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ORIGINAL SUBMISSION

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Soni & Associates Inc.

749 46th Square
Vero Beach, FL 32968, USA
Telephone: 772-299-0746
Facsimile: 772-299-5381
E-mail: msoni@soniassociates.net

GRN 000569

February 6, 2015

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Subject: GRAS Notification for Galacto-oligosaccharide (Infant Formula Use)

Dear Sir/Madam:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), New Francisco Biotechnology Corporation, through Soni & Associates Inc. as its agent, hereby provides notice of a claim that the food ingredient galacto-oligosaccharide preparations (King-Prebiotics®) for its use in infant formula and follow-on formula as described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be Generally Recognized As Safe (GRAS), based on scientific procedures.

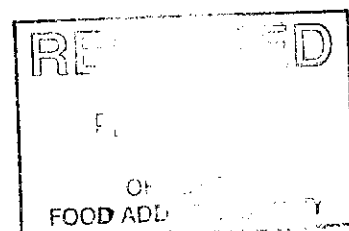
As required, please find enclosed three copies of the notification. If you have any questions or require additional information, please feel free to contact me by phone at 772-299-0746 or by email at sonim@bellsouth.net.

Sincerely,

(b) (6)

Madhu G. Soni, Ph.D.

www.soniassociates.net



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I. Claim of GRAS Status

A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

New Francisco Biotechnology Corporation (NFBC), Yunfu City, Guangdong Province, China, has determined that galacto-oligosaccharides derived from lactose is Generally Recognized As Safe under the conditions of its intended use in infant formula and, therefore, is exempt from the requirement of premarket approval. This determination is based on scientific procedures as described in the following sections. NFBC is also of the opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

Signed,

(b) (6)



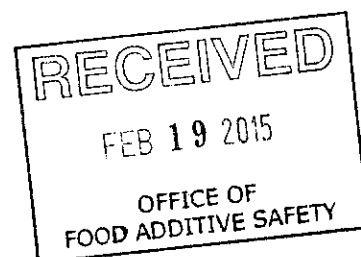
February 6, 2015

Madhu G. Soni, Ph.D., FATS, FACN

Date

Agent for:

New Francisco Biotechnology Corporation
Yunfu City, Guangdong Province,
CHINA



B. Name and Address:

Hank Tsai, Ph.D.
New Francisco Biotechnology Corporation
Swan-kan-chiau Industrial District
Kaofong Village, Yunfu City,
Guangdong Province,
CHINA 527343

Tel: +86 766-8750999 / 8750777

Fax: +86 766-8750800

Email: hank.tsai@king-prebiotics.com

C. Common or usual name of the GRAS substance:

The common name of the substance of this GRAS assessment is galacto-oligosaccharides (GOS). Depending on the customer requirements, GOS for uses in infant formula will be marketed as standardized powder or syrup under the trade-name King-Prebiotics® (GOS-1000-P, GOS-900-P, GOS-700-P, GOS-570-S or GOS-270-P).

D. Conditions of use:

NFBC intends to market their galacto-oligosaccharides (GOS) as an ingredient for addition to term infant formula, and follow-on formula at a use level providing up to 7.2 g of GOS/L of the reconstituted or ready to consume product. In addition to infant formula, NFBC GOS will be added to foods such as cereals, baby foods, and yogurt that are occasionally consumed by infants. These foods have been considered in previous GRAS notices, including GRN 495 and GRN 286. GOS is intended for addition to term infant formula and follow-on formula as a dietary source of non-digestible oligosaccharides. These oligosaccharides are generally recognized as safe and suitable alternative to non-digestible human milk oligosaccharides that are found at high concentrations in human milk from lactating women.

E. Basis for GRAS Determination:

In accordance with 21 CFR 170.30, GOS has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. A comprehensive search of the scientific literature was utilized for this determination. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data to determine safety-in-use for GOS. In recent years, GOS has been the subject of nine GRAS notifications (GRN 518; GRN 495; GRN 489; GRN 484; GRN 334; GRN 286; GRN 285; GRN 236; and GRN 233). The GRAS notice GRN 233 was for a combination of GOS and polydextrose. In response to all of these notices, FDA did not question the conclusions that the use of GOS is GRAS under the conditions of use described in the notices. The most recent notice to FDA, GRN 518 was submitted by NFBC. Among these GRAS notices, the following seven were related to use of GOS in infant formula: GRN 495; GRN 489; GRN 334; GRN 286; GRN 285 (certain baby, infant, and toddler foods); GRN 236; and GRN 233 (combination of GOS and polydextrose).

The safety determination of GOS for the present GRAS assessment is based on the totality of the available scientific evidence that includes human observations, a variety of preclinical studies, and clinical studies in infants and adults. An Expert Panel was assembled to evaluate the health aspects of GOS. Based on the available safety-related information, the Expert Panel concluded that the intended uses of GOS in term infant formula and follow-on formulas described herein are safe.

F. Availability of Information:

The data and information that forms the basis of NFBC's GOS GRAS determination will be available for the Food and Drug Administration's review and copying at the following address or will be provided to the agency upon request:

Madhu G. Soni, Ph.D., FATS
Soni & Associates Inc.,
749 46th Square,
Vero Beach FL, 32968
Phone: (772) 299-0746; E-mail: sonim@bellsouth.net

II. Detailed Information About the Identity of the GRAS Substance:

GOS is derived from lactose via a transgalactosylation catalyzed by β -galactosidase enzyme that has been determined to be safe.

A. Synonyms and Trade Name:

Galacto-oligosaccharide; transgalactosylated oligosaccharide; transgalacto-oligosaccharide; and oligogalactosyl-lactose.

The terms transoligosaccharide (TOS) and trans-galacto-oligosaccharide (TGOS) are synonymously used for GOS. However, as the term GOS is used most frequently, in this dossier, it is used.

The subject of this GRAS assessment will be marketed under the trade-name King-Prebiotics® GOS (GOS-1000-P, GOS-900-P, GOS-700-P, GOS-570-S or GOS-270-P).

B. Physical Characteristics

Off white light yellow powder or off white syrup

C. Chemical Abstract Registry (CAS) Number

The GOS that is the subject of this GRAS determination is primarily comprised of 4'-galacto-oligosaccharides. The CAS Registry Number for this specific type of oligosaccharide is 6587-31-1. In general, "oligosaccharides" (comprising carbohydrates, sugars, oligosaccharides, β -oligosaccharides and oligomeric monosaccharides) has a CAS number of 66455-21-8.

D. Chemical Formula and Molecular Weight

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GOS is a mixture of β -linked polymers in various $\beta(1-3)$, $\beta(1-4)$, $\beta(1-6)$ configurations, and the average number of galactose moieties in the GOS molecules is approximately 2.31. It consists of di- to octa-saccharides composed of 1-7 galactose units linked to a glucose molecule at the reducing end. Among different saccharides found in GOS, the trisaccharide [O-beta-D-galactopyranosyl-(1-4)-O-beta-Dgalactopyranosyl-(1-4)-beta-D-glucose] is the major one. The molecular weight of the individual oligosaccharides ranges from 342 (disaccharide) to 1315 (octasaccharide) Daltons.

E. Structure

The general chemical structure of GOS is presented in Figure 1. In the figure, p represents 0 to 6 groups.

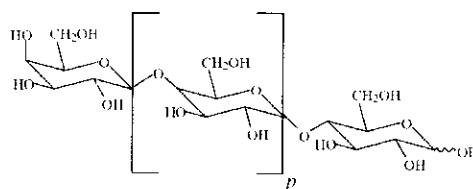


Figure 1. General Chemical Structure of GOS (p = 0 to 6)

F. Manufacturing process

GOS is manufactured according to current good manufacturing practices (cGMP) and ISO standards, as outlined in Figure 2, at New Francisco (Yunfu) Biotechnology Corporation (NFBC) facilities located at Swan-kan-chiau Ind. Dist., Kaofong Village, Yunfu City, Guangdong, Zip: 527343, China. The manufacturing details of different products form of powder (GOS-1000-P, GOS-900-P, GOS-700-P and GOS-270-P) and syrup (GOS-570-S) are shown in Figure 2.

In general, GOS is manufactured through a multistage process from food grade lactose via a transgalactosylation reaction catalyzed by a β -galactosidase enzyme obtained from the non-toxicogenic non-pathogenic microorganism. King-Prebiotics® GOS is prepared from edible lactose, isolated from sweet whey (derived from cow's milk). The lactose is subjected to the action of β -galactosidases which offer three kinds of activities: hydrolysis for breaking the galactose- $\beta(1-4)$ glucose bond to release glucose and galactose, transgalactosylation for converting lactose into galacto-oligosaccharides, and isomerization for the formation of galactose- $\beta(1-3)$ and $1-6$ glucose bonds. The β -galactosidase is derived from a non-toxicogenic non-pathogenic microorganism, *Bacillus circulans* strain¹, commonly used in food processing. The enzymatic reaction produces galacto-oligosaccharides with increasing chain lengths by a series of transgalactosylation reactions.

¹ Proprietary information, additional information if required will be shared separately

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The lactose solution is prepared by dissolving food-grade lactose in deionized water at an elevated temperature. The enzyme, β -galactosidase, derived from *B. circulans* is added to lactose solution in a fermentor. The enzymatic reaction between lactose and β -galactosidase is initiated by sodium carbonate (for pH adjustment) and subsequently terminated by citric acid. This process results in the formation of solution containing at least 57% of GOS. All final products containing different levels of GOS (from 27 to 99%) are prepared from this solution.

For the preparation of GOS-570-S syrup, following the termination of enzymatic reaction the solution is decolorized with activated carbon, filtered through perlite filter and purified using ion exchange resin. The purified GOS is evaporated resulting in the formation of GOS-570-S syrup. For the preparation of GOS-270-S, the 57% GOS solution is blended with maltodextrin followed by decolorization, filtration, purification, evaporation, spray drying and packaging.

For preparation of high purity GOS products such as GOS-700-P, GOS-900-P and GOS-1000-P (70, 90 and 99% GOS), following the termination of enzymatic reaction, GOS concentration of the solution is further increased through fermentation by addition of yeast and yeast extract. The yeast is derived from a non-toxicogenic non-pathogenic microorganism, *Kluyveromyces lactis* strain², commonly used in food processing. Both the microorganism strains (*B. circulans* and *K. lactis*) used in the GOS production are registered with ATCC and are the same as those mentioned in GRN 518. Citric acid is added to inactivate the yeast. A disc separator is employed to remove the yeast and yeast extract, followed by decolorization with activated carbon, and filtration using a Perlite filter.

The adsorption and filtration characteristics of activated carbon and Perlite, respectively, are responsible for removal of the enzyme and other impurities from the product. These filtering aids are removed from the product by filtration and purification with ion exchange resins. Excess water in the product is removed by evaporation to produce GOS syrup. The syrup thus obtained is then spray dried into a powder resulting in the production of GOS-700-P, GOS-900-P and GOS-1000-P.

All raw materials and processing aids used in the manufacture of GOS are suitable food-grade materials and/or are used in accordance with applicable U.S. federal regulations for such uses. The manufacturing facility is registered with FDA under the number: 19919474440. Additionally, the facility is ISO certified: ISO9001 2008(2003/08) and ISO 22000 HACCP (2005/08). Furthermore, NFBC has over 20-years experience in saccharide production and as per various international quality management systems, including QS Production, HALAL, OU Kosher, GMO-FREE IP, and SA8000 certification that guarantee premium quality of a series of international-grade oligosaccharide (King-Prebiotics®) products that are manufactured from food grade lactose.

