in the dossier, manufactured according to current Good

Manufacturing Practice (cGMP), are safe for human consumption. These determinations are made based on a combination of historic use of the microorganism in food products with support from scientific procedures.

The individual endorsement pages follow hereunder.

ENDORSEMENT BY MARY C. MULRY, PH.D. CFS

I, Mary Mulry, hereby affirm that ***Bifidobacterium infantis* CBT BT1** is Generally Recognized as Safe by Self-determination based upon my review and participation in the appointed Expert Panel.

Signature: [redacted] Date: 3/18/21

Mary C. Mulry, Ph.D. CFS
FoodWise One LLC

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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

SECTION A INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No._____ ☐ Supplement to GRN No. _____

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

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd):* 2021-12-06

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

SECTION B INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person Myung-jun Chung	Position or Title CEO		
	Organization *(if applicable)* Cell Biotech Co. Ltd.			
	Mailing Address *(number and street)* 50 Agibong-ro, 409 Beon-gil			
City Wolgot-myeon, Gimpo	State or Province Gyeonggi-do	Zip Code/Postal Code	Country Korea, Republic of	
Telephone Number +82 31 987 6205	Fax Number	E-Mail Address ceo@cellbiotech.com		
1b. Agent or Attorney *(if applicable)*	Name of Contact Person Jim Lassiter	Position or Title COO		
	Organization *(if applicable)* REJIMUS, INC.			
	Mailing Address *(number and street)* 600 W Santa Ana Blvd Suite 1100			
City Santa Ana	State or Province California	Zip Code/Postal Code 92701	Country United States of America	
Telephone Number 9492290072	Fax Number	E-Mail Address jim@rejimus.com		

SECTION C GENERAL ADMINISTRATIVE INFORMATION

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

Bifidobacterium infantis CBT BT1

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

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☒ Paper

If applicable give number and type of physical media

1 DVD+R

3. For paper submissions only:

Number of volumes 1

Total number of pages 35

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

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

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

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

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

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

SECTION D INTENDED USE

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

The intended use of Bifidobacterium infantis CBT BT1 is a food ingredient for inclusion in dairy products where standards of identity do not preclude such use. The intended addition level to these foods is up to 1 × 10^11 CFU per serving.

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

(Check one)

☐ Yes ☒ No

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

(Check one)

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

SECTION E PARTS 2 7 OF YOUR GRAS NOTICE

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

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

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

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

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

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

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

Other Information

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

☒ Yes ☐ No

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

☒ Yes ☐ No

SECTION F SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Cell Biotech Co. Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Bifidobacterium infantis CBT BT1
(name of notified substance)

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

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

50, Agibong-ro, 409 Beon-gil
(address of notifier or other location)

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

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Jim Lassiter Digitally signed by Jim Lassiter Date: 2022.05.09 12:09:16 -07'00'	Jim Lassiter, President/COO	05/09/2022

SECTION G LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	GRASNotice_II964.1-CBI.1.4_Bifidobacterium_infantis_CBT_BT1_2022-05-09.pdf	Administrative
	Cell_Biotech_Co_Ltd_B_infantis_CBT_BT1_2018.pdf	GRAS Notice
	Bazanella_2017.pdf	GRAS Notice
	Candela_2007.pdf	GRAS Notice
	CDER_Starting_dose_in_Initial_Clinical_Trials_and_Therapeutics_in_Adult_Healthy_Volunteers_2005.pdf	GRAS Notice
	Del_Giudice_2017.pdf	GRAS Notice
	Dirar_1992.pdf	GRAS Notice
	Escribano_2018.pdf	GRAS Notice

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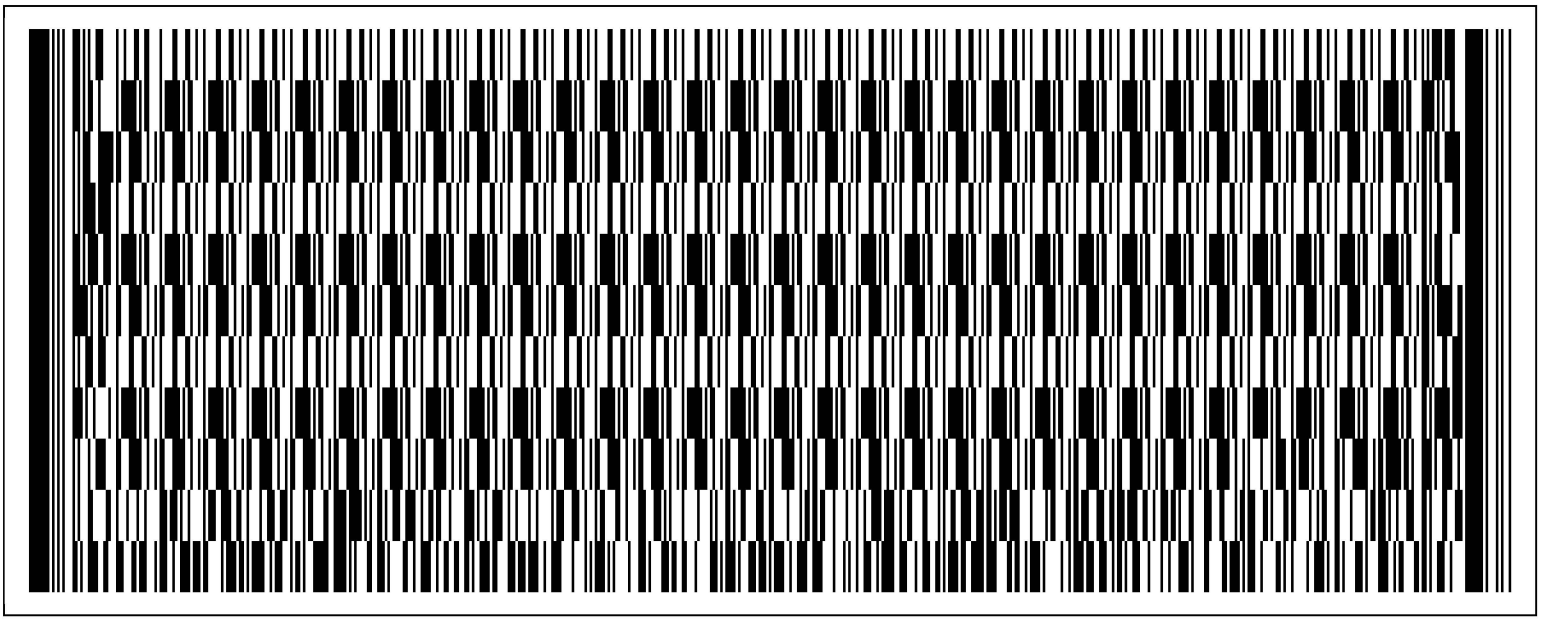
Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	EFSA_2012.pdf	GRAS Notice
	EFSA_Scientific_Opinion_on_the_Update_of_the_list_of_QPS-recommended_biological_agents.pdf	GRAS Notice
	Groeger_2013.pdf	GRAS Notice
	Health_Canada_Probiotics.pdf	GRAS Notice
	Hesseltine_1981.pdf	GRAS Notice
	Hill_2014.pdf	GRAS Notice
	Hod_2017.pdf	GRAS Notice
	Hod_2018.pdf	GRAS Notice
	Hoyos_1999.pdf	GRAS Notice

SECTION G LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Nout_1992.pdf	GRAS Notice
	Smilowitz_2017.pdf	GRAS Notice
	Steinkraus_1992.pdf	GRAS Notice
	Toure_2003.pdf	GRAS Notice
	Turroni_2014.pdf	GRAS Notice
	Ventura_2007.pdf	GRAS Notice
	Whorwell_2006.pdf	GRAS Notice



From: Joel Villareal
To: Hice, Stephanie
Cc: Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Kent Phan; Livia Consedine
Subject: FW: [EXTERNAL] Re: GRN 001081 - Questions for Notifier
Date: Saturday, April 15, 2023 10:50:03 PM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png
image007.png
image008.png
II964.1-CBI.3.pdf

Dear Dr. Hice,

In response to the document "2023-03-15 GRN 1081 – Questions for Notifier" for the request for more information for GRN 001081 (*Bifidobacterium infantis* CBT BT1) and in accordance with the below correspondence, attached you will find responses to the questions/comments (II964.1-CBI.3) with the respective attachments included therein.

Please note that there are still five (5) questions that will require additional time to gather/verify information and documentation from the Sponsor. These additional information and documents will be provided to the agency for review once we have received them and we anticipate this information to be provided by Friday, 4/21/23. Please let us know if this suffices for this response.

Thank you for sending your initial feedback and if there any other questions/concerns, please let us know.

Kind Regards.

Joel Villareal | Regulatory Manager
Quality Development Services
joel@rejimus.com

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www.rejimus.com

From: Jim Lassiter <jim@rejimus.com>
Date: Monday, April 3, 2023 at 1:50 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Cc: Brandon M. Griffin <brandon@rejimus.com>, Joel Villareal <joel@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>
Subject: Re: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dr. Hice:

After careful conferring with colleagues assigned aspects of completion – we wish to avail ourselves of your kindness in allowing for complete delivery of the materials by the end of NEXT week. We will forward each individually as they are completed and reviewed. Thank you again for your assistance and efforts.

Respectfully,

--

Jim C. Lassiter | COO
jim@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Monday, April 3, 2023 at 12:17 PM
To: Jim Lassiter <jim@rejimus.com>
Subject: RE: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

Thank you for providing an update.

You mention in your email that the responses to the questions for GRN 001078, 001080, 001081, and 001082 are intended to be delivered over the course of the next week (with the responses to the questions for GRN 001079 to be issued shortly). Do you anticipate that you'll transmit each of the amendments to us by Friday, April 7, 2023? Or, are you referring to the end of next week?

Thank you in advance for your clarification.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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

Pronouns: They-Them-Their (what is this?)

From: Jim Lassiter <jim@rejimus.com>
Sent: Monday, April 3, 2023 12:58 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Subject: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dr. Hice:

Please excuse the delay in providing updates and requests concerning this filing as we are actively working to address each of the requests for each of the submissions. We are preparing the responses to the inquiries posted and will issue the GRN 001079 shortly. The inquires posed to the notices 001078, 001080, 001081 and 001082 are also intended to be delivered promptly thereafter over the course of the next week as they are completed.

The majority of the requests have resulted in inquires and clarifications common across the submissions needing input from the Sponsor of the notifications to address the last of the issues fully. We are working to address those succinctly with each update to follow.

Your continued patience in this matter is sincerely appreciated.

Respectfully,
--
Jim C. Lassiter | COO
jim@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Friday, March 31, 2023 at 11:39 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: RE: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

I wanted to follow-up to my March 15, 2023, email to see if you intended to provide responses to our questions for GRN 001078 soon? We typically request from a response within **10 business days**. If you are unable to complete the response within that time frame, you may contact me to discuss further options.

Thank you for your attention to our comments.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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

Pronouns: They-Them-Their (what is this?)

From: Hice, Stephanie
Sent: Wednesday, March 15, 2023 12:03 PM
To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

During our review of GRAS Notice No. 001078, we noted questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients
Office of Food Additive Safety

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

Pronouns: They-Them-Their (what is this?)

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4/15/2023

Stephanie Hice, PhD
Regulatory Review Scientist & Microbiology Reviewer
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
stephanie.hice@fda.hhs.gov

RE: Response to FDA Questions/Comments Regarding GRN 001081
II964.1-CBI.3

Dear Dr. Hice,

REJIMUS, INC. received your email dated 3/15/23 regarding additional FDA questions/comments to GRN 001081. This is the first response to address the majority of the questions presented. Additional documentation from the Sponsor has been requested and a follow-up response will be necessary and is expected to be provided to you by 4/21/23 to address the identified questions surrounding the intended use levels and the overall safety conclusion.

Should you have any questions or concerns with this additional information or have additional requests based on the information provided so far, please let us know, and we'll be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS/COMMENTS REGARDING GRN 001081

Question 1

On page 7, the notifier states "… originally isolated from human feces or fermented food is identified as *Bifidobacterium infantis* and has been uniquely characterized as a distinct strain known as CBT BT1 by means of genomic typing". For the administrative record, please clarify whether *B. infantis* strain KCTC 11859BP (*B. infantis* strain "CBT BT1") was originally isolated from human feces or human food.

Response

The B. infantis BT1 (KCTC 11859BP) was isolated from human feces. (Lim et al. 2021)

Attachment II964.1-CBI.3-A1

Question 2

On page 7, the notifier states "Previously designated *Bifidobacterium longum* subsp. *infantis*, *Bifidobacterium infantis* is a species of the genus Bifidobacterium", however, on page 20, the notifier states, "*Bifidobacterium infantis* is a subspecies of *Bifidobacterium longum*". Please clarify this discrepancy and provide complete and appropriate citations to the literature, as applicable.

Response

It was inappropriately described as a species of the genus Bifidobacterium. As clarification, "Bifidobacterium infantis" is also known as Bifidobacterium longum subsp. infantis, since the organism is classified as a subspecies of Bifidobacterium longum.

Question 3

On page 7, the notifier states "The gram staining morphology of Bifidobacterium can vary as long, slender rods, in clusters, pairs or even independently", however, does not describe the morphology of *B. infantis* strain KCTC 11859BP. For the administrative record, please provide a brief description of the morphology of *B. infantis* strain KCTC 11859BP.

Response

B. infantis strain KCTC 11859BP is a gram-positive non-spore forming rod. The morphology of the colony is a circular shape with raised convex and smooth surface.

Question 4

For the administrative record, please provide a brief description of *B. infantis* strain KCTC 11859BP including phenotypic characteristics (e.g., production of antimicrobials, production of secondary metabolites), and whether this poses a safety concern. For example, on page 17, the notifier states, "*Bifidobacterium infantis* CBT BT1 is not known to secrete any exotoxins or any other substances that are classified as harmful to humans" but does not describe how this was confirmed.

Response

Bifidobacterium infantis CBT BT1 is a lactic acid bacterium (LAB). LAB produce bacteriocins, small peptides 3-6 kDA in size that help protect against pathogenic invasion (Savadogo et al. 2006, Toure et al. 2003). Most bacteriocins produced by LAB are membrane active compounds that increase permeability of the cytoplasmic membrane and show a spectrum of bactericidal activity that falls within two broad groups as shown below (Savadogo et al. 2006). Characteristics of bacteriocins produced by Bifidobacterium spp. are shown below. Therefore, the phenotypic characteristics of B. infantis strain KCTC 11859BP does not pose a safety concern.

Antimicrobial peptides produced by lactic acid bacteria (Savadogo et al. 2006).

Group I: Modified bacteriocins (the lantibiotics)		Group II: Unmodified bacteriocins	
Type A	Type B	One peptide bacteriocins	Two peptide bacteriocins
Nisin	NK [a]	Pediocin-like bacteriocins [b]:	Lactococcin G
Lactocin S		Pediocin PA1, Leucocin A,	Lactacin F
Lacticin 481		Sakacin P, Curvacin A,	Plantaricin E/F
Carnocin UI 49		Mesentericin Y105,	Plantaricin J/K
Cytolysin		Carnobacteriocin BM1, Carnobacteriocin B2, Enterocin A, Piscicolin 126, Bavaricin MN, Piscicocin V1a	Lactobin A Plantaricin S[c] Pediocin L50[d] Thermophilin 13
		Nonpediocin- like bacteriocins: Lactococcin A and B, Crispacin A, Divergicin 750, Lactococcin 972, AS-48[e], Enterocin B, Carnobacteriocin A	

[a] Not known: lantibiotics of type B produced by lactic acid bacteria are presently not known
[b] References for the pediocin like bacteriocins are: Pediocin PA1 (Henderson et al.,1992 ; Marug et al., 1992), leucocin A (Hastings et al., 1991), sakacin P (Tichaczek et al., 1992), curvacin A (Tichaczek et al., 1992 ; Holck et al., 1992), mesentericin Y105 (Hechard et al., 1992), carnobacterioin BM1 and B2 (Quadri et al., 1994), enterocin A (Aymerich et al., 1996), piscicolin 126 (Jack et al. , 1996), bavaricin MN (Kaiser , Montville ,1996), piscicocin V1a (20).
[c] Reference for plantaricin S: (Tichaczek et al., 1993).
[d] originally published as a modified ine peptide bacteriocin (Cintas et al. , 1995), but recent results indicate that is an unmodified two-peptide bacteriocin (Cintas et al.unpublished results)
[e] As-48 is a cyclic antimicrobial peptide produced by *Enterococcus faecalis* (Martinez-Bueno et al. , 1994).

Bacteriocins from *Bifidobacterium* spp. and their main characteristics (Martinez et al. 2013).

Bacteriocin	Species and strain	Mol. wt. (kDa)	Heat range stability	pH range stability	Production phase	Optimal production	Inhibitory spectrum	Reference
Bifidin	*B. bifidum* NCDC 1452	(–)	(100 °C–30 min)	4.8–5.5	After 48 h	pH: 4.8	Gram-positive and Gram-negative bacteria	Anand et al. (1984, 1985)
Bifidocin B	*B. bifidum* NCFB 1454	3.3	(121 °C–15 min)	2–12	(12–18 h)	37 °C, pH 5.0–6.0	*Bacillus cereus, Enterococcus faecalis, Listeria monocytogenes, Pediococcus acidolactici, Streptococcus faecalis*, etc.	Yildirim and Johnson (1998); Yildirim et al. (1999)
Bifilong	*B. longum*	120	(100 °C–30 min)	2.5–5.0	(–)	(–)	Gram-positive and Gram-negative bacteria	Kang et al. (1989)
Bifilact Bb-46	*B. longum* Bb-46	25–127	(121 °C–15 min)	4–7	(–)	(–)	*Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, E. coli*	Saleh and El-Sayed (2004)
Bifilact Bb-12	*B. lactis* Bb-12	25–89	Unstable for high temperatures	4–7	(–)	(–)	*Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, E. coli*	Saleh and El-Sayed (2004)
Thermophilicin B67	*B. thermophilum* RBL67	5–6	(100 °C–5 min)	2–10	24 h	pH 6 and 40 °C	*Listeria sp., Lactobacillus acidophilus*	von Ah (2006)
Bifidin I	*B. infantis* BCRC 14602	3	(121 °C–15 min)	4–10	18 h	(–)	*LAB* strains, *Staphylococcus, Bacillus, Streptococcus, Salmonella, Shigella, E. coli.*	Cheikhyoussef et al. (2009a, 2010)
Lantibiotic (Bisin)	*B. longum* DJO10A	(–)	(–)	(–)	1–8 h	Auto-induction by crude lantibiotic	*Streptococcus thermophilus* ST403, *Clostridium perfringens, Staphylococcus epidermidis, Bacillus subtilis, Serratia marcescens, E. coli* DH5a.	Lee et al. (2011)

(–): not available.

Attachment(s) II964.1-CBI.3-A2 and II964.1-CBI.3-A3

Question 5

On page 26, the notifier states "The substance's potential for pathogenicity and acute toxicity tested negative". For the administrative record, please provide a statement affirming that *B. infantis* strain KCTC 11859BP is non-pathogenic and non-toxigenic.

Response

Based on the results of the toxicity studies, there were no signs of the mortality or adverse effects of the animals at levels of 1 x 10^{11} CFU/kg. In addition, according to the Pathogenicity Island Database (http://www.paidb.re.kr/about_paidb.php?m=h), there are no pathogenicity islands (PAI) observed in the genome of this strain. Therefore, it can be affirmed that B. infantis strain KCTC 11859BP is non-pathogenic and non-toxigenic.

Question 6

In Table 10, the notifier lists "nr" under the EFSA cutoff value for kanamycin (page 20). For the administrative record, please clarify if this stands for "not required".

Response

The notation "nr" in Table 10 refers to "not required" according to EFSA. Owing to the inherent characteristics of Bifidobacterium species regarding aminoglycosides, kanamycin is not required.

Question 7

For the administrative record, please state whether *B. infantis* strain KCTC 11859BP is genetically engineered.

Response

B. infantis strain KCTC 11859BP is not genetically engineered. The strain was naturally isolated from human feces (Lim et al. 2021).

Attachment II964.1-CBI.3-A1

Question 8

On pages 8 and 9, the notifier discusses various genotypic analyses performed on *B. infantis* strain KCTC 11859BP, including comparisons to six other strains of Bifidobacterium. Table 2 includes the comparisons of these seven Bifidobacteria strains, however, the accompanying legend only lists six of the strains. For the administrative record, please provide an updated copy of Table 2 with a revised legend that correctly identifies each of the seven strains.

Response

Table 2 has been updated to include the seven strains. B. breve^T (ATCC 15700) has been included as part of the seven strains.

Percent Identity

Divergence

	1	2	3	4	5	6	7
1		99.5	99.2	93.9	96.4	90.3	93.2
2	0.2		98.8	93.4	96.8	90.8	93.0
3	0.8	0.9		93.4	95.8	90.6	93.7
4	4.5	4.6	5.1		92.9	89.3	94.1
5	2.4	2.3	3.1	4.6		91.4	93.1
6	7.2	6.9	7.0	7.6	6.3		90.9
7	5.2	5.2	5.0	4.9	5.1	6.7	

1. *B. infantis* (KCTC 11859BP)
2. *B. infantis*^{T} (ATCC 15697)
3. *B. longum*^{T} (ATCC 15707)
4. *B. bifidum*^{T} (DSM 20456)
5. *B. breve*^{T} (ATCC 15700)
6. *B. lactis*^{T} (DSM 10140)
7. *B. catenulatum*^{T} (KCTC 3221)

Question 9

On page 9, the notifier describes how pulse field gel electrophoresis was performed on *B. infantis* strain KCTC 11859BP and *B. infantis* strain ATCC 15697, however, does not provide a discussion regarding the results obtained. For the administrative record, please briefly summarize the results from this analysis.

Response

The presented method for pulse field gel electrophoresis in the notification demonstrated that the DNA fragments of B. infantis strain KCTC 11859BP are different from the reference B. infantis strain ATCC 15697. Therefore, it can be indicated that B. infantis strain KCTC 11859BP is a new strain of B. infantis species.

Question 10

On page 12, the notifier states “Stock organism is prepared and tested for microbiological contaminants”. Please clarify what microbiological contaminants are analyzed for at this stage.

Response

The stock organism is analyzed for i) aerobic microbial count and ii) total yeast and mold count.

Question 11

For the administrative record, please briefly specify how the purity of *B. infantis* strain KCTC 11859BP is ensured during manufacturing, and state whether the fermentation process is conducted in a contained, sterile environment.

Response

Prior to inoculation of the organism into the prepared sterilized medium, the stock of the strain is checked for purity. As a process inspection in the cultivation of the organism, a bacterial morphology under microscopy is performed.

The fermentation process is conducted in a contained, sterile environment. The broth storage tank and its components used in the fermentation process are steam sterilized prior to use. During the fermentation process, the bottom valve of the broth storage tank is opened, and the cultivated broth is transferred to a separator that is cleaned via Clean-in-place (CIP) procedures.

Question 12

In Table 3, the notifier provides a list of raw materials used during the manufacturing process (pages 11 and 12). The CAS numbers provided for yeast extract powder, sodium acetate, monobasic potassium phosphate, and corn starch do not appear to correspond to the correct substances. For the administrative record, please provide the correct CAS numbers for these substances. In addition, we note that the correct names for the ingredients designated by CAS numbers 6106-04-3, 10034-99-8, and 6100-05-6 are monosodium L-glutamate monohydrate, magnesium sulfate heptahydrate, and potassium citrate monohydrate, respectively. Please confirm the names of these ingredients.

Response

The CAS numbers for the following raw materials have been corrected.

Ingredient	CAS No.
Yeast Extract Powder	[8013-01-2]
Sodium acetate	[127-09-3]
Potassium Phosphate, Monobasic	[7778-77-0]
Corn starch	[977050-51-3]

The CAS number has been corrected for Monosodium L-glutamate as CAS No. 142-47-2.

According to the U.S. Food and Drug Administration Substances Added to Food database (screenshot below), Magnesium sulfate has an identified CAS Number of 10034-99-8 as shown in the screenshot below. It is acknowledged that Magnesium sulfate heptahydrate does have the same CAS number.

MAGNESIUM SULFATE

CAS Reg. No. (or other ID)*:	10034-99-8
Substance*:	MAGNESIUM SULFATE
Other Names:	♦ MAGNESIUM SULFATE ♦ EPSOM SALT ♦ MAGNESIUM SULFATE HEPTAHYDRATE ♦ SULFURIC ACID MAGNESIUM SALT (1:1), HEPTAHYDRATE ♦ MAGNESIUM SULFATE (1:1), HEPTAHYDRATE
Used for*† (Technical Effect):	ANTICAKING AGENT OR FREE-FLOW AGENT, EMULSIFIER OR EMULSIFIER SALT, FORMULATION AID, LUBRICANT OR RELEASE AGENT, MALTING OR FERMENTING AID, NUTRIENT SUPPLEMENT, PH CONTROL AGENT, PROCESSING AID, STABILIZER OR THICKENER
Food additive and GRAS regulations (21 CFR Parts 170-186)*:	184.1443

According to the U.S. Food and Drug Administration Substances Added to Food database (screenshot below), Potassium citrate has an identified CAS Number of 6100-05-6 as shown in the screenshot below. It is acknowledged that Potassium citrate monohydrate does have the same CAS number.

POTASSIUM CITRATE

CAS Reg. No. (or other ID)*:	6100-05-6
Substance*:	POTASSIUM CITRATE
Other Names:	♦ POTASSIUM CITRATE ♦ POTASSIUM CITRATE MONOHYDRATE ♦ TRIPOTASSIUM CITRATE MONOHYDRATE ♦ CITRIC ACID, TRIPOTASSIUM SALT, MONOHYDRATE ♦ 1,2,3-PROPANETRICARBOXYLIC ACID, 2-HYDROXY-, TRIPOTASSIUM SALT, MONOHYDRATE ♦ TRIPOTASSIUM 2-HYDROXY-1,2,3-PROPANETRICARBOXYLATE MONOHYDRATE ♦ CITRATE, POTASSIUM
Used for*† (Technical Effect):	EMULSIFIER OR EMULSIFIER SALT, FLAVOR ENHANCER, FLAVORING AGENT OR ADJUVANT, NUTRIENT SUPPLEMENT, PH CONTROL AGENT, SEQUESTRANT
Food additive and GRAS regulations (21 CFR Parts 170-186)*:	175.300 184.1625
Food labeling and standards regulations (21 CFR Parts 100-169):	133.169 , 133.173 , 133.179

Question 13

In Table 3, the notifier provides a list of raw materials used during the manufacturing process (pages 11 and 12). The reference provided for manganese sulfate (21 CFR 182.5461) does not correspond to a regulation in the CFR. For the administrative record, please provide a clarified reference for this substance. Further, the references provided for dextrose monohydrate (21 CFR 168.111), soy protein isolate (21 CFR 184.1553), trehalose (FEMA No. 4600), monobasic potassium phosphate (21 CFR 175.105), and corn starch (21 CFR 182.70/21 CFR 182.90) either do not appear to be applicable references for these

substances based on the intended use or correspond to different substances than those listed in the table. Based on these intended uses, more appropriate references would be 21 CFR 184.1857, SCOGS Report No. 101, GRN 000045, SCOGS Report No. 32, and SCOGS Report No. 115, respectively. For the administrative record, please provide a statement of affirmation.

Response

The regulatory references for the following raw materials have been corrected and are affirmed.

Ingredient	Reference
Manganese sulfate	21 CFR§184.1461
Dextrose monohydrate	21 §CFR 184.1857
Soy protein isolate	SCOGS Report No. 101
Trehalose	GRN 000045
Potassium Phosphate, Monobasic	SCOGS Report No. 32
Corn starch	SCOGS Report No. 115

Question 14

In Table 3, the notifier lists the components of the fermentation media, and other raw materials, including soy peptone and soy protein isolate (pages 11 and 12). Per the Food Allergen Labeling and Consumer Protection Act, soy is one of the major food allergens. Aside from these substances, please state whether any of the remaining raw materials used in the manufacturing process are major allergens or are derived from any of the nine major allergens. For any of the raw materials used that are major allergens or are derived from any of the nine major allergens, please discuss why these materials do not pose a safety concern.