² Proprietary information, additional information if required will be shared separately

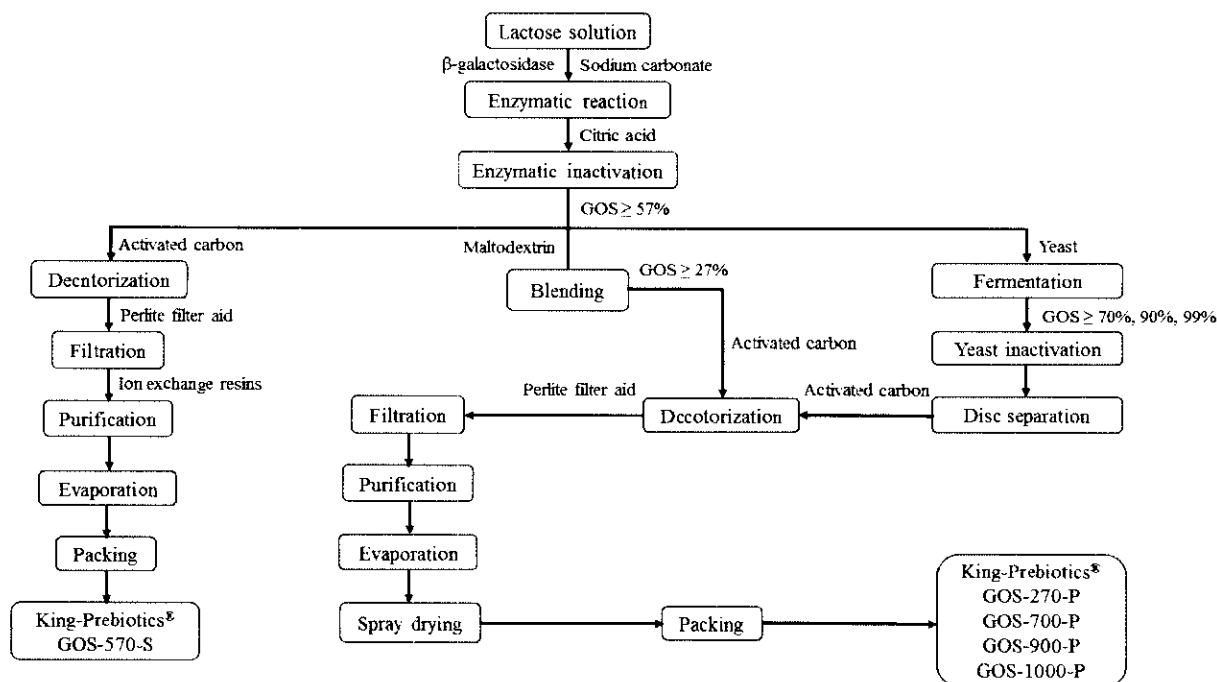


Figure 2. Manufacturing Process of GOS

G. Specifications

Specifications of GOS have been established by NFBC. GOS will be marketed in powder (GOS-1000-P, GOS-900-P, GOS-700-P and GOS-270-P) and syrup (GOS-570-S) forms. The primary difference between these different marketed products is the levels of GOS and other sugars (lactose and monosaccharide). The specifications along with results from five non-consecutive batches for all these products are presented in Tables 1 to 5. In order to demonstrate conformance with the specifications, NFBC analyzed several batches of GOS and analytical results from five non-consecutive lots (Table 1-5) suggest that GOS is consistently manufactured to meet the standard specifications. The specification parameters comprise physical appearance, purity, and GOS distribution, as well as limits for potential chemical and microbiological impurities, and contaminants. The analytical methods used are same as those mentioned in GRN 518. The subject of this GRAS determination, GOS-1000-P is the same as that described in recent FDA GRAS notice (GRN 518) by NFBC (2014). The subject of present GRAS notice is substantially equivalent to GOS that was the subject of GRAS notified substances reviewed by the FDA without any questions [including GRN 495 (Clasado, 2014a); GRN 489 (IDII, 2014); GRN 484 (Clasado, 2014b); GRN 334 (Yakult, 2010); GRN 286 (GTC Nutrition, 2009a); GRN 285(GTC Nutrition, 2009b); GRN 236 (Friesland, 2007)].

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Table 1. Specifications and Batch Data for GOS (GOS-1000-P)*

Parameters	Standard Specifications	Batch [REDACTED]	Batch (b) [REDACTED]	Batch (b) [REDACTED]	Batch (b) [REDACTED]	Batch (b) [REDACTED]
Appearance	Off white powder	Off white powder	Off white powder	Off white powder	Off white light powder	Off white powder
Taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste
Galacto-oligosaccharides	>99%	99.5%	99.6%	99.4%	99.5%	99.2%
Lactose + Monosaccharides	≤1%	1%	1%	1%	1%	1%
pH (10%)	3.0-6.0	3.5	4.2	3.8	4.5	3.7
Moisture	≤3.5%	1.8	2.3	2.1	1.9	2.5
Sulphated ash	≤0.3%	0.012	0.031	0.042	0.023	0.038
Nitrogen	≤0.032%	0.016	0.008	0.014	0.015	0.013
Nitrite	≤2 ppm	<1	<1	<1	<1	<1
Solubility	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water
Heavy metals						
Lead	≤0.02 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	≤0.05 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	≤0.1 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Total mercury	≤0.01 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological limits						
Total plate count	≤50 cfu/g	<10	<10	<10	<10	<10
Yeast	≤20 cfu/g	<10	<10	<10	<10	<10
Molds	≤20 cfu/g	<10	<10	<10	<10	<10
<i>E. coli</i> and <i>Salmonella</i>	Negative/g	Negative	Negative	Negative	Negative	Negative
<i>Salmonella</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Shigella</i>	Negative/g	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative/g	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	Negative/g	Negative	Negative	Negative	Negative	Negative
<i>Listeria</i>	Negative/50 g	Negative	Negative	Negative	Negative	Negative
<i>Bacillus cereus</i>	≤10 cfu/g	<10	<10	<10	<10	<10
<i>Cronobacter sakazakii</i>	Negative/g	Negative	Negative	Negative	Negative	Negative
<i>Sulphite-reducing clostridium</i>	≤10 cfu/g	<10	<10	<10	<10	<10

*Based on information provided by NFBC; All analytical methods used are in full compliance with Chinese Regulations

Table 2. Specifications and Batch Data for GOS (GOS-900-P)*

Parameters	Standard Specifications	Batch (b)	Batch (b)	Batch (b)	Batch (b)	Batch (b)
Appearance	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder
Taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste
Galacto-oligosaccharides	≥90%	91.1	91.5	91.0	91.4	91.5
Lactose & Monosaccharides	≤10%	8.9	8.5	9.0	8.6	8.5
pH (10%)	3.0-6.0	4.4	4.4	4.5	4.6	4.5
Moisture	≤3.5%	1.8	2.1	1.5	1.5	1.3
Sulphated ash	≤0.3%	0.013	0.012	0.010	0.015	0.013
Nitrogen	≤0.032%	0.014	0.009	0.012	0.011	0.013
Nitrite	≤2 ppm	<1	<1	<1	<1	<1
Solubility	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water
Heavy metals						
Lead	≤0.02 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	≤0.05 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	≤0.1 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Total mercury	≤0.01 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological limits						
Total plate count	≤50 cfu/g	<10	<10	<10	<10	<10
Yeast	≤20 cfu/g	<10	<10	<10	<10	<10
Molds	≤20 cfu/g	<10	<10	<10	<10	<10
<i>E. coli</i>	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0
<i>Salmonella</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Shigella</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Enterobacteriaceae</i>	<0.3MPN/g	<0.3	<0.3	<0.3	<0.3	<0.3
<i>Listeria</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Bacillus cereus</i>	<100 cfu/g	<10	<10	<10	<10	<10
<i>Cronobacter Sakazakii</i>	Negative/g	Negative	Negative	Negative	Negative	Negative

*Based on information provided by NFBC; All analytical methods used are in full compliance with Chinese Regulations

Table 3. Specifications and Batch Data for GOS (GOS-700-P)*

Parameters	Standard Specifications	Batch (b)	Batch (b)	Batch (b)	Batch (b)	Batch (b)
Appearance	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder
Taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste
Galacto-oligosaccharides	≥70%	72.0	71.8	71.4	70.7	72.6
Lactose & Monosaccharides	≤30%	28.0	28.2	28.6	29.3	27.4
pH (10%)	3.0-6.0	4.5	4.4	4.3	4.6	4.5
Moisture	≤3.5%	2.2	2.0	1.6	1.4	1.1
Sulphated ash	≤0.3%	0.015	0.008	0.010	0.017	0.011
Nitrogen	≤0.032%	0.016	0.008	0.014	0.015	0.013
Nitrite	≤2 ppm	<1	<1	<1	<1	<1
Solubility	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water
Heavy metals						
Lead	≤0.02 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	≤0.05 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	≤0.1 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Total mercury	≤0.01 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological limits						
Total plate count	≤50 cfu/g	<10	<10	<10	<10	<10
Yeast	≤20 cfu/g	<10	<10	<10	<10	<10
Molds	≤20 cfu/g	<10	<10	<10	<10	<10
<i>E. coli</i>	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0
Salmonella	Negative/25g	Negative	Negative	Negative	Negative	Negative
Shigella	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	<0.3MPN/g	<0.3	<0.3	<0.3	<0.3	<0.3
Listeria	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Bacillus cereus</i>	<100 cfu/g	<10	<10	<10	<10	<10
<i>Cronobacter sakazakii</i>	Negative/g	Negative	Negative	Negative	Negative	Negative

*Based on information provided by NFBC; All analytical methods used are in full compliance with Chinese Regulations

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Table 4. Specifications and Batch Data for GOS (GOS-570-S)*

Parameters	Standard Specifications	Batch(b)	Batch(b)	Batch(b)	Batch(b)	Batch(b)
Appearance	Off white light yellow syrup	Off white light yellow syrup	Off white light yellow syrup	Off white light yellow syrup	Off white light yellow syrup	Off white light yellow syrup
Taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste
Galacto-oligosaccharides	≥57%	57.6	57.5	57.8	57.4	57.9
Lactose	≤23%	20.9	21.3	20.5	21.0	20.2
Glucose	≤22	19.9	19.7	20.3	20.2	20.4
Galactose	≥0.8%	1.6	1.5	1.4	1.4	1.5
pH (no dilution)	2.8-3.8	3.5	3.7	3.5	3.4	3.6
Dry matter	≥74%	75.5	75.5	75.0	75.5	75.0
Sulphated ash	≤0.3%	0.016	0.024	0.012	0.013	0.015
Nitrogen	≤0.032%	0.010	0.008	0.020	0.010	0.009
Nitrite	≤2 ppm	<1	<1	<1	<1	<1
Solubility	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water
Heavy metals						
Lead	≤0.02 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	≤0.05 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	≤0.1 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Total mercury	≤0.01 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological limits						
Total plate count	≤50 cfu/g	<10	<10	<10	<10	<10
Yeast	≤20 cfu/g	<10	<10	<10	<10	<10
Molds	≤20 cfu/g	<10	<10	<10	<10	<10
<i>E. coli</i>	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0
<i>Salmonella</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Shigella</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	<0.3MPN/g	<0.3	<0.3	<0.3	<0.3	<0.3
<i>Listeria</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Bacillus cereus</i>	<100 cfu/g	<10	<10	<10	<10	<10
<i>Cronobacter Sakazakii</i>	Negative/g	Negative	Negative	Negative	Negative	Negative

*Based on information provided by NFBC; All analytical methods used are in full compliance with Chinese Regulations

Table 5. Specifications and Batch Data for GOS (GOS-270-P)*

Parameters	Standard Specifications	Batch (b)	Batch (b)	Batch (b)	Batch (b)	Batch (b)
Appearance	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder
Taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste
Galacto-oligosaccharides	≥27%	31.8	32.1	31.8	31.6	31.6
Lactose	≤12%	11.5	11.5	10.9	10.9	11.7
Glucose	≤12	10.4	10.0	10.8	10.8	9.8
Maltodextrin	40-52%	45.5	45.0	44.9	45.1	45.9
pH (10%)	3.0-6.0	4.4	4.5	4.8	4.8	4.8
Moisture	≤3.5%	2.4	2.0	1.7	2.4	2.0
Sulphated ash	≤0.3%	0.009	0.011	0.012	0.017	0.011
Nitrogen	≤0.032%	0.014	0.008	0.014	0.018	0.015
Nitrite	≤2 ppm	<1	<1	<1	<1	<1
Solubility	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water	Completely soluble in water
Heavy metals						
Lead	≤0.02 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	≤0.05 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	≤0.1 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Total mercury	≤0.01 ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological limits						
Total plate count	≤50 cfu/g	<10	<10	<10	<10	<10
Yeast	≤20 cfu/g	<10	<10	<10	<10	<10
Molds	≤20 cfu/g	<10	<10	<10	<10	<10
<i>E. coli</i>	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0
<i>Salmonella</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Shigela</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	<0.3MPN/g	<0.3	<0.3	<0.3	<0.3	<0.3
<i>Listeria</i>	Negative/25g	Negative	Negative	Negative	Negative	Negative
<i>Bacillus cereus</i>	<100 cfu/g	<10	<10	<10	<10	<10
<i>Cronobacter Sakazakii</i>	Negative/g	Negative	Negative	Negative	Negative	Negative

*Based on information provided by NFBC; All analytical methods used are in full compliance with Chinese Regulations

H. Composition

GOS are chains of galactose units, usually with a single terminal glucose molecule [galactose(Gal)*n*-glucose (Glu)]. The type of β -glycosidic linkage between the monomer units is mainly 1 \rightarrow 4 Gal, though other β -glycosidic linkages may also be present. Typical oligosaccharide distribution found in GOS (GOS-1000-P, GOS-900-P and GOS-570-S) is summarized in Table 6. The oligosaccharide distribution of GOS-700-P and GOS-270-P are similar to those of GOS-570-S.

Table 6. Typical Distribution or Fractions of GOS*

Component Name	GOS-1000-P	GOS-900-P	GOS-570-S
Galacto-oligosaccharides	99% (DM)	90%	60%
Disaccharides	-	5%	23%
Trisaccharides	39%	39%	42%
Tetrasaccharide	27%	23%	21%
Pentasaccharide	18%	13%	9%
Hexa-, hepta- and Octo-saccharide	15%	20%	5%
Lactose + Monosaccharides	1% (DM)	10%	NA
Lactose	NA	NA	18.0%
Glucose	NA	NA	20.5%
Galactose	NA	NA	1.5%
Total	100%	100%	100%

*Based on information provided by NFBC; DM = Dry matter; NA = Not applicable

III. Summary of the Basis for the Determination that GOS is GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by NFBC to determine the Generally Recognized As Safe (GRAS) status of GOS. The Expert Panel consisted of the following individuals: Professor John Thomas, Ph.D., FATS (Indiana University School of Medicine); Robert L. Martin, Ph.D. (Retired FDA Deputy Director, DBGNR); and Madhusudan G. Soni, PhD, FACN, FATS (Food Ingredient Safety Consultant).

A comprehensive search of the scientific databases for safety and toxicity information on GOS was conducted through January 2015. Additionally, safety and regulatory evaluations by national and international agencies were also searched and considered for the present assessment. The Expert Panel also reviewed all accessible information in the GRAS Notices on GOS that are in FDA's public inventory. Information related to the other GRAS Notices that is available on FDA's website is hereby incorporated by reference into this document.

Based on a critical evaluation of the pertinent data and information summarized herein, and employing scientific procedures, the Expert Panel members have individually and collectively determined that the addition of GOS to term infant formula and follow-on formula at a use level providing up to 7.2 g of GOS/L of the reconstituted or ready to consume product meeting the specification cited above and manufactured in accordance with

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current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use, as specified herein.

In arriving at this decision that GOS is GRAS, the Expert Panelists relied upon the conclusions that neither GOS nor any of its constituents pose any toxicological hazards or safety concerns at the intended use levels, as well as on published toxicology studies and other articles relating to the safety of the product. It is also the opinion of the Expert Panelists that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion. The GRAS Panel did not prepare a separate report or statement, but reviewed the entire GRAS dossier.

GOS was the subject of nine GRAS notifications to the FDA for use as a food ingredient. Of these nine GRAS notices, seven were related to use of GOS in infant formula or food. The safety information and other relevant information are hereby incorporated by reference into this document and was considered in evaluating the GRAS status of NFBC's proposed use of GOS in infant formula. A synopsis of the pertinent information in these documents is presented below.

IV. Basis for a Conclusion that GOS is GRAS for its Intended Use

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF GALACTO-OLIGOSACCHARIDES AS FOOD INGREDIENT

1. EXECUTIVE SUMMARY

At the request of New Francisco Biotechnology Corporation (NFBC), a comprehensive search of the scientific literature for safety and toxicity information on GOS was conducted through January 2015 by Soni & Associates Inc., to determine the Generally Recognized As Safe (GRAS) status of GOS as a food ingredient. NFBC intends to use GOS in infant formula providing up to 7.2 g of GOS/L of the reconstituted or ready to consume product. From the proposed uses, GOS is expected to be consumed by term infants and toddlers within the general population who may reasonably be expected to consume infant formula and/or follow-on formula products. The proposed use of GOS in infant formula at maximum use levels of 7.2 g/L will result in mean and 90th percentile estimated intake of 5.9 and 8.5 g per infant per day, respectively, for infants aged 0 through 6 months. For infants aged 7 through 12 months, the estimated dietary intakes of GOS will be 5.2 and 7.9 g per infant per day, respectively. As described below, the weight of evidence clearly supports the safety and GRAS status of GOS when it is produced in accordance with cGMP to food-grade specifications, for its intended use. No studies were identified showing any adverse effects when this amount of GOS is added to the infant formula.

1.1. Background

Group of carbohydrates composed of oligo-galactose with small amounts of lactose and glucose are collectively referred to as galacto-oligosaccharides (GOS). Recently, GOS was defined as “a mixture of those substances produced from lactose, comprising between 2 and 8 saccharide units, with one of these units being a terminal glucose and the remaining saccharide units being galactose and disaccharides comprising 2 units of galactose” (Tzortzis and Vulevic, 2009). GOS is produced commercially from lactose by using the enzyme β -galactosidase (Niittynen et al., 2007). GOS and other similar oligosaccharides occur naturally in human milk and may be one of the factors that protect human infants from gastrointestinal pathogenic bacteria (Niittynen et al., 2007). Multiple *in vitro* and *in vivo* experiments have demonstrated the lack of digestion and stability to hydrolysis by digestive enzymes, of GOS (Torres et al., 2010). However, these oligosaccharides are fermented in the colon by bacteria. Several studies in both infants and adults have shown the prebiotic, bifidogenic effect of GOS on colonic flora. The laxative effects of prebiotics, including GOS are attributed to their action as soluble fibers. Thus GOS belongs to the group of prebiotics that provide health benefit to the host mediated by the modulation of the human gut microbiota (Barile and Rastall, 2013). Given the potential health benefits of GOS, NFBC intends to use it as a food ingredient in selected food categories.

1.2. Description, Manufacturing Process and Specifications

As described earlier, the subject of this GRAS determination, GOS is an off white light yellow powder or syrup, practically odorless with a slight sweet taste. It is produced via transgalactosylation of lactose catalyzed by a well characterized galactosidase enzyme from a non-toxicogenic and non-pathogenic microorganism. Additionally, GOS is further concentrated by fermentation using a non-toxicogenic and non-pathogenic microorganism, *Kluyveromyces lactis*

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strain³, commonly used in food processing. GOS is a mixture of β -linked polymers in various configurations [$\beta(1-3)$, $\beta(1-4)$, $\beta(1-6)$], with the average number of galactose moieties in the TGOS molecules of approximately 2.31. The identity and specifications of GOS have been fully developed (see Section II). Food grade specifications of GOS products are presented in Tables 1 to 5 and compositional distribution is summarized in Table 6. The manufacturing process is summarized in Figure 2.

1.3. Natural Occurrence

Oligosaccharides resembling GOS occur naturally in human milk and colostrums, as well as in bovine milk. McVaugh and Miller (1997) reported that oligosaccharides in human milk exhibit a complex and diverse chemical profile of over 130 different compounds. Although small amounts of a few oligosaccharides have been found in the milk of other mammals, this rich diversity of sugars is unique to human milk. In mature human milk, the total concentration of oligosaccharides is variable ranging from 5-8 g/L (Kunz et al., 2000) to 15.4 g/L (Coppa et al., 1991; 1997). As compared to human milk, the chemical profile of bovine milk oligosaccharides is much less diverse, but structurally similar (Gopal and Gill, 2000). Bovine colostrum has been reported to contain 8.5 mg/L GOS (Saito et al., 1987), while mature bovine milk contains only traces of total oligosaccharides without any presence of GOS (Kunz et al., 2000; Saito et al., 1987). However, in fermented milk products, such as yogurt, low levels of GOS (0.03-0.09%) may be present due to the enzymatic activity of microbial β -galactosidases on the milk lactose (Toba et al., 1982).

1.4. Current Uses

For the modulation of the colonic microflora toward a healthy balance, GOS and other prebiotic ingredients are recognized as useful dietary tools. In terms of their prebiotic and functional properties in foods, GOS compares well to other oligosaccharides. GOS is primarily used in infant milk formula, follow-on formula, and infant foods (Playne and Crittenden, 2009; Torres et al., 2010). In infant formulas, GOS is commonly found at levels ranging from 6.0 to 7.2 g/L along with 0.6 to 0.8 g/L fructooligosaccharide (FOS). In addition to this, GOS can be incorporated into a wide variety of foods. It has been used in beverages (fruit juices and other acid drinks), meal replacers, fermented milks, flavored milks, and confectionery products (Torres et al., 2010). It is also used in several baked goods. Bread is considered suitable for GOS incorporation because during the fermentation and baking processes, GOS molecules are not cleaved or consumed. Furthermore, one of the properties of GOS is to retain high moisture which is useful to prevent excessive product drying, thus providing better taste and texture to the bread. Another potential field of GOS application is specialized foods for the elderly and hospitalized people (Torres et al., 2010). Similar to other non-digestible oligosaccharides, GOS have a pleasant taste and can increase the texture and mouth-feel of foods providing bulk properties similar to sucrose.

GOS has been used as food ingredients in Europe and Japan for about 25 years. In Europe, use of GOS is recognized as an approved ingredient by the European Union and by the governments of the United Kingdom, Italian and Dutch. The European Commission Scientific Committee on Food (SCF) reviewed the use of GOS as an ingredient for addition to infant formula, and concluded that the inclusion of up to 8 g/L of a combination of 90% GOS and 10%

³ Proprietary information, additional information if required will be shared separately

high molecular weight oligofructosyl-saccharose (inulin-derived substances) to infant formula and follow-on formula is safe (SCF, 2003). Food Standards Australia New Zealand (FSANZ) also examined the safety of the addition of GOS and inulin-derived substances to traditional foods, including infant formula and follow-on formula. The agency concluded that the addition of GOS to infant formula is safe at concentration up to 8 g/L (FSANZ, 2008).

Based on information from FDA's GRAS Notice Inventory⁴ website as of January 23, 2015, the agency has received nine notices on GOS and provided "no questions" letters to all the notifiers. The most recent notice that received the no question letter from FDA on December 22, 2014, was submitted by NFBC. In September 2007, Mead Johnson & Company submitted the first GRAS notification (GRN 233) on use of GOS and polydextrose (Mead Johnson, 2007). On September 04, 2009, FDA issued "no questions" letter for this GRAS notice (FDA, 2009c). Subsequently, eight GRAS notifications specifically on GOS were submitted to FDA by the following companies: Friesland Foods Domo (Friesland, 2007; GRN 236); GTC Nutrition (2009a; GRN 285), GTC Nutrition (2009b; GRN 286); Yakult Pharmaceutical Industry Co., Ltd. (Yakult, 2010; GRN 334); Clasado Inc. (Clasado, 2014b, GRN 484); International Dairy Ingredients, Inc. (IDII, 2014, GRN 489); and Clasado Inc. (Clasado, 2014a; GRN 495). Each of these firms received a "no questions" letter from FDA⁵. A closely related oligosaccharide, fructo-oligosaccharide, has also been determined to be GRAS for use in a variety of foods (FDA, 2000). This oligosaccharide was determined to be safe when added to a variety of foods, including baby foods, at levels of 0.1-3.6%. Inulin, yet another non-digestible carbohydrate, has been determined to be GRAS for use in a variety of foods (FDA, 2003).