Response

Aside from the noted soy peptone and soy protein isolate used in the fermentation medium, the product that is the subject of this GRAS determination does not have any other raw materials used in the manufacturing process that represent any of the major food allergens required to be listed in accordance with the Food Allergen Labeling and Consumer Protection Act, identified as milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans and sesame.

Question 15

In Table 3, the notifier provides a list of raw materials used during the manufacturing process (pages 11 and 12). For the administrative record, please clarify what “coating ingredient” means in this context.

Response

The inclusion of these materials occurs toward the end of the fermentation process. The intent of the inclusion is to encapsulate the microorganism comprising the finished ingredient for delivery in its dried and final form.

Question 16

Please clarify whether all raw materials used during the manufacturing process are food grade.

Response

All raw materials used during the manufacturing process are food grade. The raw materials used have regulatory statuses that are safe for inclusion in food.

Question 17

Figure 3 includes an "enzymatic modification" in the flow chart for the manufacturing process as the first step, however, this step is not described in any detail in the notice (page 13). Table 3 does not specify what type of enzyme or its source (pages 11 and 12). Please clarify the following:

a. the identity of the enzyme(s) used in the stated "enzymatic modification" step, including the enzyme commission number(s)

b. the intended use of the enzyme(s) during the manufacturing process

c. the source of the enzyme(s) (e.g., microbial-derived)

d. if the enzyme is produced by a microorganism, please provide clarification regarding the strain's phenotype (i.e., pathogenicity, toxigenicity), and genotype (i.e., genetically engineered)

e. how the notifier ensures that the enzyme(s) is inactivated and/or removed from the final product

Response

a. *The enzyme used in the enzymatic modification step is a protease (Alcalase) with the enzyme commission number 3.4.21.62.*

b. The intended use of the enzyme during the manufacturing process is for protein hydrolysis.

c. The source of the enzyme is from the microorganism, Bacillus licheniformis.

d. The microorganism, Bacillus licheniformis, where the enzyme is produced is a non-pathogenic strain and is not genetically engineered. In addition, protease enzymes using the non-pathogenic strain of Bacillus licheniformis are considered GRAS according to 21 CFR§184.1027 "Mixed carbohydrase and protease enzyme product."

e. After fermentation is complete, all components of the fermentation media, including the enzyme, are removed from the strain through the separator.

Question 18

The method for measuring viable cell count is listed on page 13 as USP <2022>. We note that this method is intended to be used to measure the absence of Clostridium species, Escherichia coli, Salmonella species, and/or Staphylococcus aureus in dietary supplements. Please clarify this discrepancy.

Response

The method referenced in the GRAS notification was misidentified. As a clarification, the viable cell count is performed as an in-house method. The analytical method for viable cell count is attached.

Attachment: II964.1-CBI.3-A4

Question 19

The method for measuring coliforms is listed on page 13 as USP <2023>, we note that this is not a USP method, but rather refers to "Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements". Please provide the correct method used to analyze for the presence of coliforms.

Response

Coliforms are tested according to KFDA Food Code VIII. Food Analytical Method, 4.7 Coliforms.

Question 20

In Table 4, the notifier lists specifications for microorganisms, including coliforms, but does not provide specifications for other common, notable foodborne pathogen analyses, such as Salmonella serovars (page 13). For the administrative record, please clarify if further analysis is performed to identify the genera or species of any presumptive positive result from analysis of coliforms. If further analysis is not performed, please describe why analysis for coliforms is sufficient. Additionally, please briefly describe how contamination is controlled during the manufacturing process.

Response

Microbiological testing such as E. coli, S. aureus, Salmonella, L. monocytogenes is performed and meets specifications as shown in the Certificate of Analysis for each presented batch. Testing of presumptive positive coliform results are further conducted to confirm the genus and species of any presumptive coliforms identified during the initial testing.

The contamination control program utilized during the manufacturing process includes the testing for contamination of stock organism(s), and all equipment used in the fermentation as well as the manufacturing processes, which are conducted through controlled cleaning programs. The finished ingredient testing is performed to verify purity and potency in accordance with the approved specification.

Attachment: II964.1-CBI.3-A5

Question 21

The notifier does not provide specifications for heavy metals (Table 4, page 13). We note that we typically request that, at a minimum, a limit for lead be included in the specifications for fermentation-derived ingredients. Please include a limit for lead in the specifications for *B. infantis* strain KCTC 11859BP and provide analytical results from a minimum of three non-consecutive batches to demonstrate that the ingredient can be manufactured that to meet this specification limit. Please note that the limit for lead should be as low as possible and be reflective of the results of the batch analyses. In addition, please specify the analytical method that is used to test for lead.

Response

Heavy metals are being performed as identified in the Certificate of Analysis. These include results for Lead, Arsenic, Cadmium, and Mercury in three non-consecutive batches. The limit for Lead is $\leq$ 1.0 mg/kg. Attached is the Certificate of Analysis of the three non-consecutive batches. The analytical method used for testing for lead is through ICP performed under Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal.

Attachment: II964.1-CBI.3-A5

Question 22

Please state whether all analytical methods used to analyze the batches for conformance with the stated specifications (including lead) have been validated for that particular purpose.

Response

All analytical methods used in the testing of the batches (including lead) have been validated for their respective purposes.

Question 23

On page 15, the notifier states *B. infantis* strain KCTC 11859BP is intended to be added to dairy products at concentrations needed to provide at least 10^{11} CFU per serving. According to the stability study (Table 5, page 14), the survival rate decreases ~30% during 12-months of storage. Considering the loss during storage, please provide narrative how the notifier ensures that 1×10^{11} CFU per serving remains viable over the product shelf life.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels. The response to this question will be addressed in the follow-up response.

Question 24

Please provide food subcategories included in the estimation of consumption of "dairy products" in Table 7 (page 16). In addition, please specify a serving size for each food subcategory and provide the reference that was used as the basis for determining the serving size.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels of each of the food sub-categories. The response to this question will be addressed in the follow-up response.

Question 25

Please clarify what population is represented by "all users" in the dietary exposure estimate (Table 7, page 16). If the dietary exposure estimate is not for the U.S. population aged 2 years and older, please provide mean and 90th percentile eaters-only dietary exposure estimates for U.S. population aged 2 years and older.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels and the appropriate dietary exposure. The response to this question will be addressed in the follow-up response.

Question 26

On page 16, the notifier states, "three daily servings would result in a cumulative exposure of 2.68×10^{11} CFU per day ($8.94 \times 10^{10} \times 3$)". Further, the notifier states, "the recommended levels of the cumulative exposure of 2.68×10^{11} CFU per day and the cumulative exposure at an estimated 90th percentile of 5.55×10^{11} CFU per day". Please note that the cumulative dietary exposure should consider background sources, and all current and proposed uses of *B. infantis* strain KCTC 11859BP. For the administrative record, please confirm that the term "cumulative" was incorrectly used in the statements mentioned above.

Further, on page 16 the notifier states, "The estimated 90th percentile of consumers of dairy products at this level of recommended consumption adjusted for the findings of the per capita data". We consider that the data in Table 7 (page 16) represents estimates for "users" (eaters) only, i.e., individuals consuming the proposed dairy products at least once during the survey period. Please note that "per capita" estimates would include eaters and non-eaters. For the administrative record, please confirm that the estimates in Table 7 are for the eaters-only population and explain what is meant by "the findings of the per capita data".

Response

Currently, B. infantis strain KCTC 11895BP is considered a novel ingredient in food and there are no current uses of this strain. As dairy products are the only proposed food, the dietary exposure of the ingredient is based on the dairy products only. Therefore, the term "cumulative" was inappropriately used.

The estimates used in the Table 7 is confirmed as eaters-only population. Therefore, the appropriate term should be "findings from the eaters-only population" and not "findings of the per capita data."

Question 27

Please provide an updated literature search that discusses the safety of *B. infantis*, including the safety of Bifidobacteria, this strain, or closely related strains, as applicable. Please do not limit your discussion solely to studies in human populations and include a discussion on pathogenicity and toxigenicity. Further, any reports of bacteremia, or foodborne illness involving *Bifidobacterium infantis*, should also be discussed. For example, but not limited to, please see:

- Esaiassen, E., Hjerde, E., Cavanagh, J. P., Simonsen, G. S., and Klingenberg, C.(2017). Bifidobacterium bacteremia: clinical characteristics and a genomic approach to assess pathogenicity. Journal of Clinical Microbiology, 55, 2234-2248. doi: 10.1128/JCM.00150-17

Please include the date (month and year) the literature search was performed and discuss whether there are any publications that may be contradictory to a GRAS conclusion.

Response

A PubMed and Google Scholar search was performed for "Bifidobacterium infantis", and "CBT BT1" to determine if there are any adverse events in a human populations or animal studies. Published studies are summarized below.

Reference	Study Title	Subjects	Dose	Duration	Summary of Safety
Smecuol et al. (2020)	Effect of *Bifidobacterium infantis* NLS super strain in symptomatic coeliac disease patients on long-term gluten-free diet – an exploratory study	Adult treated Celiac disease patients	2 capsules containing 2 x 10^9 CFU/capsule of *B. infantis* NLS-SS.	3 weeks	No adverse effects were observed.

Esaiassen et al. (2017) discusses the frequency and causes for bacteremia by Bifidobacterium species. A review of the publication shows that Bifidobacterium longum is the most frequent species that caused bacteremia. Regarding B. longum subsp. infantis, the authors mention that there were "putative virulence genes among the noninvasive isolates than amount the isolates causing invasive bacteremia." However, the authors specifies that these cases of bacteremia occur mainly in patients who were

immunocompromised, had a known medical condition, or a gastrointestinal tract condition. Esaissen et al. (2016) also presented three cases of probiotic bacteremia from Bifidobacterium longum subspecies infantis. The three cases involved preterm infants who have underlying conditions. Boyle et al. (2006) presented a review publication on what may cause bacteremia. However, the author mentions "all cases of bacteremia or fungemia gave occurred in patients with underlying immune compromise, chronic disease, or debilitation, and no reports have described sepsis related to probiotic use in otherwise healthy persons." Therefore, these publications conclude that food-borne illness, such as bacteremia, are typically caused by medical or external causes.

Owing to the results of the updated literature search performed on April 2022 and additional publication on the pathogenicity and toxigenicity as well as no significant adverse effects of B. infantis, none of the published studies is contradictory with the GRAS conclusion.

Attachment(s) II964.1-CBI.3-A6, II964.1-CBI.3-A7, II964.1-CBI.3-A8, II964.1-CBI.3-A9

Question 28

The notifier includes two tables in the notice, labeled as "Table 7" (pages 16 and 19). For the administrative record, please clarify this discrepancy.

Response

Table 7 on page 19 was a typographical error. The correct table number on page 19 is Table 8.

Question 29

In Tables 7 and 9, the notifier lists several GRAS notices, where the subject of the notice was a strain of *B. infantis* or Bifidobacteria, that have been submitted to FDA and have received "no questions" letters (page 19). We evaluated GRNs 000049, 000950, 000952, 000985, 001002, and 001003, and responded in letters respectively dated March 19, 2002, March 1, 2021, March 17, 2021, December 21, 2021, July 22, 2022, and April 26, 2022, stating that we had no questions at the time regarding the notifiers' GRAS conclusions. For the administrative record, please briefly discuss these GRNs in the context of the notifier's safety conclusion.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels and confirm the safety conclusion. The response to this question will be addressed in the follow-up response.

Question 30

In Table 7, "GRAS notices containing *Bifidobacterium infantis* receiving reply from FDA that it had no questions", the notifier includes GRN 000268, the subject of which was *B. longum* strain BB536 (page 19). In Table 9, "GRAS notices of Bifidobacterium organisms of species other than *infantis* receiving reply from FDA of no questions", the notifier includes GRN 000877, the subject of which was also *B. longum* strain

BB536. For the administrative record, please clarify why GRN 000268 is listed in Table 7, when the subject of the table is strains of B. *infantis*.

Response

GRN 000268 was inappropriately placed in the Table 7 owing to Bifidobacterium infantis also known as Bifidobacterium longum subsp. infantis. Therefore, this GRN has been moved to Table 9.

Question 31

In Table 9, the notifier lists the substance associated with GRN 000813 as "*Bifidobacterium bifidum* BORI", however, the substance associated with this GRAS notice is *B. longum* BORI. For the administrative record, please provide a statement of acknowledging this (page 19).

Response

It is acknowledged that the substance associated with GRN 000813 is identified as B. longum BORI.

Question 32

On page 26, the notifier states "The applicable GRAS notices, referenced in Table 8 and Table 9 within Part 6 of this notice, incorporate myriad studies demonstrating the safety of ingestion of substances closely related to *Bifidobacterium infantis* CBT BT1" but does not identify or summarize the relevant information from each GRAS notice. As each GRAS notice stands on its own, for the administrative record, please briefly summarize the information incorporated by reference from the GRAS notices listed in Tables 8 and 9.

Response

Table 8 and 9 has been updated to include a summary of each of the listed GRAS notices.

Table 8. GRAS notices containing *Bifidobacterium infantis* receiving reply from FDA that it had no questions (GRAS Notices Inventory Database).