1.5. Intended Use Levels

As mentioned earlier, NFBC intends to market GOS as an ingredient for addition to term infant formula and follow-on formula at a use level providing up to 7.2 g of galacto-oligosaccharides/L of the reconstituted or ready to consume product. These same uses of GOS and the resulting exposures from it have been estimated in the previous GRAS notices to FDA (GRN 495; GRN 334; GRN 286; GRN 236). In the previous estimates reported by GTC Nutrition (2009b), survey data from the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Survey (NHANES) were used. This analysis suggests that 80% of the U.S. infant population is expected to consume GOS from these intended food uses. The intended use of GOS in infant formula at the maximum intended use level of 7.2 g/L was estimated to result in the mean and 90th percentile dietary intakes 5.9 and 8.5 g per infant per day, respectively, for infants aged 0 through 6 months (GTC Nutrition, 2009b). For infants aged 7 through 12 months, the mean and 90th percentile all-users intakes of GOS was estimated as 5.2 and 7.9 g per infant per day, respectively. This analysis also revealed that only 3.7% of toddlers aged 1 to 2 years were estimated to consume GOS from infant formula uses with estimated intakes of 2.8 and 6.6 g per child per day for 90th percentile consumers.

In recent years, various GOS preparations, including GOS manufactured by NFBC, have successfully undergone GRAS determination for uses in a variety of conventional food that may be consumed on occasion by infants (e.g., cereals, baby food, yogurt). Potential exposure of GOS from infant formula and other dietary sources have been considered earlier in GRAS notices reviewed by FDA (GRN 286; GRN 495). In these GRAS notices, it was concluded that

⁴ Accessible at: <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true>.

⁵ Accessible at: <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true>.

consumption of these types of foods in young infants (i.e., 0 to 4 months) is limited, and, among older infants dietary intake of GOS from other foods containing GOS is likely to partially replace dietary intakes from infant formula consumption. Introduction of GOS, as manufactured by NFBC, to the US food supply will not increase dietary intake of GOS by infants and children above levels earlier determined as GRAS.

The GOS product described in GTC Nutrition (2009b) GRAS notice was reported to contain 91% of GOS, while the subject of present GRAS notice contains different products that range on GOS levels from 27% to 99%. Therefore, NFBC intends to proportionately adjust the actual use levels of the products to provide levels of GOS that are identical to those listed in GRN 286 (GTC Nutrition, 2009b; FDA, 2009b) for the respective food categories. The introduction of the GOS by NFBC to the U.S. food supply for use in term infant formula and follow-on formula as described herein will not increase dietary intake of GOS by infants and toddlers above levels previously determined to be GRAS.

2. DATA PERTAINING TO SAFETY

2.1. Preamble

In a series of comprehensive safety evaluations by national and international agencies such as FDA, SCF and FSANZ, the GOS have been extensively reviewed and demonstrated to be safe for use as an ingredient in food, including infant formula. In several published experimental studies and review articles, toxicity potentials of GOS have been summarized. These studies include metabolic (*in vitro* and *in vivo*) experiments, short- and long-term toxicity in experimental animals as well as human clinical studies in adults and infants. The currently marketed GOS products are manufactured using lactose as a starting material that is converted to GOS using β -galactosidase(s) enzymes obtained from different non-toxicogenic and non-pathogenic strains of bacteria. Given the use of similar manufacturing processes, the differences between various GOS products would be limited to minor variations in the compositional distribution of the GOS oligomers, and to differences in the residual levels of lactose. This also suggests that the safety information on GOS products can be interchangeably used. This assumption is consistent with the SCF (2001a; 2001b) and FSANZ (2008) regulatory opinions for the use of GOS in traditional food products and infant formulas. Additionally, FDA also did not question such an assumption.

In recent years, as the new safety-related data and additional uses for GOS have been requested, the regulatory agencies, particularly FDA, have also updated their evaluations. The majority of these studies are described in FDA notifications, including the most recent GRAS notice submitted by NFBC (2014). FDA did not question the acceptability and suitability of the available evidence to support the proposed uses described in nine GRAS notices and replied to all these notifications the agency received with recognition of the notifiers request and a statement that they had no questions regarding the conclusions that the GOS is GRAS for the intended applications, including uses in infant formula. Given the similarity between the FDA notices, as well as the subject of SFA scientific opinion and the subject of present GRAS assessment, it is instructive to review the information presented in these documents on GOS from a safety perspective. In the following section, an attempt has been made to present the relevant safety-related data of GOS to support its intake from the intended uses described in this dossier.

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2.2. GRAS Notices on GOS

2.2.1. GRN 518

In this GRAS notice, NFBC (2014) informed FDA that GOS is GRAS, through scientific procedures, for use as an ingredient in selected foods at concentrations ranging from 0.3 to 11 g/serving. The subject of GRN 518 is identical (identity, specifications, manufacturing) to one of the GOS products GOS-1000-P described in the current GRAS assessment. In GRN 518, NFBC proposed to use to GOS-1000-P in same food categories but at proportionately reduced actual use levels to provide levels of GOS that are identical to those listed in GRN 000334 for the respective food categories. The estimated mean and 90th percentile exposures to GOS for the total population were reported as 12.2 g/person/day (0.28 g/kg body weight (bw)/day) and 25.3 g/person/day (0.70 g/kg bw/day), respectively.

In GRN 518, NFBC described the relevant absorption, distribution, metabolism, and elimination pathways for GOS. GOS is fermented by colonic bacteria to produce innocuous metabolites (e.g., short chain fatty acids, carbon dioxide, and hydrogen gas) that are common fermentation products of the normal diet, and any unfermented GOS is excreted in the feces. NFBC summarized and incorporated by reference toxicological data from previous GRAS notices for the intended uses of GOS. These data included published animal studies, specifically subchronic oral toxicity studies in which rats received up to 2000 mg/kg/day of GOS (the highest dose tested) with no observed adverse effects, as well as published studies in which people consumed 15 g of GOS for 3 weeks and had no adverse effects. Following its review, on December 22, 2014, FDA responded to NFBC that the agency has no questions at this time regarding the conclusion that GOS (GOS-1000-P) is GRAS under the intended conditions of use.

2.2.2. GRN 495

In another recent GRAS notice, Clasado (2014a) informed FDA that GOS is GRAS, through scientific procedures, for use as an ingredient in term infant formula and follow-on formula at up to 7.2 g/L of reconstituted or ready-to-consume product. As part of this notice, Clasado (2014a) included the statement from Expert Panelists, qualified by scientific training and experience to evaluate the safety of substances added to food. The Panelists evaluated the identity, manufacturing, specifications, conditions of use, estimated dietary exposure, and safety information. Based on this review, Clasado's GRAS panel concluded that GOS produced in accordance with good manufacturing practices is GRAS under the conditions of its intended use. In this GRAS notice, Clasado described that GOS was the subject of previous GRAS notices and that slight differences in the manufacturing conditions (e.g., enzyme source organism, reaction time, temperature, pH) are employed by each manufacturer. These differences in manufacturing conditions typically yield mixtures consisting of oligosaccharides that contain between 2 and 8 sugar moieties. GOS, produced by the activity of β -galactosidases, contains β -linkages in 1 \rightarrow 2, 1 \rightarrow 3, 1 \rightarrow 4, or 1 \rightarrow 6 anomeric configurations. The specific β -galactosidase enzyme used in the reaction influences the particular linkages and molecular weight distribution observed between different preparations.

In its GRAS notice, Clasado (2014a) incorporated previous GRAS notices GRNs 000236, 000286, and 000334 by reference and included an update of the literature for the time period through August 2013. Clasado described in detail the chemical identity and structure of GOS, as well as the relevant absorption, distribution, metabolism and elimination pathways that are general scientific principles. GOS is fermented by colonic bacteria to produce innocuous

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metabolites (e.g., short chain fatty acids, CO₂ and H₂) that are common fermentation products of the normal diet. In addition, Clasado discussed published animal studies and clinical trials. Published clinical trials where pre-term and term infants have consumed infant formula with up to 7.2 g/L of GOS, have shown that GOS is safe, well-tolerated, and does not adversely affect infant growth. Clasado also described that the European Commission Scientific Committee on Foods and Food Standards Australia New Zealand have published opinions authorizing the safe use of GOS in infant formula preparations. Based on the information provided by Clasado, as well as other information available to FDA, the agency did not question the Clasado's conclusion that GOS is GRAS under the intended conditions of use.

2.2.3. GRN 489

In this GRAS notice, IDII (2014) informed FDA of its view that GOS is GRAS, through scientific procedures, for use as an ingredient in infant formula and follow-on formula at a maximum concentration of 4 g/L of formula; in follow-on foods at a maximum concentration of 0.38%; and, in yogurts, yogurt drinks, frozen yogurts, ice milks, custards and puddings; dairy shake mixes, instant breakfasts, meal replacements, carbonated and non-carbonated beverages, juice-based beverages, juice coolers, sweetened and flavored waters, ready-to-drink iced teas and coffees, and sport and isotonic drinks at a maximum concentration of 2%. IDII (2014) described information about the identity and composition of GOS. GOS was as a spray-dried powder, synthesized from food grade lactose using a transgalactosidic reaction with a β -galactosidase enzyme derived from *Aspergillus oryzae*. The enzyme catalyzes the extension of lactose to form a mixture of β -linked disaccharide, trisaccharide, tetrasaccharide, and pentasaccharide chains. GOS also contains the monosaccharides glucose, galactose, and fructose, and the disaccharides lactose, maltose, and sucrose. The specifications included the minimum levels of dry matter (≥ 73.5 %) and total GOS (≥ 26.0 %). The specifications also included limits on moisture (≤ 26.5 %), protein (≤ 0.2 %), lactose (≤ 18 % of DM), glucose (≤ 22.0 % of DM), galactose (≥ 8.0 % of DM).

In its response letter, FDA noted that as the uses and proposed concentrations by IDII (2014) are not exactly the same as those in previous GRAS Notices(GRNs 000236, 000286, and 000334), the uses and estimates, while generally substitutive for those in the previous notices, are lower because of lower use levels. The estimates are not additive to the previous estimated exposures in GRN 334 and suggest that there would be no increase in exposure to GOS from IDII (2014) intended uses. Based on the information provided by IDII (2014), as well as other information available to FDA, the agency provided a no questions letter to IDII for use of GOS under the intended conditions of use.

2.2.4. GRN 484

In this GRAS notice, Clasado (2014b) informed FDA that GOS is GRAS, through scientific procedures, for use as an ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, coffee and tea, dairy product analogs, frozen dairy desserts and mixes, grain products and pastas, milk and milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, snacks, and sweet sauces at levels ranging from 0.8 to 3.0 g/serving. The subject of this notice is similar to the one described in the above GRAS notice (GRN 495) by the same company (Clasado, 2014a). In this GRAS notice, Clasado (2014b) provided specifications for GOS that included minimum levels of dry matter ($\geq 70\%$ in the syrup and $\geq 95\%$ in the powder) and total GOS (46 to 60% in the syrup and 55 to 80% in the powder). The specifications also include limits for other parameters such as moisture, protein, lactose,

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total monosaccharides, heavy metals and limits for microbial contaminants for both the syrup and powder.