GRAS No.	Date of Closure	Substance	Intended Use	Amount
758	8/20/18	*Lactobacillus helveticus* strain R0052, *Bifidobacterium longum* subsp. *infantis* strain R0033, and *Bifidobacterium bifidum* strain R0071	Powdered infant formulas	5 x 10^7 CFU/g of powder in formulas with hydration rates of 12.5 to 13.5 g/100 ml

Table 9. GRAS notices of Bifidobacterium organisms of species other than infantis receiving reply from FDA of no questions (GRAS Notices Inventory Database)

GRAS No.	Date of Closure	Substance	Intended Use	Amount
877	12/26/19	*Bifidobacterium longum* BB536	In term infant formula at a level of 1 x 10^8 CFU per gram of product.	1 x 10^8 CFU per gram of product.
872	12/9/19	*Bifidobacterium animalis* subsp. *lactis* UABIa-12	Foods generally, excluding infant formula and foods under the authority of USDA	10^9 to 10^{11} CFU per serving
856	12/9/19	*Bifidobacterium animalis* subsp. *lactis* strain BB012	Conventional foods for use by the general population, excluding foods subject to regulation by the USDA	5 x 10^{11} CFU per serving
855	2/5/20	*Bifidobacterium animalis* subsp. *lactis* strain R0421	Exempt powdered milk-based infant formula intended for healthy term infants	5 x 10^9 CFU/800 ml of formula as prepared.
814	6/25/19	*Bifidobacterium bifidum* BGN4	Powdered non-exempt term infant formula Fermented milk; includes buttermilk and kefir; flavored milk beverages mixes, dried milk powder; imitation milk; yogurt; baby cereals and foods, powder form; meal replacement powder and nutrition drink mix powder; and sugar substitute, powder form	10^8 CFU per gram of powdered formula Up to 10^9 CFU per serving

GRAS No.	Date of Closure	Substance	Intended Use	Amount
813	6/21/19	*Bifidobacterium bifidum* BORI	Powdered non-exempt term infant formula Fermented milk; includes buttermilk and kefir; flavored milk beverages mixes, dried milk powder; imitation milk; yogurt; baby cereals and foods, powder form; meal replacement powder and nutrition drink mix powder; and sugar substitute, powder form	Up to 10^8 CFU per gram of powdered formula. Up to 10^9 CFU per serving.
455	9/30/13	*Bifidobacterium breve* M-16V	Exempt term powdered amino acid-based formulas	Up to 10^8 CFU per gram of infant formula powder
454	9/27/13	*Bifidobacterium breve* M-16V	Non-exempt powdered term infant formulas (milk- or soy-based) and exempt powdered term infant formula containing partially hydrolyzed milk or soy proteins	Up to 10^8 colony forming units per gram of infant formula powder
453	9/27/13	*Bifidobacterium breve* M-16V	Baked goods, breakfast cereals, fruit juices and nectars, fruit ices, vegetable juices, milk-based drinks and powders, dairy product analogs, frozen dairy desserts, processed cheese, imitation cheese, cheese	Up to 5 x 10^9 colony forming units per serving

GRAS No.	Date of Closure	Substance	Intended Use	Amount
			spreads, butter-type products, snack foods, gelatin, pudding, fillings, meal replacements, snack bars, nut and peanut spreads, hard and soft candies, cocoa-type powder, and condiment sauces at levels	
445	4/10/13	*Bifidobacterium animalis* subsp. *lactis* strains HN019, Bi-07, BI-04 and B420	Ready-to-eat breakfast cereals, bars, cheeses, milk drinks and milk products, bottled water and teas, fruit juices, fruit nectars, fruit 'ades' and fruit drinks, chewing gum, and confections	Maximum level of 2 x 10^{11} colony forming units per serving
377	9/29/11	*Bifidobacterium animalis* subsp. *lactis* strain Bf-6	Intended foods include: dairy foods such as fluid milks, yogurt, milk-based desserts and gravies and cheeses; dry seeds, nuts, and nut butters; grain products such as flour, yeast breads, quickbreads, cakes, cookies, pies, pastries, crackers, pancakes, waffles, French toast, crepes, pasta, cooked and ready-to-eat cereals, grain mixtures, and meat substitutes; fruits and fruit beverages; dark-green vegetables, olives, pickles, relishes, and	Maximum level of 10^{11} colony forming units (cfu) per serving.

GRAS No.	Date of Closure	Substance	Intended Use	Amount
			vegetable soups; salad dressings; sugars and sugar substitutes, syrups, honey, molasses, jellies, jams, preserves, gelatin desserts, ices, and popsicles, candies, and chewing gum; and carbonated soft drinks, sports drinks, energy drinks, and water	
268	7/8/09	*Bifidobacterium longum* strain BB536	Breads/baked goods, cereals, dairy products/dairy-based foods and dairy substitutes, fruit products, candy, chewing gum, cocoa powder, condiment sauces, flavored beverage syrups, fruit flavored powder beverage mixes, gelatin desserts, gravies, margarine, peanut and other nut butter/spreads, snack foods, weaning foods Milk based powdered infant formula	$1x10^{10}$ colony forming units (cfu) per serving $1x10^{10}$ cfu per gram of infant formula powder

Question 33

The notifier lists the intended use of *B. infantis* strain KCTC 11859BP as up to 10^{11} CFU/serving in dairy products. FDA has evaluated and issued "no questions" letters to four previous GRAS notices, where the subject of the notice was a strain of *B. infantis* with various intended uses. The highest intended use level evaluated was up to 10^{10} CFU/serving. For the administrative record, please briefly discuss the 1-log increase in use level in the context of the notifier's safety conclusion.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels and confirm the safety conclusion. The response to this question will be addressed in the follow-up response.

Question 34

On page 20, the notifier states "While the conclusion of general recognition of safety (GRAS) is based upon scientific procedures, there is a history of use of *Bifidobacterium infantis* CBT BT1 in foreign countries and in multiple food products" but does not provide a summary of these food products. For the administrative record, please provide a brief summary of these food products.

Response

Below is a table of food products that contain Bifidobacterium infantis CBT BT1 in foreign countries.

Product	**Availability**	**Ingredients**	**Amount per Serving**
DUOLAC® Balance Baby 	Korea https://www.ebay.com/itm/Duolac-Baby-Probiotics-Powder-30-days-Dual-Coated-Bifidus-Triplets-Kid-Child-/232069774531	*B. infantis* BT1 *B. breve* BR3 *L. rhamnosus* LR5 *L. plantarum* LP3 *B. longum* BG7 *B. bifidum* BF3	7.56×10^8 CFU 7.56×10^8 CFU 9.89×10^8 CFU 9.89×10^8 CFU 7.56×10^8 CFU 7.56×10^8 CFU 5.0×10^9 Total CFU / Stick
DUOLAC® Duo-D Drops 	Denmark https://www.duolac.dk/products/duolac-duo-d-draaber/	*B. infantis* BT1 *B. breve* BR3 *B. bifidum* BF3 *B. longum* BG7	1.25×10^8 CFU 1.25×10^8 CFU 1.25×10^8 CFU 1.25×10^8 CFU 5.0×10^8 Total CFU / 6 Drops
NBL Probiotic D3 Drop	Turkey https://www.nblprobiotic.com/nbl-probiotic-ailesi/cocuk/nbl-probiotic-drop/	*B. infantis* BT1 *B. breve* BR3 *B. bifidum* BF3 *B. longum* BG7	1.25×10^8 CFU 1.25×10^8 CFU 1.25×10^8 CFU 1.25×10^8 CFU

			5.0 x 10^8 Total CFU / 6 Drops

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions submitted so far as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions or requests on the above responses or the prior responses, please let us know at your earliest convenience and we will do everything we can to address those promptly. We look forward to completing the follow up response to the Agency addressing the remaining items that are identified herein as “in progress” promptly with final inputs from the Sponsor.

Attachments

II964.1-CBI.3-A1	Lim TJ, Lim S, Yoon JH, Chung MJ (2021). Effects of multi-species probiotic supplementation on alcohol metabolism in rats. *Journal of Microbiology* (2021) Vol. 59, No. 4, pp. 417-425.
II964.1-CBI.3-A2	Savadogo A, Ouattara CAT, Bassole IHN, Traore SA. Bacteriocins and lactic acid bacteria- a minireview. *African Journal of Biotechnology* Vol. 5(9), pp. 678-683, 2 May 2006.
II964.1-CBI.3-A3	Martinez FAC, Balciunas EM, Converti A, Cotter PD, de Souza Oliveira RP (2013). Bacteriocin production by *Bifidobacterium* spp. A review. *Biotechnology Advances* 31 (2013) 482-488.
II964.1-CBI.3-A4	In-house analytical method for Viable Cell Count
II964.1-CBI.3-A5	Certificate of Analysis
II964.1-CBI.3-A6	Smecuol E, Constante M, Temprano de la Paz M, Costa ML, Pinto-Sanchez MI, Vazquez H, Stefanolo JP, Gonzalez AF, D'Adamo CR, Niveloni SI, Maurino E, Verdu EF, Bai JC (2020). Effect of *Bifidobacterium infantis* NLS superstrain in symptomatic coeliac disease patients on long-term gluten-free diet – an exploratory study. *Beneficial Microbes,* 2020.
II964.1-CBI.3-A7	Esaissen E, Hjerde E, Cavanagh JP, Simonsen GS, Klingenberg C, Norwegian Study Group on Invasive Bifidobacterial Infections (2017). *Bifidobacterium* bacteremia: Clinical characteristics and a genomic approach to assess pathogenicity. *J Clin Microbiol* 55:2234-2248.
II964.1-CBI.3-A8	Esaissen E, Cavanaugh P, Hjerde E, Simonsen GS, Stoen R, Klingenberg C. (2016). *Bifidobacterium longum* subspecies *infantis* bacteremia in 3 extremely preterm infants receiving probiotics. *Emerging Infectious Diseases* Vol. 22, No. 9, September 2016.

II964.1-CBI.3-A9	Boyle RJ, Robins-Browne RM, Tang MLK (2006). Probiotic use in clinical practice: what are the risks? *Am J Clin Nutr* 2006; 83:1256-64.

The following attachments been removed in accordance with copyright laws:

II964.1-CBI.3-A1	Lim TJ, Lim S, Yoon JH, Chung MJ (2021). Effects of multi-species probiotic supplementation on alcohol metabolism in rats. *Journal of Microbiology* (2021) Vol. 59, No. 4, pp. 417-425.
II964.1-CBI.3-A2	Savadogo A, Ouattara CAT, Bassole IHN, Traore SA. Bacteriocins and lactic acid bacteria- a minireview. *African Journal of Biotechnology* Vol. 5(9), pp. 678- 683, 2 May 2006.
II964.1-CBI.3-A3	Martinez FAC, Balciunas EM, Converti A, Cotter PD, de Souza Oliveira RP (2013). Bacteriocin production by *Bifidobacterium* spp. A review. *Biotechnology Advances* 31 (2013) 482-488.
II964.1-CBI.3-A6	Smecuol E, Constante M, Temprano de la Paz M, Costa ML, Pinto-Sanchez MI, Vazquez H, Stefanolo JP, Gonzalez AF, D'Adamo CR, Niveloni SI, Maurino E, Verdu EF, Bai JC (2020). Effect of *Bifidobacterium infantis* NLS superstrain in symptomatic coeliac disease patients on long-term gluten-free diet – an exploratory study. *Beneficial Microbes,* 2020.
II964.1-CBI.3-A7	Esaissen E, Hjerde E, Cavanagh JP, Simonsen GS, Klingenberg C, Norwegian Study Group on Invasive Bifidobacterial Infections (2017). *Bifidobacterium* bacteremia: Clinical characteristics and a genomic approach to assess pathogenicity. *J Clin Microbiol* 55:2234-2248.
II964.1-CBI.3-A8	Esaissen E, Cavanaugh P, Hjerde E, Simonsen GS, Stoen R, Klingenberg C. (2016). *Bifidobacterium longum* subspecies *infantis* bacteremia in 3 extremely preterm infants receiving probiotics. *Emerging Infectious Diseases* Vol. 22, No. 9, September 2016.
II964.1-CBI.3-A9	Boyle RJ, Robins-Browne RM, Tang MLK (2006). Probiotic use in clinical practice: what are the risks? Am J Clin Nutr 2006; 83:1256-64.

The following 2 attachments remain:

- Attachment II964.1-CBI.3-A4 in-house analytical method for Viable Cell Count
- Attachment II964.1-CBI.3-A5 Certificate of Analysis

Attachment
II964.1-CBI.3-A4

Analytical Method of Viable Cell Count

Materials :

1. The diluent (Buffered peptone water)

Composition	g/L
Peptone	10
Sodium chloride	5
Disodium phosphate	3.5
Monopotassium phosphate	1.5
Tween 80	0.5
Sterilized water	979.5
pH	6.8~7.0

* Adjust pH with 0.1N NaOH

Method:

1. Dissolve precisely 1 g of the specimen in 15 mL falcon tube filled with 9 mL of the sterilized diluent (pH: 6.8 ~ 7.0)
2. Auto-vortex for 20 min. using tube adaptor at room temperature to remove the coating materials completely. If the tube adaptor is not equipped, semiauto-vortex for 20 min. in a pattern of 2-minute-vortexing-and-3-minute-resting.

   * Vortex or vortexing of the followings means semiauto-vortex or semiauto-vortexing.
3. Prepare approx. 10 glass tubes containing 9 mL of the diluent respectively. And perform the first serial dilution with a 1 in 10 (1:9) dilution method.
4. After diluting the first glass tube, vortex 3 min. and check the bacterial cells by microscope (×1,000). If the bacteria are not released completely, repeat this procedure.
5. Vortex the first glass tube for 10 sec. and continue serial dilution with a 1 in 10 (1:9) dilution method until the expected final dilution, at which 30 colonies are formed in the final culture plate. The operation between the two tubes must be done within one minute.

Dilution factor	Vortex for
10^{-1}	20 min
10^{-2}	3 min
10^{-3}	1 min
10^{-4}	30 sec
10^{-5}~	15 sec

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6. Select the last 3 tubes and vortex one tube for 10 sec. and put 1.0 mL of the diluted solution into the sterilized culture plate (Petri-dish). Pour about 20 mL of the readymade culture media (MRS or BL) carefully into the plate, cap it with the plate cover and shake the plate smoothly (clockwise 5 times and then counterclockwise 5 times). Mark the dilution ratio on the plate cover. Perform the same procedure for the other 2 tubes.
   * MRS agar for Lactobacillus, Lactococus, Enterococcus and Streptococcus species
   * BL agar for Bifidobacterium species or for total viable cell count.
   * CBT uses MRS agar and BL agar manufactured by Difco.
7. Leave the plates at room temp. until the media become hard. And then incubate the culture plate at 37℃ for 72 hrs in an aerobic incubator (for MRS agar) or for 72 hrs in an anaerobic incubator (for BL agar).
8. Select the plate at which 30~300 colonies are formed and calculate viable cells inversely using the following formula.