Clasado (2014b) described that the intended use of GOS will be substitutional for other GOS preparations currently used in foods. Additionally, Clasado (2014b) also proposed uses in coffee and tea. Clasado discussed the current dietary exposure to GOS from intended uses described in previous GRAS notices for a wide variety of foods and beverages (e.g. GRNs 000236, 000285, and 000334). The highest estimated 90th percentile exposure to GOS from these intended uses is 33 g/person/day for male teenagers and 25.3 g/person/day for the total population. For infants and children, 90th percentile exposures were estimated to be up to 26.8 and 30.4 g/person/day, respectively. Clasado estimates the daily exposure to GOS from the intended uses in coffee and tea as 5.0 g/person/day for adult males and 4.4 g/person/day for the total population (users only). Clasado concludes that exposure to GOS from the intended use in coffee and tea will be substitutional for other GOS containing foods, and that the cumulative exposure to multiple GOS containing products at the highest use levels on a chronic basis is unlikely to occur. In a response letter to the notifier on May 8, 2014, FDA stated that the agency has no questions regarding the conclusion that GOS is GRAS under the intended conditions of use.

2.2.5. GRN 334

In this most recent GRAS notice, Yakult Pharmaceutical Industry Co., Ltd. informed FDA that GOS is GRAS, through scientific procedures, for use as an ingredient in term infant formula at a concentration of 7.2 g/L and in the following food categories: milk and milk products, soups, bakery products, cereals, fruit and vegetable juices, sugars and sweets, and non-alcoholic beverages at levels ranging from 0.3 to 9.5 g/serving (Yakult, 2010; FDA, 2010). The GOS described in the notice was reported to be manufactured according to GMP using food-grade lactose that was subjected to the action of two β -galactosidases from *Sporobolomyces singularis* and *Kluyveromyces lactis* leading to the formation of GOS. β -Galactosidase derived from *S. singularis* possesses transgalactosylation activity; this enzyme is used primarily to catalyze the production of GOS with increasing chain length by a series of transglycosylation reactions. The enzyme derived from *K. lactis* primarily degrades unreacted lactose. The GOS is manufactured in syrup and powder forms; both forms contain GOS, residual lactose, glucose, and galactose. The notifier also provided information on the identity and composition of GOS. GOS was reported as a mixture of di- to hexasaccharides composed of 1 to 5 galactose units linked to a glucose molecule at the reducing end. The major saccharide in the GOS fraction was reported as the trisaccharide. The molecular weights of the individual oligosaccharides ranged between 342 (disaccharide) and 991 (hexasaccharide) Da. The GOS was reported to primarily contain 4'-galacto-oligosaccharides. The product was reported to include minimum levels of GOS at 55% (dry weight).

The notifier estimated the mean and 90th percentile daily intake of GOS in infants up to 1 year of age as 14.7 and 26.8 g/person/day, while for the total population it was estimated as 12.2 and 25.3 g/person/day. The notifier discussed absorption, distribution, metabolism (including *in vitro*) and excretion studies of GOS. Published information has shown that GOS is indigestible by gastric juice and α -amylase, but it is fermented when it reaches the colon (Yakult, 2010). In the colon, GOS was reported to be metabolized by colonic microflora to fermentation products (short-chain fatty acids, carbon dioxide, methane and hydrogen gases). The notifier stated that the safety of GOS is supported by published subchronic and genotoxicity studies conducted in

animals. The notifier also discussed published clinical studies in adults and infants including pre-term and term infants to further support its view that GOS is GRAS for the intended uses in infant formula. In a response letter to the notifier dated October 27, 2010, FDA stated that the agency has no questions regarding the conclusion that GOS is GRAS under the intended conditions of use (FDA, 2010).

2.2.6. GRN 286

The subject of this GRAS notice is GOS for use in term infant formula and follow-on formula at a level of 7.2 g GOS/liter (GTC Nutrition, 2009b; FDA, 2009b). The identity, composition, specifications and manufacturing process for GOS were identical to those described in the above described notice (GRN, 285; section 2.2.3). Based on the intended use levels the estimated mean and 90th percentile intake in infants ages 0 to 6 months was determined as 5.9 and 8.5 g/person/day; for infants ages 7 to 12 months as 5.2 and 7.9 g/person/day; and for toddlers ages 1 to 2 years as 2.8 and 6.6 g/person/day, respectively. The notifier discussed absorption, distribution, metabolism, and excretion (ADME) of GOS and noted the indigestibility of GOS by humans as evidenced by lack of hydrolysis by human salivary amylase or pancreatic juices. GOS passes undigested and unabsorbed to the colon where it is metabolized by colonic microflora to normal metabolites of fermentation (short-chain fatty acids, carbon dioxide, methane and hydrogen gases); any unfermented dietary GOS will be excreted in the feces. GOS has been shown to be non-genotoxic in published *in vitro* and *in vivo* genetic toxicity studies. The notifier also discussed the published animal studies, including a 90 day rodent study, and published human studies. The human investigations included infants studies that show that 7.2 g/L GOS in combination with 0.8 g/L fructo-oligosaccharide have no adverse effects. Based on the totality of the scientific evidence, the notifier concluded that GOS is safe for its intended use at a level of 7.2 g/L. Based on the information provided in the notification, as well as other information available to the FDA, the agency did not question the conclusion that GOS is GRAS under the intended conditions of use in term infant formula and follow-on formula.

2.2.7. GRN 285

In 2009, GTC Nutrition, submitted a GRAS notice for use of GOS in certain baby, infant, and toddler foods at levels ranging from 0.86 to 1.28 g/serving; and, in certain beverages and beverage bases, dairy product analogs, milk products, bakery products, cereal and other grain products, desserts, dessert toppings and fillings, fruit and fruit juices, snacks, soups, and soft and hard candy at a level of 1.28 g/serving (GTC Nutrition, 2009a; FDA, 2009a). The notifier provided information about the identity and composition of GOS. GOS was reported as a spray-dried white powder produced from food-grade lactose via a transgalactosylation enzyme reaction using β -galactosidase from *B. circulans*. The final product was reported to contain at least 90% GOS (dry weight basis), with the remaining material characterized primarily as lactose (7-10%), water, and trace amounts of dextrose and galactose. The GOS component was reported as a mixture of β -linked GOS in various β (1-3), β (1-4), β (1-6) configurations, having a degree of oligomerization ranging between 3 and 5.

Based on the intended use levels, the estimated mean and 90th percentile intake was determined as 9.3 and 15.4 g/person/day, respectively. For 0 to 2 year olds, the mean and 90th percentile intakes were reported as 5.7 and 9.8 g/person/day, respectively. The notifier described absorption, distribution, metabolism, and excretion of GOS and concluded that GOS is not hydrolyzed by human salivary amylase or pancreatic juices (GTC Nutrition, 2009a). GOS passes

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undigested and unabsorbed to the colon where it is metabolized by colonic microflora to normal metabolites of fermentation (short-chain fatty acids, carbon dioxide, methane and hydrogen gases). The notifier stated that any unfermented dietary GOS will be excreted in the feces. In genetic toxicity studies, GOS has been shown to be non-genotoxic. The notifier also discussed the published animal studies, including a 90 day rodent study, and published human studies. Based on the information provided by the notifier, as well as other information available to FDA, the agency did not question the conclusion that GOS is GRAS under the intended conditions of use (FDA, 2009a).

2.2.8. GRN 236

In this notice, Friesland Foods Domo informed FDA that GOS is GRAS, through scientific procedures, for use as an ingredient in term infant formula at a level of 5 g/L and in selected food categories such as dairy products, fruit drinks and waters/quenchers, fruit preparations and milk beverages (Friesland, 2007; FDA, 2008). The GOS was manufactured from food-grade lactose via a transgalactosylation enzyme reaction using β -galactosidase from *Bacillus circulans*. The notifier described the identity and composition of its GOS ingredient. GOS is a mixture of di- to octasaccharides composed of 1 to 7 galactose units linked to a glucose molecule at the reducing end. The major saccharide in the GOS fraction of the preparation was reported as the trisaccharide. The molecular weights of the individual oligosaccharides was reported to range between 342 (disaccharide) and 1315 (octasaccharide) Da. The average molecular weight of the GOS fraction is 522.28 Da. The GOS that was the subject of this GRAS notice was reported to primarily contain 4'-galacto-oligosaccharides. The notifier provided product specifications for its GOS ingredient with minimum levels of total GOS ($\geq 57\%$ dry matter) and galactose ($\geq 0.8\%$), and maximum levels of lactose ($\leq 23\%$) and glucose ($\leq 22\%$). The estimated mean and 90th percentile intake of GOS from the proposed uses was determined as 8.0 and 16.8 g/person/day, respectively, for eaters only, two and greater years of age. The mean estimated intake by infants zero to 5 months, 6 to 11 months, and 12 to 23 months of age was determined as 5.3, 6.1, and 5.3 g/infant/day, respectively.

The notifier discussed published studies showing that human milk contains a complex mixture of oligosaccharides (Friesland, 2007). The concentration of complex oligosaccharides in mature human milk was estimated to range from 5 to 8 g/L. The oligosaccharide concentrations as high as 25.6 g/L have been reported in human colostrums and 15.4 g/L in mature milk. The bovine milk oligosaccharides have been reported to be structurally similar to those found in human milk. The notifier discussed published and unpublished studies conducted with their preparation and similar GOS-containing formulations. These studies included *in vitro* and *in vivo* investigations, a 90-day animal study, and adult, term and preterm infant clinical trials. Based on these studies, the notifier concluded that there is no evidence in the available literature of any adverse effects of GOS used at the intended levels. In a response letter to the notifier on July 28, 2008, FDA stated that the agency has no questions regarding the conclusion that GOS is GRAS under the intended conditions of use (FDA, 2008).

Recently, on April 4, 2013, Friesland informed FDA about the addition of new food categories for the intended use of GOS and an increase in the use level in infant formula. Friesland proposed new uses of GOS in baby cereals at a level of 3 g/serving, adult cereals at a level of 5 g/serving, milk drinks (yogurt drinks and fermented milk drinks including kefir) at a level of 7.5 g/serving, dairy based desserts (puddings, custards, and mousses) at a level of 3 g/serving, and juices at a level of 5 g/serving; and, in term infant formula at a level up to 7.2 g/L.

On April 24, 2014, FDA responded to the Friesland supplemental request stating that the agency has no questions regarding the conclusion that GOS is GRAS under the intended conditions of use (FDA, 2014).

2.2.9. GRN 233

This GRAS notice relates to the use of a combination of GOS and polydextrose as an ingredient in milk-based term infant formula at levels not to exceed 2 g/L for GOS and 2 g/L for polydextrose (Mead Johnson, 2007; FDA, 2009c). The notifier described the identity and composition of both GOS and polydextrose. As regards GOS, it was reported as a mixture of di- to octa-saccharides composed of 1 to 7 galactose units linked to a glucose molecule at the reducing end; primarily containing 4'-galacto-oligosaccharides. The average molecular weight of the GOS fraction was approximately 522 Da. The product was reported to contain galacto-oligosaccharides, lactose, glucose, and a small amount of galactose. GOS was produced through the enzymatic conversion of edible lactose isolated from sweet whey (derived from cow's milk) with an enzyme β -galactosidase. The estimated 90th percentile intake of GOS was determined as 0.4 g/kg bw/day. The notifier discussed the safety of GOS from published and unpublished studies, including *in vitro* studies, studies in different animal models, as well as studies in human adults and infants. These studies suggest that administration of these oligosaccharides to experimental animals does not cause any adverse effects on microfloral populations, nutrient absorption and retention, weight gain, or food consumption. Based on these studies, the notifier concluded that consumption of polydextrose and GOS by human adults does not cause any long- or short-term adverse effects and that consumption of up to 1.6 g GOS/kg bw/day by human infants does not cause any adverse effects on microfloral populations, nutrient absorption, blood biochemistry, or growth parameters. The FDA reviewed the notice and responded to the notifier that, based on the information provided in the notification, as well as other information available to the FDA, the agency has no questions at this time regarding the conclusion that GOS and polydextrose is GRAS under the intended conditions of use.