Formula: Viable cells (cfu/g) = Colony number × Dilution Factor

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Attachment
II964.1-CBI.3-A5

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Certificate of Analysis

Product Name : ***Bifidobacterium infantis***

Place of Production: KOREA

Batch(Lot) No. : BT1 08R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 03 May. 2017

Exp. Date: 02 May. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)	≤ 1.0 mg/kg	Passes test
Cadmium (Cd)	≤ 0.3 mg/kg	Passes test
Mercury (Hg)	≤ 0.1 mg/kg	Passes test
Arsenic (As)	≤ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

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CELL BIOTECH

Certificate of Analysis

Product Name : ***Bifidobacterium infantis***

Place of Production: KOREA

Batch(Lot) No. : BT1 01R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 04 Apr. 2017

Exp. Date: 03 Apr. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	$\leq$ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)	$\leq$ 1.0 mg/kg	Passes test
Cadmium (Cd)	$\leq$ 0.3 mg/kg	Passes test
Mercury (Hg)	$\leq$ 0.1 mg/kg	Passes test
Arsenic (As)	$\leq$ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

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Certificate of Analysis

Product Name : ***Bifidobacterium infantis***

Place of Production: KOREA

Batch(Lot) No. : BT1 44Q

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 16 Nov. 2016

Exp. Date: 15 Nov. 2017

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	$\leq$ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)	$\leq$ 1.0 mg/kg	Passes test
Cadmium (Cd)	$\leq$ 0.3 mg/kg	Passes test
Mercury (Hg)	$\leq$ 0.1 mg/kg	Passes test
Arsenic (As)	$\leq$ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

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From:	Joel Villareal
To:	Hice, Stephanie
Cc:	Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Kent Phan; Livia Consedine
Subject:	Re: [EXTERNAL] Re: GRN 001081 - Questions for Notifier
Date:	Friday, April 21, 2023 1:12:25 AM
Attachments:	image001.png image002.png image003.png image004.png image005.png image006.png image009.png image007.png II964.1-CBI.4 .pdf

---

Dear Dr. Hice,

Thank you for granting us additional time to provide further information regarding the five remaining questions in document "2023-03-15 GRN 1081 – Questions for Notifier" for GRN 001081 (*Bifidobacterium infantis* CBT BT1). Attached you will find responses to the remaining questions/comments (II964.1-CBI.4).

Thank you for sending your initial feedback and if there any other questions/concerns, please let us know.

Kind Regards.

Joel Villareal | Regulatory Manager
Quality Development Services
joel@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Monday, April 17, 2023 at 6:12 AM
To: Joel Villareal <joel@rejimus.com>
Cc: Jim Lassiter <jim@rejimus.com>, Brandon M. Griffin <brandon@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>, Kent Phan <kent@rejimus.com>, Livia Consedine <livia@rejimus.com>
Subject: RE: [EXTERNAL] Re: GRN 001081 - Questions for Notifier

Dear Mr. Villareal,

Thank you for your attention to our comments. I am confirming receipt. We look forward to receiving the remaining five responses by April 21, 2023.

We will let you know if we have further questions.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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

Pronouns: They-Them-Their (what is this?)

From: Joel Villareal <joel@rejimus.com>
Sent: Saturday, April 15, 2023 10:48 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Cc: Jim Lassiter <jim@rejimus.com>; Brandon M. Griffin <brandon@rejimus.com>; Kenneth Cairns <kenneth@rejimus.com>; Kent Phan <kent@rejimus.com>; Livia Consedine <livia@rejimus.com>
Subject: FW: [EXTERNAL] Re: GRN 001081 - Questions for Notifier

Dear Dr. Hice,

In response to the document "2023-03-15 GRN 1081 – Questions for Notifier" for the request for more information for GRN 001081 (*Bifidobacterium infantis* CBT BT1) and in accordance with the below correspondence, attached you will find responses to the questions/comments (II964.1-CBI.3) with the respective attachments included therein.

Please note that there are still five (5) questions that will require additional time to gather/verify information and documentation from the Sponsor. These additional information and documents will be provided to the agency for review once we have received them and we anticipate this information to be provided by Friday, 4/21/23. Please let us know if this suffices for this response.

Thank you for sending your initial feedback and if there any other questions/concerns, please let us know.

Kind Regards.

Joel Villareal | Regulatory Manager
Quality Development Services
joel@rejimus.com

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From: Jim Lassiter <jim@rejimus.com>
Date: Monday, April 3, 2023 at 1:50 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Cc: Brandon M. Griffin <brandon@rejimus.com>, Joel Villareal <joel@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>

Subject: Re: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dr. Hice:

After careful conferring with colleagues assigned aspects of completion – we wish to avail ourselves of your kindness in allowing for complete delivery of the materials by the end of NEXT week. We will forward each individually as they are completed and reviewed. Thank you again for your assistance and efforts.

Respectfully,

--

Jim C. Lassiter | COO
jim@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Monday, April 3, 2023 at 12:17 PM
To: Jim Lassiter <jim@rejimus.com>
Subject: RE: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

Thank you for providing an update.

You mention in your email that the responses to the questions for GRN 001078, 001080, 001081, and 001082 are intended to be delivered over the course of the next week (with the responses to the questions for GRN 001079 to be issued shortly). Do you anticipate that

you'll transmit each of the amendments to us by Friday, April 7, 2023? Or, are you referring to the end of next week?

Thank you in advance for your clarification.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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

Pronouns: They-Them-Their (what is this?)

From: Jim Lassiter <jim@rejimus.com>
Sent: Monday, April 3, 2023 12:58 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Subject: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dr. Hice:

Please excuse the delay in providing updates and requests concerning this filing as we are actively working to address each of the requests for each of the submissions. We are preparing the responses to the inquiries posted and will issue the GRN 001079 shortly. The inquires posed to the notices 001078, 001080, 001081 and 001082 are also intended to be delivered promptly thereafter over the course of the next week as they are completed.

The majority of the requests have resulted in inquires and clarifications common across the submissions needing input from the Sponsor of the notifications to address the last of the issues fully. We are working to address those succinctly with each update to follow.

Your continued patience in this matter is sincerely appreciated.

Respectfully,

--

Jim C. Lassiter | COO
jim@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Friday, March 31, 2023 at 11:39 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: RE: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

I wanted to follow-up to my March 15, 2023, email to see if you intended to provide responses to our questions for GRN 001078 soon? We typically request from a response within **10 business days**. If you are unable to complete the response within that time frame, you may contact me to discuss further options.

Thank you for your attention to our comments.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Their (what is this?)

From: Hice, Stephanie
Sent: Wednesday, March 15, 2023 12:03 PM
To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

During our review of GRAS Notice No. 001078, we noted questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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Pronouns: They-Them-Their (what is this?)

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4/20/2023

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RE: Second Response to FDA Questions/Comments Regarding GRN 001081
II964.1-CBI.4

Dear Dr. Hice,

This is a second response following the prior response issued on 4/15/23 to address the remaining questions regarding FDA questions/comments with respect to GRN 001081.

Should you have any questions or concerns with this additional information or have additional requests based on the information provided so far, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
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jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS/COMMENTS REGARDING GRN 001081

Question 23

On page 15, the notifier states *B. infantis* strain KCTC 11859BP is intended to be added to dairy products at concentrations needed to provide at least 10^{11} CFU per serving. According to the stability study (Table 5, page 14), the survival rate decreases ~30% during 12-months of storage. Considering the loss during storage, please provide narrative how the notifier ensures that 1×10^{11} CFU per serving remains viable over the product shelf life.

Response

After additional review and re-consideration by the Sponsor relative to the available safety information included in this notification as well as the prior notices cited, and current products in the marketplace as well as published clinical studies, on of B. infantis KCTC 11859BP, the intended use levels have been updated to 1 x 10^9 CFU/serving in the identified food categories, allowing for additional margin of safety.

With respect to the modified intended use level of 1 x 10^9 CFU/serving, there should not be a concern over the viability of the ingredient over a 12-months shelf-life owing to the original stability study performed at 10^{11} CFU/serving level. Even at an approximate 30% decrease in the survival rate over the identified storage period (12 months), the ingredient is capable of meeting the modified intended level of use. Furthering this, we acknowledge that the stability of the microorganism within a food matrix is quite variable and such a determination is to be made ultimately by the manufacturer of the final food product.

Question 24

Please provide food subcategories included in the estimation of consumption of "dairy products" in Table 7 (page 16). In addition, please specify a serving size for each food subcategory and provide the reference that was used as the basis for determining the serving size.

Response

Below is a table of food subcategories used in the estimation of consumption with the respective food code from NHANES as well as the respective serving size.

Food Code	Food Subcategories	Serving Size	Food Serving
11100000	Milk, NFS	1 x 10^9 CFU/serving	8 fl oz or 240mL
11111000	Milk, whole	1 x 10^9 CFU/serving	8 fl oz or 240mL
11111100	Milk, low sodium, whole	1 x 10^9 CFU/serving	8 fl oz or 240mL
11111150	Milk, calcium fortified, whole	1 x 10^9 CFU/serving	8 fl oz or 240mL
11111160	Milk, calcium fortified, low fat (1%)	1 x 10^9 CFU/serving	8 fl oz or 240mL
11111170	Milk, calcium fortified, fat free (skim)	1 x 10^9 CFU/serving	8 fl oz or 240mL
11112110	Milk, reduced fat (2%)	1 x 10^9 CFU/serving	8 fl oz or 240mL
11112210	Milk, low fat (1%)	1 x 10^9 CFU/serving	8 fl oz or 240mL
11113000	Milk, fat free (skim)	1 x 10^9 CFU/serving	8 fl oz or 240mL

Food Code	Food Subcategories	Serving Size	Food Serving
11114300	Milk, lactose free, low fat (1%)	1 x 10^9 CFU/serving	8 fl oz or 240mL
11114320	Milk, lactose free, fat free (skim)	1 x 10^9 CFU/serving	8 fl oz or 240mL
11114330	Milk, lactose free, reduced fat (2%)	1 x 10^9 CFU/serving	8 fl oz or 240mL
11114350	Milk, lactose free, whole	1 x 10^9 CFU/serving	8 fl oz or 240mL

The serving size of each of the food subcategories has been modified to 1 x 10^9 CFU/serving. Based on clinical studies provided in the GRAS notification, intended levels of previous GRAS notifications, updated literature search, and current products in the marketplace outside the United States, the serving size of 1 x 10^9 CFU/serving is reasonable to be safe for consumption.

Question 25

Please clarify what population is represented by "all users" in the dietary exposure estimate (Table 7, page 16). If the dietary exposure estimate is not for the U.S. population aged 2 years and older, please provide mean and 90th percentile eaters-only dietary exposure estimates for U.S. population aged 2 years and older.

Response

*The intended population known as "all users" is for eaters-only. The dietary exposure estimate in the GRAS submission was for 3 years and older. Therefore, the dietary exposure estimates were re-evaluated to include the total U.S population aged 2 years and older. Further evaluation with three daily servings was performed with the modified intended levels of 10^9 CFU/serving. Based on the mean eaters-only data, the dietary exposure with the suggested three daily servings would be 2.68 x 10^9 CFU/day (*8.94 x 10^8 x 3*). Based on the eaters-only at the 90th percentile, the dietary exposure with the suggested three daily servings is 5.55 x 10^9 CFU/day (*1.85 x 10^9 x 3*). Below is a summary of the updated dietary exposure of B. infantis CBT BT1 in dairy products including the mean and 90th percentile eaters-only with the modified 10^9 CFU/serving.*

Population Group	Age Group	Eaters only (CFU/day)	
		Mean	90th Percentile
Total Population	2 years old and older	2.68 x 10^9	5.55 x 10^9

Question 29

In Tables 7 and 9, the notifier lists several GRAS notices, where the subject of the notice was a strain of *B. infantis* or Bifidobacteria, that have been submitted to FDA and have received "no questions" letters (page 19). We evaluated GRNs 000049, 000950, 000952, 000985, 001002, and 001003, and responded in letters respectively dated March 19, 2002, March 1, 2021, March 17, 2021, December 21, 2021, July 22, 2022, and April 26, 2022, stating that we had no questions at the time regarding the notifiers' GRAS conclusions.

For the administrative record, please briefly discuss these GRNs in the context of the notifier's safety conclusion.

Response

Below is a summary of the 6 GRAS notification identified in this question. Based on the summary, the strains in the identified GRNs are used in similar foods intended for B. infantis CBT BT1. Owing to intended food involving dairy products and not infant formula, the level of 1 x 10^9 CFU/serving is considered safe for consumption due to the levels in the previous notifications specific to dairy products.

GRAS No.	Date of Closure	Substance	Intended Use	Amount
000049	3/19/02	*Bifidobacterium lactis* strain Bb12, *Streptococcus thermophilus* strain Th4	Ingredients in milk-based infant formula that is intended for consumption by infants four months and older, at levels not to exceed good manufacturing practice.	*B. lactis* strain Bb12: 1 x 10^7 – 1 x 10^8 CFU/g. *S. thermophilus* strain Th4: 1 x 10^7 – 1 x 10^8 CFU/g
000950	3/1/21	*Bifidobacterium longum* subsp. *infantis* DSM 33361	Ingredient in cow milk-, soy-, and partially hydrolyzed protein-based, non-exempt infant formula. In conventional foods , including but not limited to milk and dairy products; plant-based dairy alternatives; beverages; bars; confectionary; and cereals.	Up to 1 x 10^{10} CFU/g Up to 2.8 x 10^{10} CFU/g

GRAS No.	Date of Closure	Substance	Intended Use	Amount
000952	3/17/21	*Bifidobacterium animalis* subsp. *lactis* strain AD011	Ingredient in non-exempt powdered infant formula (milk and soy based). Fermented milk, including buttermilk and kefir, flavored milk beverage mixes, dried milk powder, imitation milk, yogurt , powdered baby cereals and foods, meal replacement and nutritional drink mix powders, and powdered sugar substitutes.	Up to 10^8 CFU/g Up to 10^{10} CFU/g
000985	12/21/21	*Bifidobacterium longum* subsp. *infantis* strain ATCC SD 6720	Cow milk and soy based non-exempt powdered infant formula and powdered toddler formula	Up to 10^8 CFU/g
001002	7/22/22	*Bifidobacterium breve* strain MCC1274	Baked goods, breakfast cereals, fruits (juices and nectars, ices, vegetable juices, frozen fruit, frozen juice bats), milk-based drinks and powders, yogurt, dairy product analogs, frozen dairy desserts, cheeses, condiments and spreads, nut and peanut spreads, gelatins and puddings, milk and non-milk meal replacements, soft and hard candies, and snack foods.	Up to 5 x 10^{10} CFU/serving

GRAS No.	Date of Closure	Substance	Intended Use	Amount
001003	4/26/22	*Bifidobacterium longum* subsp. *infantis* M-63	Non-exempt cow milk- and soy-based infant formula for term infants	Up to 1 x 10^8 colony forming units (CFU)/g of powdered formula
			Breads and baked goods; ready-to-eat and hot breakfast cereals; fruit juices, nectars, and blends; dairy products and dairy substitutes; candy; condiment sauces; gelatin desserts; peanut and other nut butters and spreads; snack foods; and infant and toddler foods.	Up to 1.25 x 10^{10} CFU per serving

Question 33

The notifier lists the intended use of *B. infantis* strain KCTC 11859BP as up to 10^{11} CFU/serving in dairy products. FDA has evaluated and issued "no questions" letters to four previous GRAS notices, where the subject of the notice was a strain of *B. infantis* with various intended uses. The highest intended use level evaluated was up to 10^{10} CFU/serving. For the administrative record, please briefly discuss the 1-log increase in use level in the context of the notifier's safety conclusion.