2.3. EFSA

The European Union (EU) Scientific Committee on Food (SCF) reviewed the use of GOS as an ingredient for addition to infant formula, and concluded that the inclusion of up to 8 g/L of a combination of 90% GOS and 10% high molecular weight oligofructosyl-saccharose (inulin-derived substances) to infant formula and follow-on formula is safe (SCF, 2003). The agency also noted that it was not practical to develop specifications for the use of these products in traditional food products or infant formula, and a generic approval of the use of these products has been granted. The Committee concluded that it has no major concerns on the inclusion of up to 0.8 g/100 mL of a combination of 90% oligogalactosyl-lactose and 10% high molecular weight oligofructosyl-saccharose to infant formulae and follow-on formulae (SCF, 2001a). Subsequently, the Committee reviewed additional data from four clinical studies and concluded that the additional information made available, in particular with respect to growth and markers of water balance, does not provide any indication of adverse effects from the use of a formula with up to 0.8 g/100 mL of a combination of 90% oligogalactosyl-lactose and 10% high molecular weight oligofructosyl-saccharose.

2.4. FSANZ

Food Standards Australia New Zealand (FSANZ) also reviewed the safety of the addition of GOS and inulin-derived substances to traditional foods, including infant formula and follow-

on formula. Following its assessment, the agency concluded that the addition of GOS to infant formula up to a concentration of 8 g/L is safe (FSANZ, 2008). The agency estimated the baseline intakes of inulin derived substances and GOS, based on natural sources and added sources at the mean and 90th percentile as 17 and 42 g/person/day for infants age 1 to 3 years. FSANZ concluded that infant and follow-on formula containing up to 8 g/L of inulin-derived substances and/or GOS, singularly or combined, in any ratio, are unlikely to pose a risk to infants. This conclusion was based on data from clinical trials, in which infants were provided formulas supplemented with up to 10 g/L of inulin-derived substances and GOS and no adverse effects were noted. The available data also indicated that these oligosaccharides were fermented to a similar or greater extent than human milk oligosaccharides. The safety at use levels of 8 g/L is further supported by the presence of higher levels of human milk oligosaccharides, up to 25 g/L in breast milk.

2.5. Recent Safety Publications

A literature search of recent publications from scientific databases such as PubMed and Toxline was conducted on GOS to determine whether any additional or new publications appeared during the past one year since the submission of the GRAS notice GRN 518 (NFBC, 2014). The literature search did not reveal any significant new safety-related studies. Hence, all data and information used in support of this GRAS affirmation is the same as that presented in previous nine GRAS notices (see sections 2.2.1. to 2.2.9.) and the data made available to EFSA and FSANZ. Pertinent information related to the safety, primarily from recent publications, is summarized in the following sections.

2.5.1. Summary of Safety Studies

2.5.1.1. Infant Studies

In recent years, consumption of GOS containing infant formulas has been extensively investigated in infants. The addition of GOS to infant formula is intended to provide a dietary source of oligosaccharides that are representative of oligosaccharides found in human milk from lactating mothers (Veereman-Wauters, 2005; Oozeer et al., 2013; Vandenplas et al., 2014). In over 50 published studies, nutritional and physiological effects or safety of GOS containing infant formulas in premature infants, term infants, and infants with atopic disorders has been studied. The majority of these published studies GOS was administered in a 9:1 ratio with long-chain FOS. In several of the studies with GOS/FOS in a ratio of 9:1 in infant milk formulas, positive effects of this mixture on stool characteristics such as stool consistency and stool frequency were observed (Scholtens et al., 2014). Additional studies included use of GOS alone and the co-administration of GOS with acidic oligosaccharide preparations or polydextrose. All these studies suggest that the addition of GOS to infant formula at use levels up to 7.2 g/L is well-tolerated and safe. As described above in several GRAS notices and other international agencies assessments, these studies have been the subject of comprehensive and critical reviews.

The safety evaluations by National and International agencies were performed as per the Institute of Medicine (IOM, 2004) and the American Academy of Pediatrics (AAP, 1988) recommendations. These assessments include the following endpoints within the study design: inclusion of pre-term or term infants; feeding initiated within the first two weeks of life; feeding duration of at least 3 months; inclusion of anthropometric safety evaluations and recruitment of sufficient study subjects to provide adequate statistical power to detect clinically meaningful changes in weight gain. Using these criteria, the safe use of GOS in infant formula in term

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infants at a concentration of up to 7.2 g/L was primarily based on findings by Schmelzle et al. (2003) and Moro et al. (2006). Additional studies by other investigators (referenced in the above described GRAS notices and tabulated in Appendix I) corroborated the findings that the addition of GOS to infant formula is safe, well-tolerated, and does not adversely affect infant growth. The available information indicate that consumption of GOS supplemented infant formula provides physiological changes in gastro-intestinal tract such as promotes *Bifidobacteria* growth, increases production of short-chain fatty acids and corresponding reductions in stool pH, and overall changes in stool consistency that are similar to those observed in infants consuming human milk from lactating women (Veereman-Wauters, 2005; Roberfroid et al., 2010; Oozeer et al., 2013).

The search of recent literature revealed few new studies related to the effect of GOS consumption by infants. Available clinical studies of GOS in infants are summarized in Appendix I. In these studies, GOS was administered alone, or in combination with other oligosaccharides such as FOS, polydextrose, acidic oligosaccharides and/or probiotic ingredients (Appendix I). In a majority of these studies, safety related parameters such as anthropometric indices of growth and monitoring of adverse events and gastrointestinal tolerance were investigated. Additionally, in some studies, the potential immunomodulatory effects of GOS in term-infants have also been investigated (Raes et al., 2010; van der Aa et al., 2012). The findings from the newly published studies on the effect of dietary consumption of GOS supplemented infant formula are consistent with the previous studies suggesting that consumption of infant formula containing GOS at a concentration of up to 7.2 g/L is safe and well tolerated in term infants. In these studies, no evidence of osmotic effects resulting in the development of diarrhea among infants consuming GOS supplemented formulas has been reported.

Sierra et al. (2015) investigated the effects of GOS-containing infant formula (4.4 g/L of GOS) and the subsequent feeding of a GOS-containing follow-on formula (5.0 g/L of GOS). In this recent multicentre, randomized, double-blind and placebo-controlled trial, 365 healthy term infants were enrolled before 8 weeks of age and randomly assigned to a formula with or without GOS, until 12 months of age. The incidence of infections and allergy manifestations, the antibiotics prescribed and fecal characteristics were recorded up to 12 months of age, while fecal samples were collected up to 4 months for the measurement of secretory immunoglobulin A, short-chain fatty acids and microbiota. A prebiotic effect on the fecal analysis was observed at 4 months of life. The GOS group showed a lower fecal pH, a lower decreasing trend in secretory immunoglobulin A, lower butyric acid concentration and an increase in *Bifidobacterium* counts. Changes in fecal characteristics involved greater frequency and softer consistency. The incidence of infections or allergic manifestations during the first year of life was similar in both groups, with no statistical differences. The investigators concluded that feeding of GOS-containing infant formula produced a definite prebiotic effect consisting of changes in fecal composition and microbiota, and in fecal consistency and the frequency of defecation. No changes in the incidence of infection or allergic manifestation during the first year of life were noted.

In another recent study, Meli et al. (2014) compared the growth in infants fed formula supplemented with a mixture of bovine milk-derived oligosaccharides (BMOS) with infants fed standard formula. BMOS was generated from whey permeate, contains GOS and other oligosaccharides from bovine milk, such as 3'- and 6'-sialyllactose. In this study, healthy term infants ≤ 14 days old were randomly assigned to standard formula (control; n = 84); standard formula with BMOS (IF-BMOS; n = 99); or standard formula with BMOS and probiotics (*Bifidobacterium longum*, *Lactobacillus rhamnosus*) (IF-BMOS + Pro; n = 98). The total

oligosaccharides supplemented was 7.3 ± 1.0 g/100 g of powder formula. A breastfed reference group was also enrolled ($n = 30$). The primary outcome was mean weight gain/day from enrollment to age 4 months. Additionally, gastrointestinal tolerability, stool bacterial counts, and occurrence of adverse events were measured. The investigators concluded that infant formula containing BMOS either with or without probiotics provides adequate nutrition for normal growth in healthy term infants. During the study 125 (45%) infants had at least one adverse event during the study: 36 (46%) in the control group, 39 (39%) in the IF-BMOS group, 47 (48%) in the IF-BMOS + Pro group, and 8 (26.7%) in the breastfed group. No significant differences in the frequency of adverse events were observed between groups. A total of 26 serious adverse events were reported in 25 infants during the 4-month intervention period. None of these were considered related to the study formulae. Hematology and blood biochemical analyses (performed in about 1/3 of formula-fed infants) were normal.

In two separate studies, immunomodulatory effects of GOS were investigated (Raes et al., 2010; van der Aa et al., 2012). In the double-blind, randomized, placebo-controlled intervention trial from clinical sites in Belgium, Raes et al. (2010) recruited a group of 215 pregnant women and their infants. In this study, the effect of an infant milk formula with 6 g/L short-chain galacto- and long-chain fructo-oligosaccharides (scGOS/lcFOS, ratio 9:1) on basal immune parameters in 215 healthy, term infants during the first 26 week of life were investigated. After birth, the infants received breast milk or were randomized to receive an infant formula with or without scGOS/lcFOS. Blood samples were collected at the age of 8 and 26 week for the analysis of serum immunoglobulins, lymphocyte subpopulations, and cytokines. A breast fed group was included as a reference. In total, 187 infants completed the study. No significant differences were noted between both formula groups in the different studied immune parameters at weeks 8 and 26. The findings from this study indicates that supplementation of infant formula with a mixture of prebiotic oligosaccharides did not change the basal level of the measured parameters of the developing immune system in healthy infants with a balanced immune system during the first 6 months of life in comparison to feeding a standard infant formula and in comparison to exclusive breastfeeding.

In another trial, van der Aa et al. (2012) who evaluated the effect of an infant formula supplemented with a mixture in a group of 90 infants (0 to 7 months of age) with atopic dermatitis reported similar findings. In this study, eligible infants from participating mothers were randomized to a control group administered standard infant formula or a symbiotic treatment group administered the same infant formula supplemented with GOS/FOS (8 g/L; 9:1 ratio) in combination with *Bifidobacterium breve* M-16V (1.3×10^{10} CFU/L) for a duration of 12 weeks. Blood samples were obtained at baseline and week 12 for measurement of various atopic disease markers, ex vivo cytokine production, and circulating regulatory T cell percentage. No clinically relevant changes in various measure of the immune system function were observed in infants consuming the symbiotic formula during the study.

2.5.1.2. Human Studies

In several human studies, the effects of GOS in human subjects have been investigated. These studies are summarized in the FDA GRAS notices as well as in the EFSA and FSANZ reports. In a double-blind cross-over trial adolescent girls, Whisner et al. (2013) investigated the dose-response relationship of GOS supplementation on calcium absorption during growth and to assess changes in colonic microbiota to better understand the mechanism by which GOS is acting. In this study, a total of 31 healthy girls (10-13 years of age) consumed smoothie drinks

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twice daily with 0 (control), 2.5 or 5.0 g GOS for three week periods in a random order. The total daily of GOS was 0, 5 and 10 g/day. Fractional calcium absorption was calculated based on urinary calcium isotope excretion (over 48 hour at the end of each 3-week period) and expressed as a ratio of excess ^{44}Ca and ^{43}Ca . Similarly, fecal microbiota and bifidobacteria were assessed. Fractional calcium absorption after the 48 hour treatment with 0, 5 and 10 g GOS/day was 0.393, 0.444 and 0.419, respectively. As compared to the control, a significant increase in calcium absorption was noted in both low and high dose treated GOS groups. However, the increase was not dose-related. The increase in calcium absorption was highest in the urine collected after 24 hours, which is consistent with lower gut absorption. Fecal bifidobacteria increased (control- 10.89, 5 g GOS- 22.80 and 10 g GOS- 11.54) with the GOS treatment. The results suggest that daily consumption of 5 g GOS increases calcium absorption, which may be mediated by the gut microbiota, specifically bifidobacteria. No adverse effects were reported.