Response

After additional review and re-consideration by the Sponsor relative to the available safety information included in this notification as well as the prior notices cited, and current products in the marketplace as well as published clinical studies, on of B. infantis KCTC 18159BP, the intended use levels have been updated to 1 x 10^9 CFU/serving in the identified food categories, allowing for additional margin of safety. The findings, use and inclusion of this strain of the microorganism at levels up to this affirmed amount have been determined safe.

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions submitted so far as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions or requests on the above responses or the prior responses, please let us know at your earliest convenience and we will do everything we can to address those promptly.

REJIMUS

From:	Joel Villareal
To:	Hice, Stephanie
Cc:	Jim Lassiter; Kenneth Cairns; Brandon M. Griffin; Kent Phan; Livia Consedine
Subject:	[EXTERNAL] FW: GRN 001081 - Questions for Notifier
Date:	Tuesday, June 13, 2023 8:51:15 PM
Attachments:	image001.png image002.png image003.png image004.png image005.png image006.png image007.png II964.1-CBI.5.pdf

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Dear Dr. Hice,

In response to the document "2023-05-26 GRN 001081 - Questions for Notifier" for the request for more information for GRN 001081 (*Bifidobacterium infantis* CBT BT1), attached you will find responses to the questions/comments (II964.1-CBI.5) with the respective attachments included therein.

As similar to the response to GRN 001078, please note that there is one (1) question that will require additional time to gather/verify information and documentation from the Sponsor. These additional information and documents will be provided to the agency for review once we have received them and we anticipate this information to be provided by Monday, 6/19/23. Please let us know if this suffices for this response.

Thank you for sending your feedback and if there any other questions/concerns, please let us know.

Kind Regards.

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Friday, May 26, 2023 at 11:37 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 001081 - Questions for Notifier

Dear Mr. Lassiter,

During our evaluation of GRAS Notice No. 001081, we noted additional questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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6/13/2023

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RE: First Response to FDA Questions/Comments Regarding GRN 001081 Received on 5/26/23
II964.1-CBI.5

Dear Dr. Hice,

REJIMUS, INC. received your email dated 5/26/23 regarding additional FDA questions/comments to GRN 001081. This is the first response to address the questions presented. Additional documentation from the Sponsor has been requested and a follow-up response will be necessary and is expected to be provided to you by 6/19/23 to address the identified question surrounding the heavy metal testing.

Should you have any questions or concerns with this additional information based on the information provided so far, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
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TABLE OF CONTENTS

FDA QUESTIONS/COMMENTS REGARDING GRN 001081 RECEIVED ON 5/26/23

Question 1

1. In Part 1 of GRN 001081, the notifier states that the intended addition level of the ingredient is *up to* 1 x 10^{11} CFU/serving; however, in Part 3, the notifier states that the intended use of the ingredient in dairy products is to provide *at least* 1 x 10^{11} CFU/serving. In our questions dated March 15, 2023, considering the loss of viability during storage, we requested that the notifier provide a narrative on how the notifier ensures that 1 x 10^{11} CFU/serving remains viable over the shelf life of the dairy products (question 23).

   In the amendment dated April 21, 2023, the notifier stated that the intended use level of 1 x 10^{11} CFU/serving has been lowered to 1 x 10^{9} CFU/serving in the identified food category (i.e., milk). Accordingly, the notifier also revised the dietary exposure estimate based on 1 x 10^{9} CFU/serving.

   However, the results of the stability study presented in Part 3 (Table 5) demonstrate that the viable cell counts of the ingredient decline during storage. Therefore, it is expected that if the ingredient is added to milk at the revised use level of 1 x 10^{9} CFU/serving, the viable cell count of the ingredient would decline over the milk shelf life and be less than 1 x 10^{9} CFU/serving. Based on the notifier's amendment, we believe that the proposed lower level of 1 x 10^{9} CFU/serving may represent the target level of viable cells in the milk, not the intended *maximum use level* (i.e., maximum addition level). It is likely that an overage amount of the ingredient may be needed to compensate for the loss of viable cells added to provide the target level of *at least* 1 x 10^{9} CFU/serving over the shelf life of the milk.

   Please clarify the intended *maximum use level* of the ingredient as well as the target level over the shelf life of the milk. Accordingly, please provide estimates of the dietary exposure to the ingredient based on the intended food uses and the intended *maximum use level* (not the target level over the milk shelf life).

Response

The Sponsor intends to market the ingredient as a bulk ingredient only. The intended maximum use level of the ingredient is up to 1 x 10^{9} CFU/serving. The producer of the milk product is responsible for determinations regarding inclusion of this microorganism, but the limits of inclusion as established in this notification remain at not higher than 1 x 10^{9} CFU/serving. Based on the intended food uses and the intended maximum use level of up to 1 x 10^{9} CFU/serving, the estimated dietary exposure, based suggested three daily servings, remains as presented in the previous amendment and is shown below:

Population Group	Age Group	Eaters only (CFU/day)	
		Mean	90th Percentile
Total Population (eaters-only)	2 years old and older	2.68×10^9	5.55×10^9

Question 2

2. In the amendment dated April 21, 2023, (response to question 24), the notifier provides the serving size of the dairy products. For the administrative record, please clarify if the serving size of 8 fl oz (240 mL) for the milk category is based on the reference amounts customarily consumed (RACC) specified in 21 CFR 101.12(b).

Response

The serving size of 8 fl. oz (240 mL) for the milk category is based on the reference amounts customarily consumed (RACC).

Question 3

3. In the April 21, 2023, amendment, in response to question 33 the notifier mentions *B. infantis* KCTC 18159BP; however, the subject of the notice is *B. infantis* KCTC 11859BP. For the administrative record, please clarify this discrepancy.

Response

As a clarification, the strain identification to question 33 is a typographical error. The corrected ingredient is B. infantis KCTC 11859BP.

Question 4

4. In the April 15, 2023, amendment to the notice, the notifier lists the date the literature search was performed as April 2022. For the administrative record, please confirm whether this should be April 2023.

Response

The updated literature search is confirmed as April 2023.

Question 5

5. In the April 15, 2023, amendment (response to question 21), the notifier provides a requested specification limit for lead as well as the limits for arsenic, cadmium, and mercury along with the results (reported as "Passes test") from the analyses of three non-consecutive batches. We note that we typically do not see limits for lead as high as ≤1 mg/kg (the limit proposed by the notifier) for fermentation derived ingredients manufactured in accordance with good manufacturing practices. In addition, we would like to bring to your attention a relevant FDA's "Closer to Zero" initiative that focuses on reducing the levels of heavy metals in foods consumed by infants and young children.

   Please specify the limit of detection (LOD) and/or limit of quantitation (LOQ) for the analytical method(s) used to test for heavy metals and provide the results for heavy metals as the actual measured levels or state that the levels are below the specified LOQ or LOD.

   If ≤1 mg/kg is the LOQ or LOD of the analytical method used to test for lead, we recommend that the notifier use a more sensitive method to measure the actual levels of lead in the ingredient and propose a specification limit that reflects the results of the batch analyses and is as low as possible. If a new method is employed, please provide a statement that it is validated for its purpose

Response

In-progress

REJIMUS is currently working with the Sponsor on obtaining this information. We anticipate providing this information in a second response by 6/19/23. For clarification purposes, "passes test" in this case means that the analytical results were at or below the established specification limits.

Question 6

6. In the April 15, 2023, amendment, the notifier provides several specifications for various microbial analyses, including yeast and mold, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* serovars, and *Listeria monocytogenes*. The notifier affirms that all the analytical methods employed are validated for their intended purpose; however, does not provide the citation for the analytical methods used for the listed microbial specifications. For the administrative record, please provide complete citations for the analytical methods used for the above listed analyses.

Response

The analytical methods used for the above listed analysis are shown below and are attached:

Test	*Test Method*
Yeast and Mold	*In-house test method*
Escherichia coli	*KFDA Food Code, VIII. Food Analytical Method, 4.8 E. coli*
Staphylococcus aureus	*In-house test method*
Salmonella	*In-house test method*
Listeria monocytogenes	*In-house test method*

Attachment(s): II964.1-CBI.5-A1, II964.1-CBI.5-A2, II964.1-CBI.5-A3, II964.1-CBI.5-A4, II964.1-CBI.5-A5

Question 7

7. A general comment; response not requested. In the April 15, 2023, and April 21, 2023, amendments to the notice, the notifier summarizes several previously submitted GRAS notices for Bifidobacteria used in various conventional foods. The notifier reiterates the intended uses displayed in the online GRAS Notice Inventory. We note that some details in the notifier's summaries do not accurately reflect the information contained in the response letters to the GRAS notices. For future submissions, we recommend that the notifier refer to the response letters when summarizing previously submitted GRAS notices.

Response

For future submissions, we will summarize the GRAS notices based on the response letters.

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions presented as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any further questions or requests, please let us know at your earliest convenience and we will do everything we can to address those promptly.

Attachments

II964.1-CBI.5-A1	Analytical method for Yeast and Mold
II964.1-CBI.5-A2	Analytical method for *Escherichia coli*
II964.1-CBI.5-A3	Analytical method for *Staphylococcus aureus*
II964.1-CBI.5-A4	Analytical method for *Salmonella*
II964.1-CBI.5-A5	Analytical method for *Listeria monocytogenes*

Attachment
II964.1-CBI.5-A1

Analytical Method of Yeast and Mould

1. SCOPE

This work instruction defines the procedures for counting yeast and mould colony-forming units.

2. MEDIA AND REAGENTS

Dichloran rose Bengal chloramphenicol agar (DRBC)

3. METHODS

1. Prepare a 1 in 5 dilution of sample by emulsifying 10 grams in 40 mL of 0.1% peptone water.
2. Pipette 0.2mL of the (1 in 5) diluted sample onto 3 plates of DRBC.
3. Incubate upright at 25°C for 5 days.
4. Examine each plate and count yeast and mould colonies.

4. RESULTS

0.2mL of a 1 in 5 dilution sample is spread onto 3 DRBC plates, so the "limit of detection", one colony out of the 3 plates, is equivalent to 8 colony-forming units per gram.

Attachment
II964.1-CBI.5-A2

Analytical Method of Coliform and E.coli

1. Test Method Summary

This test method defines the procedures for isolation and identification of Coliforms and E.coli in 1 gram of sample using most probable number technique and for E.coli in 1 gram of sample.

2. Media and Reagents

2.1 Single-strenth BGLB broth

2.2 Double-strenth BGLB broth

2.3 Eosin methylene blue agar (EMB)

2.4 EC broth (ECB)

2.5 Tryptone water

2.6 Kovac's reagent

3. Test Method

3.1 Prepare a 1 in 10 dilution of sample by emulsifying 10 grams in 90 ml of 0.1% peptone water. Also prepare a 1 in 100 dilution by transferring 1 ml of the initial suspension into 9 ml of 0.1% peptone water.

3.2 Take three tubes of double-strength BGLB broth. Using a sterile pipette, transfer to each of these tubes 10 ml of the 1 in 10 diluted sample.

3.3 Then take three tubes of single-strength BGLB broth. Using a fresh sterile pipette transfer to each of these tubes 1 ml of the 1 in 10 diluted samples.

3.4 Then take three tubes of single-strength BGLB broth. Using a fresh sterile pipette transfer to each of these tubes 1 ml of the 1 in 100 diluted samples.

3.5 Incubate all tubes at 37℃ for 2 days if neither gas formation nor opacity preventing the observation of gas formation is observed at this stage for 3 days.

3.6 Steak any presumptive positives (i.e. positive in BGLB) onto EMB agar and incubate at 37℃ for one day.

3.7 Examine for coliforms. Typical coliform colonies on EMB are dark purple. They may also have a green metallic sheen or be mucoid and pink on the surface but are dark purple when viewed from the back of the plate. Record any dark colonies as coliform positive.

3.8 Subculture from EMB into EC broth and tryptone water and incubate in a water bath at 44.0℃ to 44.5℃ for up to 48 hours.

3.9 Tap the tubes gently before reading reading to counter ant gas supersaturation. E.coli produce gas in ECB at 44.5℃.

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3.10 Test the tryptone water cultures for indole production by adding about 0.2 ml Kovac's reagent. E.coli is indole positive at 44.5℃.

4. Result

4.1 If no gas formation is observed in the BGLB tube, the result is reported as not detected in samples for E.coli.

Reference: KFDA Food Code, Ⅷ. Food Analytical Method, 4.7 Coliforms, 4.8 E.coli

Attachment
II964.1-CBI.5-A3

Analytical Method of *S. aureus*

1. Add 25g or 25mL of Test Solution to 225mL of Tryptic Soy Broth(BD REF 211825) with 10% NaCl concentration and cultivate at 35~37℃ for 18~24 hours. Then, Inoculate the cultured solution to Baird-Parker agar (BD REF 276840) and cultivate at 35~37℃ for 18~24 hours. Conduct confirmatory test on the agar if glossy black colonies surrounded by transparent rim or black colonies surrounded by opaque circles as a result of cultivation on Baird-Parker agar.

2. Suspected Staphylococcus-positive when black colonies proliferate on Baird-Parker agar then move those onto normal agar (BD REF 213000) and cultivate at 35~37℃ for 18~24 hours. Do Gram staining to check Gram positive coccus which has Staphylococcus pattern, then if found, conduct coagulase test to determine coagulation within 24 hours.

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Attachment
II964.1-CBI.5-A4

Analytical Method of Salmonella

1. SCOPE

This work instruction defines the procedures for isolation and identification of Salmonella.

2. MEDIA AND REAGENTS

- Buffered peptone water (BPW)
- Muller-Kaufman tetrathionate/novobiocin broth (MKTTn broth)
- Rapport Vassiliadis medium with soya (RVS broth)
- XLD(xylose lysine desoxycholate) medium
- API20E

3. METHODS

1. Inoculate 25g of sample into 225mL of BPW and incubate at 37^{0}C for 16-20 hours. This is known as the pre-enrichment stage.
2. Transfer 1ml of pre-enrichment into 10ml MKTTn broth, and another 0.1ml of pre-enrichment into 10ml of RVS broth.
3. Incubate MKTTn broth at 37℃ and incubate RVS broth at 42℃, both for 24 hours.
4. Streak MKTTn an RVS selective enrichment broths onto one plate XLD agar.
5. Invert the dishes and place in the incubator set at 37℃ for 1-2 days for XLD agar.
6. Examine the plate for the presence of typical colonies of Salmonella and atypical colonies that may be Salmonella. Typical colonies of Salmonella grown on XLD agar have a black center and a slightly transparent zone of reddish color due to the color change of the indicator. Confirm any pink colonies.

   Note: Salmonella H_2S negative variants grown on XLD agar are pink with a darker pink center. Lactose-positive Salmonella grown on XLD agar are yellow with or without blackening.
7. If the API20E result shows that Salmonella is very unlikely, the result should be reported as Salmonella-negative, quoting the API 20E result code, regardless of whether a unique identification is achieved.

4. RESULTS

Report result as presence or absence for Salmonella in 25g sample.

Attachment
II964.1-CBI.5-A5

Analytical Method of *L.monocytogenes*

1. SCOPE

This work instruction defines the procedures for isolation and identification of *Listeria*.

2. MEDIA AND REAGENTS

- Buffered listeria enrichment broth (BLEB)
- Oxford Agar
- Tryptone soya yeast extract agar (TSYEA)
- Tryptone soya yeast extract broth (TSYEB)
- API Listeria
- Motility medium
- Hydrogen peroxide solution 3% (v/v)

3. METHODS

1. Inoculate 25g of sample into 225mL of BLEB and incubate at 30^0C for 46-50 hours.

2. Using a technique ensuring isolated colonies, streak the enrichment broth onto Oxford agar, and incubate at 37^0C for 48±2 hours.

3. Examine each plate for typical Listeria colonies, which are small dark colonies with possible greenish sheen and are about 2mm in diameter with black halos and sunken centres.

4. Streak each suspect colonies onto tryptone soya yeast extract agar (TSYEA), and incubate at 37^0C for 24 hours or until growth is satisfactory.

5. Perform a Gram stain on each suspect culture.
   a) *Listeria* spp. are Gram-positive slim rods.
   b) If the Gram result is convincingly atypical, report the culture as *Listeria*-negative, otherwise continue.

6. Perform a catalase test on each of the suspect culture:
   a) *Listeria* spp. are catalase positive.
   b) If the culture is catalase-negative, report as *Listeria*-negative, otherwise continue.

7. Perform a motility test on each suspect culture; using the stabbing technique and or using a hanging drop technique to determine typical tumbling motility.
   a) *Listeria* are motile, with a typical umbrella like growth pattern in motility medium and an unmistakable tumbling motion in fresh hanging drops preparations.
   b) If the culture is non-motile, report as *Listeria*-negative, otherwise continue.

8. Report presumptive *Listeria* identification immediately, if the Gram, catalase and motility results are atypical.

9. Confirm the genus *Listeria* and identify the species using API *Listeria* kit.

4. RESULTS

Report result as presence or absence for *Listeria* in 25g sample.

Motility test

Take a typical colony obtained on the TSYEA and suspend in a tube containing TSYEB.

Incubate at 25^{0}C for 8 - 24h until a cloudy medium is observed.

Deposit a drop of the above culture using a loop onto a clean glass microscope slide. Place a cover slip on top and examine it with the microscope. *Listeria* spp. appears slim, short rods with tumbling motility.

Cultures grown above 25^{0}C may fail to exhibit this motion. Always compare to known culture. Cocci, large rods, or rods with rapid swimming motility are not *Listeria* spp.

As an alternative test for motility, using an inoculating needle, stab the motility agar with a culture from a typical colony on TSYEA. Incubate for 48h at 25^{0}C.

Examine for growth around the stab. *Listeria* spp. are motile, giving a typical umbrella-like growth pattern. If growth is not sufficient, incubate for up to an additional 5 days and observe the stab again.

From:	Joel Villareal
To:	Hice, Stephanie
Cc:	Jim Lassiter; Kenneth Cairns; Brandon M. Griffin; Kent Phan; Livia Consedine
Subject:	Re: [EXTERNAL] FW: GRN 001081 - Questions for Notifier
Date:	Thursday, June 22, 2023 8:49:51 PM
Attachments:	image008.png image009.png image010.png image011.png image012.png image013.png image015.png image016.png image017.png image018.png image019.png image020.png image001.png II964.1-CBI.6 .pdf

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Dear Dr. Hice,

In response to the document "2023-05-26 GRN 001081 - Questions for Notifier" for the request for more information for GRN 001081 (*Bifidobacterium infantis* CBT BT1), attached you will find the response to the remaining questions (II964.1-CBI.6) with the respective attachments included therein.

Thank you for sending your feedback and if there any other questions/concerns, please let us know.

Best Regards

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

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Main: 949.485.2112 | Fax: 949.200.8546
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2510-2521 and is legally privileged.

From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Wednesday, June 14, 2023 at 4:53 AM
To: Joel Villareal <joel@rejimus.com>
Cc: Jim Lassiter <jim@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>, Brandon M. Griffin <brandon@rejimus.com>, Kent Phan <kent@rejimus.com>, Livia Consedine <livia@rejimus.com>
Subject: RE: [EXTERNAL] FW: GRN 001081 - Questions for Notifier

Dear Mr. Villareal,

Thank you for your attention to our comments. I am confirming receipt. We will let you know if we have any questions. Further, receiving a response to the remaining outstanding question (to accompany the amendment to GRN 001081) no later than Monday, June 19, 2023 is acceptable.

Thank you, and please let me know if I can clarify anything.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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

Pronouns: They-Them-Their (what is this?)

From: Joel Villareal <joel@rejimus.com>
Sent: Tuesday, June 13, 2023 8:51 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Cc: Jim Lassiter <jim@rejimus.com>; Kenneth Cairns <kenneth@rejimus.com>; Brandon M. Griffin <brandon@rejimus.com>; Kent Phan <kent@rejimus.com>; Livia Consedine <livia@rejimus.com>
Subject: [EXTERNAL] FW: GRN 001081 - Questions for Notifier

Dear Dr. Hice,

In response to the document "2023-05-26 GRN 001081 - Questions for Notifier" for the request for more information for GRN 001081 (*Bifidobacterium infantis* CBT BT1), attached you will find responses to the questions/comments (II964.1-CBI.5) with the respective attachments included therein.

As similar to the response to GRN 001078, please note that there is one (1) question that will require additional time to gather/verify information and documentation from the Sponsor. These additional information and documents will be provided to the agency for review once we have received them and we anticipate this information to be provided by Monday, 6/19/23. Please let us know if this suffices for this response.

Thank you for sending your feedback and if there any other questions/concerns, please let us know.

Kind Regards.

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

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Santa Ana, CA 92701
Main: 949.485.2112 | Fax: 949.200.8546
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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Friday, May 26, 2023 at 11:37 AM

To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 001081 - Questions for Notifier

Dear Mr. Lassiter,

During our evaluation of GRAS Notice No. 001081, we noted additional questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients
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Pronouns: They-Them-Their (what is this?)

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6/22/2023

Stephanie Hice, PhD
Regulatory Review Scientist & Microbiology Reviewer
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
stephanie.hice@fda.hhs.gov

RE: Second Response to FDA Questions/Comments Regarding GRN 001081 Received on 5/26/23
II964.1-CBI.6

Dear Dr. Hice,

REJIMUS, INC. received your email dated 5/26/23 regarding additional FDA questions/comments to GRN 001081. The first response was submitted on 6/13/23 to address the majority of the questions. This is the second response to address the remaining question presented.

Should you have any questions or concerns with this additional information based on the information provided so far, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS/COMMENTS REGARDING GRN 001081 RECEIVED ON 5/26/23

Question 5

5. In the April 15, 2023, amendment (response to question 21), the notifier provides a requested specification limit for lead as well as the limits for arsenic, cadmium, and mercury along with the results (reported as "Passes test") from the analyses of three non-consecutive batches. We note that we typically do not see limits for lead as high as ≤1 mg/kg (the limit proposed by the notifier) for fermentation derived ingredients manufactured in accordance with good manufacturing practices. In addition, we would like to bring to your attention a relevant FDA's "Closer to Zero" initiative that focuses on reducing the levels of heavy metals in foods consumed by infants and young children.

   Please specify the limit of detection (LOD) and/or limit of quantitation (LOQ) for the analytical method(s) used to test for heavy metals and provide the results for heavy metals as the actual measured levels or state that the levels are below the specified LOQ or LOD.

   If ≤1 mg/kg is the LOQ or LOD of the analytical method used to test for lead, we recommend that the notifier use a more sensitive method to measure the actual levels of lead in the ingredient and propose a specification limit that reflects the results of the batch analyses and is as low as possible. If a new method is employed, please provide a statement that it is validated for its purpose

Response

A limit of detection (LOD) and Limit of quantitation (LOQ) for the analytical method used to test for heavy metals is provided in the attached Certificate of Analysis. The same Certificate of Analysis provides the test results in actual measured levels and all test results met specifications regarding the level of these heavy metals.

The established LOD and LOQ for Lead for this analytical method used is 0.017 ppb (0.000017 mg/kg) and 0.050 ppb (0.00005 mg/kg), respectively. Owing to the very low LOQ and LOD, the analytical method used is sensitive enough to detect or quantify a small amount of Lead in the product. In addressing the specification of Lead at ≤ 1mg/kg, the specification in the attached COAs was based on production from 2016 - 2017. However, based on more current batch analysis results and in recognition of FDA's "Closer to Zero" initiative, future production batches of this ingredient will have an updated Lead specification of ≤ 10 ppb (≤ 0.01 mg/kg).

Attachment(s): II964.1-CBI.6-A1

Conclusion

We sincerely appreciate this opportunity to clarify the additional question submitted as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions or requests on the above response or the prior responses, please let us know at your earliest convenience and we will do everything we can to address those promptly.

Attachments

II964.1-CBI.6-A1	Certificate of Analysis

Attachment
II964.1-CBI.6-A1

CELL BIOTECH

Certificate of Analysis

Product Name : ***Bifidobacterium infantis***

Place of Production: KOREA

Batch(Lot) No. : BT1 01R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 04 Apr. 2017

Exp. Date: 03 Apr. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	$\leq$ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)*	$\leq$ 1.0 mg/kg	0.0086 mg/kg
Cadmium (Cd)**	$\leq$ 0.3 mg/kg	0.0025 mg/kg
Mercury (Hg)***	$\leq$ 0.1 mg/kg	0.0011 mg/kg
Arsenic (As)****	$\leq$ 0.1 mg/kg	0.0072 mg/kg

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

* LOD: 0.017 ppb, LOQ: 0.050 ppb ** LOD: 0.026 ppb, LOQ: 0.080 ppb

*** LOD: 1.400 ppb, LOQ: 5.400 ppb **** LOD: 0.049 ppb, LOQ: 0.148 ppb

Director, Head of Quality Management Division

CELL BIOTECH Co., Ltd.
Headquarters : 50, Aegibong-ro 409 beon-gil, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
Manufacturer : 397, Aegibong-ro, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
PHONE +82 31 987 8107 | FAX +82 31 987 6216 | www.cellbiotech.com

CELL BIOTECH

Certificate of Analysis

Product Name : ***Bifidobacterium infantis***

Place of Production: KOREA

Batch(Lot) No. : BT1 44Q

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 16 Nov. 2016

Exp. Date: 15 Nov. 2017

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	≥ 1.0×10^{11} CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)*	≤ 1.0 mg/kg	0.0019 mg/kg
Cadmium (Cd)**	≤ 0.3 mg/kg	0.0042 mg/kg
Mercury (Hg)***	≤ 0.1 mg/kg	0.0023 mg/kg
Arsenic (As)****	≤ 0.1 mg/kg	0.0047 mg/kg

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

* LOD: 0.017 ppb, LOQ: 0.050 ppb ** LOD: 0.026 ppb, LOQ: 0.080 ppb

*** LOD: 1.400 ppb, LOQ: 5.400 ppb **** LOD: 0.049 ppb, LOQ: 0.148 ppb

Director, Head of Quality Management Division

CELL BIOTECH Co., Ltd.
Headquarters : 50, Aegibong-ro 409 beon-gil, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
Manufacturer : 397, Aegibong-ro, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
PHONE +82 31 987 8107 FAX +82 31 987 6216 www.cellbiotech.com

CELL BIOTECH

Certificate of Analysis

Product Name : ***Bifidobacterium infantis***

Place of Production: KOREA

Batch(Lot) No. : BT1 08R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 03 May. 2017

Exp. Date: 02 May. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	$\leq$ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)*	$\leq$ 1.0 mg/kg	0.0044 mg/kg
Cadmium (Cd)**	$\leq$ 0.3 mg/kg	0.0005 mg/kg
Mercury (Hg)***	$\leq$ 0.1 mg/kg	0.0002 mg/kg
Arsenic (As)****	$\leq$ 0.1 mg/kg	0.0014 mg/kg

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

* LOD: 0.017 ppb, LOQ: 0.050 ppb ** LOD: 0.026 ppb, LOQ: 0.080 ppb

*** LOD: 1.400 ppb, LOQ: 5.400 ppb **** LOD: 0.049 ppb, LOQ: 0.148 ppb

[redacted]

Director, Head of Quality Management Division

CELL BIOTECH Co., Ltd.
Headquarters : 50, Aegibong-ro 409 beon-gil, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
Manufacturer : 397, Aegibong-ro, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
PHONE +82 31 987 8107 | FAX +82 31 987 6216 | www.cellbiotech.com

From: Joel Villareal
To: Hice, Stephanie
Cc: Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Kent Phan; Livia Consedine
Subject: [EXTERNAL] FW: GRN 001081 - Questions for Notifier
Date: Friday, June 30, 2023 3:28:40 PM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png
image007.png
II964.1-CBI.7.pdf

Dear Dr. Hice,

In response to the two additional questions received on 6/27/23 regarding GRN 001081 (*Bifidobacterium infantis* CBT BT1), attached you will find the responses to these questions (II964.1-CBI.7).