In a randomized, double-blind, placebo-controlled, crossover study, Vulevic et al. (2013) assessed the effect of a GOS mixture on markers of metabolic syndrome, gut microbiota, and immune function. In this study, 47 subjects (mean age of 44.6 years; 16 M, 29 F; overweight with 3 or more risk factors associated with metabolic syndrome) received GOS (content of 48%) at a dose level of 5.5 g/day. Whole blood, saliva, feces, and anthropometric measurements, including adverse event reports, were taken at the beginning, week 6, and end of each 12-week intervention period. GOS increased the number of fecal bifidobacteria at the expense of less desirable groups of bacteria. Increases in fecal secretory IgA and decreases in fecal calprotectin, plasma C-reactive protein, insulin, total cholesterol (TC), triglyceride, and the TC:HDL cholesterol ratio were also observed. The investigators concluded GOS may be a useful candidate for the enhancement of gastrointestinal health, immune function, and the reduction of metabolic syndrome risk factors in overweight adults. No adverse effects were noted.

In another study, Vo et al. (2012) investigated the cause of acute allergic reactions (itchy rash and some with breathing difficulties) in Vietnamese children following (shortly after) the drinking of a new milk product. A case-series was conducted to generate hypotheses on the possible causes of the illness and was followed by a case-control study to test the hypothesis. Parents of all cases and controls were interviewed face-to-face. The association between food items and the allergy was tested using conditional logistics regression. From 9 to 28 October 2009, 19 cases fulfilled the case definition, and 16 of the 17 cases included in the study had consumed milk supplemented with GOS shortly before the onset of illness. Age-matched neighborhood controls (n=51) were enrolled into the case control study. Of the 30 food items consumed by study participants in the preceding 24 hours, only the odds ratio (OR) of milk supplemented with GOS was statistically significant: OR=34.0 (95% CI=3.9, 294.8). Analysis of this milk product did not reveal any unusual properties, chemicals, or other toxic substances. This is the first report of an acute allergic reaction to fresh milk supplemented with GOS. However, the specific allergen in this product was not identified. Once the product was withdrawn from the market, further cases were not reported. It is unlikely that GOS played any role in allergic reaction as oligosaccharides, including GOS, are added to infant food for their potential to prevent sensitization of infants to dietary allergens (Osborn and Sinn, 2013).

In a multi-center, double-blind, parallel-designed, gender-stratified prospective study, Ashley et al. (2012) randomized 419 infants to receive either a marketed routine cow's milk-based infant formula (Control; Enfamil® LIPIL®) (n = 142) or one of two investigational formulas from 14 to 120 days of age. Investigational formulas were supplemented with 4 g/L

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(1:1 ratio) of a prebiotic blend of polydextrose (PDX) and GOS (PDX/GOS; n = 139) or 4 g/L of GOS alone (GOS; n = 138). No group differences in growth rate from 14 to 120 days of age were noted. Discontinuation rates were not significantly different among study groups. No differences in formula intake or infant fussiness or gassiness were observed. During study weeks 1 and 2 and at 60 days of age stool consistency ratings were higher (i.e., softer stools) for infants in the PDX/GOS and GOS groups versus Control and remained higher at 120 days for the PDX/GOS group. The overall incidence of medically-confirmed adverse events was similar among groups. The investigators concluded that infant formulas supplemented with 4 g/L of either a prebiotic blend of PDX and GOS or GOS alone were well-tolerated and supported normal growth.

Walton et al. (2012) investigated the effects of GOS (content 59%) on modulation of fecal microbiota, fermentation characteristics and fecal water genotoxicity in men and women of 50 years and above. In this randomized, double-blind, placebo-controlled crossover trial, 39 subjects (ages 50 to 81 years; mean age of 58.9±5.9 years; healthy) were recruited and 37 volunteers that completed the study received juice containing 4 g GOS and placebo twice daily for 3 weeks, preceded by 3-week washout periods. *In vivo*, following GOS intervention, bifidobacteria were significantly more compared to post-placebo. No changes in fecal water genotoxicity were observed. No adverse events in parameters such as in stool consistency, intestinal bloating, abdominal discomfort, or flatulence severity and frequency were reported.

Davis et al. (2010) investigated the effect of different doses of GOS on the fecal microbiota of healthy adults. In this single-blinded study, 18 subjects consumed GOS-containing chocolate chews at four increasing dosage levels (0, 2.5, 5, and 10 g) for 3 weeks, with a two-week baseline period preceding the study and a two-week washout period at the end. An increase in bifidobacteria populations was noted as the GOS dosage increased to 5 or 10 g. The results of this study showed that a high purity GOS, administered in a confection product at doses of 5 g or higher, was bifidogenic, while a dose of 2.5 g showed no significant effect. However, the results also showed that even when GOS was administered for many weeks and at high doses, there were still some individuals for which a bifidogenic response did not occur. No adverse effects of GOS were reported.

2.5.1.3. Metabolism

Available metabolism-related studies demonstrate that GOS is not hydrolyzed by human salivary or pancreatic enzymes and passes undigested and unabsorbed to the colon where it is fermented by colonic microflora to short-chain fatty acids, carbon dioxide, methane, and hydrogen gases. The unfermented dietary GOS will be excreted in the feces. In a review article on metabolism of oligosaccharides, Ganzle and Follador (2012) reported that the only GOS occurring widely in nature is lactose [Gal-β-(1-4)-Glu], which is present in the milk of mammals at concentrations of 2-10%. Tri- and tetrasaccharides are present only in trace amounts in humans and most non-human mammals.

In another *in vitro* study, Rodriguez-Colinas et al. (2013) investigated fermentation of several purified GOS products, specifically the trisaccharides 4'-galactosyl-lactose and 6'-galactosyl-lactose and a mixture of the disaccharides 6-galactobiose and allolactose. In this study, the bifidogenic effect of GOS at 1% (w/v) as compared to a commercial GOS mixture (Bimuno-GOS) was studied in a pH-controlled batch culture fermentation system inoculated with healthy adult human feces. Bifidobacteria increased after 10 hours fermentation for all the

GOS substrates, but the changes were only significant for the mixture of disaccharides and Bimuno-GOS. Acetic acid, whose formation is consistent with bifidobacteria metabolism, was the major small chain fatty acid (SCFA) synthesized. The acetate concentration at 10 hours was similar with all the substrates and significantly higher than that observed for formic, propionic and butyric acids. All the purified GOS could be considered bifidogenic under the assayed conditions, displaying a selectivity index in the range 2.1-3.0, which was slightly lower than that determined for the commercial mixture Bimuno-GOS.

Ladirat et al. (2013) compared the impact of GOS, in an *in vitro* fermentation screening-platform, on adult gut microbiota composition and activity upon treatment with four antibiotics at two doses. These investigators noted that the changes in the relative abundance of bacteria upon antibiotic treatment and the growth of *Bifidobacterium* and *Lactobacillus* upon GOS addition were antibiotic and dose dependant. The combination of GOS-Amoxicillin showed a decrease of *Bifidobacterium* levels, followed by a recovery of mainly *Bifidobacterium longum* that could be correlated to specific degradation patterns of GOS. As compared to non-treated microbiota, in antibiotic-treated microbiota different degradation profiles of individual GOS oligosaccharides, an accumulation of monosaccharides and intermediate organic acids was noted. The results of this study show that GOS was utilized and beneficial bacteria could grow in 3 out of 4 antibiotics tested. However, the metabolic activity of an antibiotic-treated microbiota was still disturbed as compared to the non-treated microbiota.

2.5.1.4. Animal and Other Studies

Marin-Manzano et al. (2013) investigated the effects of GOS derived from lactulose on the growth of *Bifidobacterium animalis* in the large intestine of growing rats. In this study, the differential modulatory effects of GOS derived from lactulose (GOS-Lu) in comparison with GOS derived from lactose (GOS-La) in gut microbiota of growing rats (5 weeks old) were studied. Rats were fed either a control diet or diets containing 1% (w/w) of GOS-Lu or GOS-La, and cecal and colonic contents were collected after 14 days of feeding. Compared to controls, GOS-Lu had significantly more bifidobacteria within the large intestine, showing a significant and selective increase of *B. animalis* in the cecum and colon. However, no significant differences in the number of bifidobacteria among GOS-Lu and GOS-La groups were observed. Both types of GOS significantly increased the number of the *Eubacterium rectale/Clostridium coccoides* group.

Hernández-Hernández et al. (2012) compared the *in vivo* ileal digestibility and changes in fecal microbiota following administration of GOS-Lu and GOS-La to growing rats. Weaned male Wistar rats were fed either a control diet or diets containing 1% of GOS-Lu or GOS-La for 14 days. Quantitative analysis of carbohydrates from dietary and ileal samples demonstrated that the trisaccharide fraction of GOS-Lu was significantly more resistant to gut digestion than that from GOS-La, as indicated by their ileal digestibility rates of $12.5 \pm 2.6\%$ and $52.9 \pm 2.7\%$, respectively, whereas the disaccharide fraction of GOS-Lu was fully resistant to the extreme environment of the upper digestive tract. The low ileal digestibility of GOS-Lu was due to the great resistance of galactosyl-fructoses to mammalian digestive enzymes, highlighting the key role played by the monomer type and linkage involved in the oligosaccharide chain. The partial digestion of GOS-La trisaccharides showed that glycosidic linkages (1→6) and (1→2) between galactose and glucose monomers were significantly more resistant to *in vivo* gastrointestinal digestion than the linkage (1→4) between galactose units. The absence of GOS-La and GOS-Lu digestion-resistant oligosaccharides in fecal samples indicated that they were readily fermented

within the large intestine, enabling both types of GOS to have a potential prebiotic function. As compared with controls, the GOS-Lu group showed higher bifidobacteria in fecal samples after 14 days of treatment.

In a series of tests, Kobayashi et al. (2009) investigated the safety of GOS, produced from lactose by a two-step enzymatic process. As part of the genotoxicity studies, bacterial reverse mutation and chromosomal aberration tests, with or without metabolic activation, were performed. These tests did not reveal mutagenesis as evaluated by the Ames assay or in *Escherichia coli* WP2uvrA, and no chromosomal aberrations in cultured fibroblast cells from Chinese hamster lungs were noted. Oral administration of GOS to mice did not induce micronuclei in the reticulocytes of peripheral blood. In a 90-day repeated oral dose toxicity study in rats, GOS was administered at 0, 500, 1000 and 2000 mg/kg bw to male and female Sprague-Dawley rats. There were no GOS-related changes in clinical signs, body weight, water intake, feed intake, urinalysis, ophthalmology, hematology, blood chemistry, organ weights, gross pathology or histopathology in any of the treatment groups compared to the control group. The no observed adverse effect level (NOAEL) of GOS was determined as 2000 mg/kg/day, the highest dose tested. These studies are extensively discussed in the FDA GRAS notification as unpublished investigations (Yakult, 2010).

Desbwards et al. (2012) investigated the effects of GOS in pregnant mice and their offspring. In this study, pregnant BALB/cj mice were fed a control diet (n=13) or a diet supplemented with a prebiotic mixture consisting of a GOS and inulin (9:1) (n=12) throughout gestation and lactation. Based on reported maternal feed intake, body weight values, and the GOS content of the test article, the resulting dose was equivalent to 1620 mg GOS/kg bw/day and 400 mg inulin/kg bw/day. At weaning, male offspring were separated from their mothers, and weaned to the same test diet as their dams. The offspring were monitored and then killed on Day 48 after weaning. No significant differences in maternal body weight gain or feed intake during pregnancy were noted between the groups. Additionally, no significant differences in the number of offspring per dam were reported. Male pups administered GOS:inulin exhibited significantly higher body weights at weaning, and at Days 2, 40, and 48 after weaning compared to the control group. In male pups administered GOS:inulin, body length, colon length, and relative thigh muscle weights were significantly higher compared to the control group. No other developmental or reproductive toxicological endpoints were examined.