Thank you for sending your feedback and if there any other questions/concerns, please let us know.

Best Regards.

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Tuesday, June 27, 2023 at 9:27 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 001081 - Questions for Notifier

Dear Mr. Lassiter,

During our evaluation of GRAS Notice No. 001081, we noted additional questions that need to be addressed and are below.

1. In the April 15, 2023, amendment to the notice, the notifier states, "*Microbiological testing such as E. coli, S. aureus, Salmonella, L. monocytogenes is performed and meets specifications as shown in the Certificate of Analysis for each presented batch". The accompanying COAs list the specifications for S. aureus and L. monocytogenes as absent in 1 g and absent in 10 g, respectively. In the June 13, 2023, amendment, the notifier provides the analytical methods for the microbial specifications. Per the provided analytical methods, the analyses for both S. aureus and L. monocytogenes are performed on 25 g samples, not 1 g and 10 g, respectively. Further, the analytical method for L. monocytogenes states, "Report result as presence or absence for Listeria in 25 g sample". Therefore, for the administrative record, please clarify the sample size for both S. aureus and L. monocytogenes.*

2. For the administrative record, please provide a revised copy of Table 4 for all specifications, including microorganisms (i.e., coliforms, yeast and mold, *E. coli*, *S. aureus*, *Salmonella* serovars, and *L. monocytogenes*) and heavy metals. Please include the most recent revisions made to the specifications (e.g., revision of the lead specification).

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
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stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Their (what is this?)

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6/30/2023

Stephanie Hice, PhD
Regulatory Review Scientist & Microbiology Reviewer
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
stephanie.hice@fda.hhs.gov

RE: Response to FDA Questions Regarding GRN 001081 Received on 6/27/23
II964.1-CBI.7

Dear Dr. Hice,

REJIMUS, INC. received your email dated 6/27/23 regarding additional FDA questions to GRN 001081. This is the response to address the two questions presented.

Should you have any questions or concerns with this additional information based on the information provided, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS REGARDING GRN 001081 RECEIVED ON 6/27/23

Question 1

1. In the April 15, 2023, amendment to the notice, the notifier states, "Microbiological testing such as *E. coli*, *S. aureus*, *Salmonella*, *L. monocytogenes* is performed and meets specifications as shown in the Certificate of Analysis for each presented batch". The accompanying COAs list the specifications for *S. aureus* and *L. monocytogenes* as absent in 1 g and absent in 10 g, respectively. In the June 13, 2023, amendment, the notifier provides the analytical methods for the microbial specifications. Per the provided analytical methods, the analyses for both *S. aureus* and *L. monocytogenes* are performed on 25 g samples, not 1 g and 10 g, respectively. Further, the analytical method for *L. monocytogenes* states, "Report result as presence or absence for *Listeria* in 25 g sample". Therefore, for the administrative record, please clarify the sample size for both *S. aureus* and *L. monocytogenes*.

Response

For the administrative record, the sample size for both S. aureus and L. monocytogenes is 25g.

Question 2

2. For the administrative record, please provide a revised copy of Table 4 for all specifications, including microorganisms (i.e., coliforms, yeast and mold, *E. coli*, *S. aureus*, *Salmonella* serovars, and *L. monocytogenes*) and heavy metals. Please include the most recent revisions made to the specifications (e.g., revision of the lead specification).

Response

Below is the revised Table 4 that includes all specifications for the ingredient:

Parameter	***Limits***	***Method***
Appearance	*Light brown powder*	*Visual*
Viable Cell Count	*≥ 1.0 x 10^{11} CFU/g*	*In-house test method*
Coliforms	*Absent*	*Korean FDA Food Code VIII. Food Analytical Method, 4.7 Coliforms*
Yeast and Mold	*≤ 10 CFU/g*	*In-house test method*
E. coli	*Absent in 1g*	*KFDA Food Code, VIII. Food Analytical Method, 4.8 E. coli*
S. aureus	*Absent in 25g*	*In-house test method*
Salmonella	*Absent in 25g*	*In-house test method*
L. monocytogenes	*Absent in 25g*	*In-house test method*
Lead	*≤ 0.01 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*
Cadmium	*≤ 0.3 mg/kg*	*KFDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*
Mercury	*≤ 0.1 mg/kg*	*KFDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*

Parameter	***Limits***	***Method***
Arsenic	*≤ 0.1 mg/kg*	*KFDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions submitted as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions or requests on the above response or the prior responses, please let us know at your earliest convenience and we will do everything we can to address those promptly.

From:	Joel Villareal
To:	Hice, Stephanie
Cc:	Jim Lassiter; Kenneth Cairns; Brandon M. Griffin; Kent Phan; Livia Consedine
Subject:	[EXTERNAL] Re: GRN 001081 - Questions for Notifier
Date:	Monday, August 7, 2023 4:13:35 PM
Attachments:	image001.png image002.png image003.png image004.png image005.png image006.png image007.png image008.png II964.1-CBI.8.pdf

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Dear Dr. Hice,

In response to the two additional questions received on 7/24/23 regarding GRN 001081, attached you will find the response to these questions (II964.1-CBI.8).

Thank you for sending your feedback and if there any other questions/concerns, please let us know.

Sincerely,

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

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From: Joel Villareal <joel@rejimus.com>
Date: Wednesday, July 26, 2023 at 2:53 PM

To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Cc: Jim Lassiter <jim@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>, Brandon M. Griffin <brandon@rejimus.com>, Kent Phan <kent@rejimus.com>, Livia Consedine <livia@rejimus.com>
Subject: FW: GRN 001081 - Questions for Notifier

Dear Dr. Hice,

Thank you for your email. This is to acknowledge receipt of the two questions below for GRN 001081. We are working on addressing the questions and intend to provide a response within 10 business days of the issuance of these questions. Therefore, the response is anticipated to be provided by Monday, 8/7/23.

Sincerely,

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Monday, July 24, 2023 at 5:56 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 001081 - Questions for Notifier

Dear Mr. Lassiter,

During our evaluation of GRAS Notice No. 001081, we noted additional questions that need to be addressed and are below.

1. In the June 30, 2023, amendment, the notifier provided a revised Table 4 that included the proposed specification of "absent" for coliforms. For the administrative record, please clarify the sample size for coliforms (e.g., "absent in 10 g").

2. In the June 30, 2023, amendment, the notifier provided a revised Table 4 that included the proposed specification of ≤ 0.3 mg/kg for cadmium and ≤ 0.1 mg/kg for arsenic and mercury. However, we note that the results of the batch analyses provided for cadmium in the June 22, 2023, amendment were similar to those for arsenic and mercury. For consistency with the specifications proposed for arsenic and mercury and in keeping with FDA's Closer to Zero initiative for heavy metals, please consider lowering the specification for cadmium to at least ≤ 0.1 mg/kg. Please include the revised Table 4 in your response.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients
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stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Their (what is this?)

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8/7/2023

Stephanie Hice, PhD
Regulatory Review Scientist & Microbiology Reviewer
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
stephanie.hice@fda.hhs.gov

RE: Response to FDA Questions Regarding GRN 001081 Received on 7/24/23
II964.1-CBI.8

Dear Dr. Hice,

REJIMUS, INC. received your email dated 7/24/23 regarding additional FDA questions to GRN 001081. This is the response to address the two questions presented.

Should you have any questions or concerns with this additional information based on the information provided, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS REGARDING GRN 001081 RECEIVED ON 7/24/23

Question 1

1. In the June 30, 2023, amendment, the notifier provided a revised Table 4 that included the proposed specification of "absent" for coliforms. For the administrative record, please clarify the sample size for coliforms (e.g., "absent in 10 g").

Response

For the administrative record, the sample size for coliform testing is, "absent in 10 g."

Question 2

2. In the June 30, 2023, amendment, the notifier provided a revised Table 4 that included the proposed specification of ≤ 0.3 mg/kg for cadmium and ≤ 0.1 mg/kg for arsenic and mercury. However, we note that the results of the batch analyses provided for cadmium in the June 22, 2023, amendment were similar to those for arsenic and mercury. For consistency with the specifications proposed for arsenic and mercury and in keeping with FDA's Closer to Zero initiative for heavy metals, please consider lowering the specification for cadmium to at least ≤ 0.1 mg/kg. Please include the revised Table 4 in your response.

Response

For consistency with the specifications proposed for arsenic and mercury and in keeping with FDA's Closer to Zero initiative for heavy metals, the specification for cadmium has been updated to ≤ 0.1 mg/kg. Below is the revised Table 4 that includes all specifications for the ingredient:

Parameter	***Limits***	***Method***
Appearance	*Light brown powder*	*Visual*
Viable Cell Count	*≥ 1.0 x 10^{11} CFU/g*	*Analytical Method of Viable Cell Count (In-house test method)*
Coliforms	*Absent in 10 g*	*Korean FDA Food Code VIII. Food Analytical Method, 4.7 Coliforms*
Yeast and Mold	*≤ 10 CFU/g*	*Analytical Method of Yeast and Mold (In-house test method)*
E. coli	*Absent in 1 g*	*Korean FDA Food Code, VIII. Food Analytical Method, 4.8 E. coli*
S. aureus	*Absent in 25 g*	*Analytical Method of S. aureus (In-house test method)*
Salmonella	*Absent in 25 g*	*Analytical Method of Salmonella (In-house test method)*
L. monocytogenes	*Absent in 25 g*	*Analytical Method of L. monocytogenes (In-house test method)*
Lead	*≤ 0.01 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*
Cadmium	*≤ 0.1 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*

Parameter	***Limits***	***Method***
Mercury	*≤ 0.1 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*
Arsenic	*≤ 0.1 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions submitted as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions or requests on the above response or the prior responses, please let us know at your earliest convenience and we will do everything we can to address those promptly.

From: Joel Villareal
To: Hice, Stephanie
Cc: Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Livia Consedine; Kent Phan
Subject: [EXTERNAL] FW: GRN 001081 - Questions for Notifier
Date: Monday, October 2, 2023 3:11:54 PM
Attachments: image001.png

Dear Dr. Hice,

Thank you for your email. We would like to respectfully respond to the question below and confirm the estimated dietary exposure of the ingredient.

1. In the amendment dated April 20, 2023 (response to Question 25), the notifier multiplied the values of 8.94 x 10^8 CFU/person (p)/d and 1.85 x 10^9 CFU/p/d by three (i.e., by the number of suggested daily servings) to obtain the eaters-only estimate of dietary exposure at the mean and 90th percentile, respectively. We note that based on the information provided in Table 7 of GRN 001081, the values of 8.94 x 10^8 CFU/p/d and 1.85 x 10^9 CFU/p/d already account for the number of servings consumed per person day that were estimated based on food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES). Therefore, we consider that multiplying these values by three suggested daily servings was inappropriate. Please confirm that the estimated eaters-only dietary exposure to the ingredient would be 8.94 x 10^8 CFU/p/d at the mean and 1.85 x 10^9 CFU/p/d at the 90th percentile for the U.S. population aged 2 years and older and that these updated dietary exposure estimates would not affect the notifier's GRAS conclusion.

Response:
We confirm for GRN 001081 that the estimated eaters-only dietary exposure of the ingredient for the U.S. population aged 2 years and older is 8.94 x 10^8 CFU/p/d at the mean and 1.85 x 10^9 CFU/p/d at the 90th percentile. As such, the updated dietary exposure estimates would not affect the GRAS conclusion for GRN 001081.

If there are any questions regarding this response, please let us know and we will be sure to address that promptly.

Sincerely,

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

signature_1575762594

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Friday, September 29, 2023 at 2:30 PM
To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 001081 - Questions for Notifier

Dear Mr. Lassiter,

During our evaluation of GRAS Notice No. 001081, we noted an additional question that needs to be addressed and is attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

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

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
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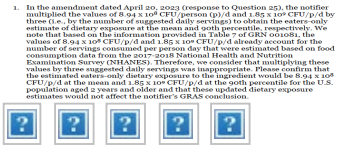
Pronouns: They-Them-Their (what is this?)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From: Joel Villareal
To: Highbarger, Lane A
Cc: Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Livia Consedine; Kent Phan
Subject: [EXTERNAL] FW: Wash step in GRNs 1078-1088
Date: Friday, October 6, 2023 7:53:13 PM
Attachments: image001.png

Dear Dr. Highbarger,

Thank you for your email. Below is the response to the following question.

Request:
Is there a wash step in the purification process in GRNs 1078-1088 after the microorganisms are separated by filtration?

Response:
There is no wash step in the purification process after the microorganisms are separated.

The Sponsor has brought to our attention a translation issue concerning the separation process. As a clarification, microorganisms are separated not by filtration, but by using a centrifugation method. During this process, the microorganisms are spun down and concentrated. Following this step, all fermentation medium is removed and the microorganisms are transferred into the blending process. Please note that this process applies to all notified microorganisms from Cell Biotech Co. Ltd.

If there are any questions regarding this response, please let us know and we will be sure to address that promptly.

Sincerely,

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

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