2.6. Safety of Bacterial Enzyme

The subject of the present GRAS assessment, GOS, is produced from food grade lactose via a transgalactosylation reaction catalyzed by a β -galactosidase enzyme derived from *B. circulans* a member of the Bacillus genus of Gram-positive, rod-shaped bacteria. This genus contains a large number of bacterial strains that have been used industrially in the preparation of a number of enzymes that are used in food production (Schallmey et al., 2004). In the published literature, limited information on the potential pathogenic or toxigenic effects of *B. circulans* was found. In general, with the exception of *Bacillus cereus*, illness from Bacillus spp. is rare.

Rowan et al. (2001) reported isolation of *B. circulans* from blood samples of patients with sepsis. Additionally, some species of *B. circulans* have been reported to contain genes encoding enterotoxigenic compounds (Beattie and Williams, 1999; Rowan et al., 2001; Phelps and McKillip, 2002). In a natural isolate of bacteria, Phelps and McKillip (2002) reported isolation of a strain of *B. circulans* from whole milk that showed the presence of a number of

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genes encoding hemolytic and non-hemolytic enterotoxigenic proteins (hblC, hblD, MA; nheA, nheB). These genes were shown to display functional activity as β -hemolysis and experiments with sheep blood agar plates supported this notion (Phelps and McKillip, 2002). The presence of virulence factors in some strains of *B. circulans* does not preclude the use of enzymes isolated from the species in the production of food ingredients. However, this suggests the need to properly characterize the safety of the specific *B. circulans* strain.

In the European Union, as per Commission Directive 2003/95/EC cyclodextrin is produced by cyclodextrin transferase enzyme derived from *B. circulans* is approved in the production of β -cyclodextrin. In response to two GRAS notices 236 and 285, FDA did not question the use of GOS in various foods and infant formula produced from lactose using a β -galactosidase, derived from *B. circulans* LOB 377. Additionally, several enzymes derived from *Bacillus* species, such as α -amylase derived from *Bacillus licheniformis*, pullulanase from *Bacillus subtilis* and *Bacillus licheniformis*; and pectate lyase from *Bacillus subtilis* are considered GRAS. Furthermore, carbohydrase and protease enzymes derived from *Bacillus subtilis* are affirmed as GRAS for use as direct food ingredients, and α -acetolactate decarboxylase from recombinant *Bacillus subtilis* is currently regulated by the FDA as a secondary direct food additive permitted for use in food for human consumption.

The enzyme, β -galactosidase, used for the enzymatic reaction in the production of GOS (subject of current GRAS assessment), is obtained by fermentation of a *B. circulans* strain in a fermentor. Unpublished safety studies have shown that the β -galactosidase is obtained from a nonpathogenic and nontoxigenic microorganism. Additional steps used in enzyme preparations and use of the enzyme further supports the safety. The enzyme is isolated using standard procedures for the enzymatic reaction with lactose. The constituents from the enzyme preparation are unlikely to become part of the product. The manufacture of GOS involves extensive purification steps such as activated carbon filtration, ion-exchange and chromatography separation stages, that are likely to remove potential metabolic impurities and/or toxin(s) produced during fermentation. The *B. circulans* strain used in the β -galactosidase preparation is nonpathogenic and nontoxigenic. Additionally, *Kluyveromyces lactis* strain is also used to further increase the GOS content. This strain is also non-toxigenic non-pathogenic. In order to remove the yeast and yeast extract a disc separator is employed, followed by decolorization, filtration, purification, and evaporation. Under 21 CFR 184.1388, use of lactase enzyme preparation derived from the non-pathogenic, non-toxicogenic yeast *Kluyveromyces lactis* (previously named *Saccharomyces lactis*) is considered as GRAS.

3. SUMMARY AND DISCUSSION

New Francisco Biotechnology Corporation (NFBC) intends to market galacto-oligosaccharides (GOS) as ingredients for addition to term infant formula and follow-on formula at a use level providing up to 7.2 g of GOS/L of the reconstituted or ready to consume product. The manufacturing of GOS involves a multistage process in which food grade lactose is converted via a transgalactosylation reaction catalyzed by a β -galactosidase enzyme obtained from the non-toxigenic, non-pathogenic microorganism, *B. circulans*. The GOS are prepared using raw materials and processing aids that are food-grade and comply with applicable U.S. federal regulations. GOS is manufactured according to cGMP and NFBC has established food grade specifications for GOS. Depending on the customer requirements, GOS for uses in infant formula will be marketed as standardized powder or syrup under name King-Prebiotics® (GOS-

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1000-P, GOS-900-P, GOS-700-P, GOS-570-S or GOS-270-P). The final product consists of di- to octa-saccharides composed of 1-7 galactose units linked to a glucose molecule at the reducing end. Among different saccharides, trisaccharide is the major one, followed by tetrasaccharide.

Based on available information, currently, GOS are used in a variety of foods. Multiple GOS preparations produced from lactose using food grade microbial derived beta-galactosidases, including NFBC GOS, have been previously determined as GRAS, for use in food and beverage products. Additionally, use of GOS in infant formula and follow on formula also has been determined to be GRAS. In addition to these critical reviews by Expert Panelists and FDA for safety-in-use of GOS preparations in infant formula and follow on formula, general recognition of the safety of GOS for use in infant formula is further established by opinions authorizing the safe use of GOS in infant formula preparations issued by the European Commission and FSANZ.

There is sufficient qualitative and quantitative scientific evidence to determine the safety-in-use of GOS in term infant formula and follow-on formula at a use level providing up to 7.2 g of GOS/L of the reconstituted or ready to consume product. Oligosaccharides resembling GOS occur naturally in human milk. Additionally, manufactured GOS products have been used in food for over 25 years, and in infant formulas for over 10 years, with no evidence of adverse effects related to the safety of its use. GOS has been the subject of nine GRAS notices to FDA. FDA did not question the safety of GOS for the intended food uses, including infant formula uses. Of the nine GRAS notices, six were related to use of GOS in infant formula. In these submissions, the intended use of GOS in infant formula at the maximum intended use level of 7.2 g/L was estimated to result in the 90th percentile dietary intakes 8.5 and 7.9 g GOS/infant/day, for infants aged 0-6 months and 7-12 months, respectively.

The subject of this present GRAS determination is substantially equivalent to GOS that has been the subject of FDA GRAS notified substances, including its use in infant formula. The use of a similar manufacturing process in the preparation of GOS that is the subject of this GRAS assessment and those that has been the subject of FDA notifications suggests that the differences between various GOS products would be limited to minor variations in the compositional distribution of the GOS oligomers, and to differences in the residual levels of lactose. These observations also suggest that the safety information on GOS products can be interchangeably used. The FDA responses to GRAS notifications on GOS indicate that the agency is satisfied with the safety-in-use of GOS in foods as well as in infant formula.

The available metabolism related information of GOS demonstrate that GOS is not digested by human gastric juice or pancreatic enzymes and passes undigested and unabsorbed to the colon where it is fermented by colonic microflora to short-chain fatty acids, carbon dioxide, methane and hydrogen gases. Any unfermented dietary GOS will be excreted in the feces. Several published studies of GOS are described in the GRAS notices submitted to FDA. In genetic toxicity studies, GOS has been shown to be non-genotoxic. The published animal studies, including a 90 day rodent study, and published human studies supports the safety of GOS. The findings from these studies reveal that intake of GOS does not cause any adverse effects on microfloral populations, nutrient absorption and retention, weight gain, or food consumption.

In addition to pre-clinical and clinical studies, consumption of GOS containing infant formulas has been extensively investigated in infants. There is evidence that addition of prebiotics in infant formula alters the gastrointestinal microbiota resembling that of breastfed infants. These prebiotics, including GOS are added to infant formula because of their presence in

breast milk. In several published clinical studies, nutritional and physiological effects or safety of GOS containing infant formulas in premature infants, term infants, and infants with atopic disorders has been investigated. Findings from these studies demonstrate that addition of GOS to infant formula at use levels up to 7.2 g/L is well-tolerated and safe. Recent studies that appeared subsequent to the most recent FDA GRAS notification also did not reveal any significant findings that affect the safety conclusion from the GRAS notices. The safety determination of GOS is based on the totality of available evidence, including current approved uses, *in vitro* and *in vivo* metabolism studies, human observations and a variety of animal studies that supports the safety-in-use of GOS.

In summary, on the basis of scientific procedures⁶, use of GOS, derived from lactose and produced according to current good manufacturing practices (cGMP), in term infant formula and follow-on formula at levels providing up to 7.2 g of GOS/L of the reconstituted or ready to consume product resulting in a maximum daily intake of 8.5 g GOS/infant/day is considered safe.

⁶ 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members, whose signatures appear below, have individually and collectively concluded that galacto-oligosaccharides (GOS), meeting the appropriate food grade specifications, and manufactured in accordance with current Good Manufacturing Practice, is generally recognized as safe (GRSA) for use in term and follow-on infant formula at a use level providing galacto-oligosaccharides (GOS) at a concentration of up to 7.2 g/L in the reconstituted and/or ready-to-serve formula.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that galacto-oligosaccharides (GOS), when used as described, is GRAS, based on scientific procedures.

Signatures

(b) (6)

John A. Thomas, Ph.D., F.A.T.S., D.A.T.S.

2/2/15
Date

(b) (6)

Robert L. Martin, Ph.D.

Jan. 31, 2015
Date

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.

February 5, 2015
Date

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6. APPENDIX I

Clinical Studies of GOS in Infants*

Reference and Study Design	Subjects ^a	Dose	Duration	Results ^b	
Studies Conducted Using GOS					
Prasad et al., 2013; Sazawal et al., 2010 Randomized, controlled	613 children (1 to 3 years old; body weight NR; M and F; healthy) (128 children with severe anemia)	Treatment: <i>Bifidobacterium lactis</i> HN019 and GOS 2.4 g/d Control: Standard formula All children with severe anemia (n=128) were given a therapeutic dose of iron for 3 months in addition to milk supplement.	12 months	Growth	↑ Weight gain
				Adverse event reporting	↓ Rate of diarrhea in children >24 months old NSD in rate of diarrhea in children 12 to 24 months old ↓ Rate of dysentery ↓ Incidence of pneumonia and ALRI (wide confidence interval) ↓ Days with severe illness ↓ Days with high fever ↓ Antibiotics usage
				Effects on anemia	Lower risk of being anemic and iron deficient NSD on iron status indicators (hemoglobin, zinc protoporphyrin, serum transferrin and ferritin)
				Fecal microflora	NSD in <i>bifidobacteria</i> NSD in cultivable <i>lactobacilli</i> NSD in total bacterial counts
Maldonado et al., 2012 Randomized, double-blind, controlled	188 infants (term ^c ; 6 months old; body weight NR; 94 M, 94 F; Healthy)	Treatment: 1. <i>Lactobacillus fermentum</i> CECT5716 and GOS (source NR) (4 g/L) 2. GOS (4 g/L)	6 months	Growth	NSD in weight, length, head circumference, and growth rate
				Adverse event reporting	No adverse effects No upper GI symptoms
				Infections	↓ Incidence rate of GI infections, URTI ↓ Total and recurrent respiratory infections ↓ Total number of infections NSD in incidence rates of otitis, UTI, and other infections NSD in antibiotic treatment and fever episodes
				Fecal microflora	↑ <i>Bifidobacteria</i> and <i>lactobacilli</i> NSD in other bacteria
				Stool effects	NSD in fecal IgA concentrations NSD in diarrhea NSD in SCFA in stool
Studies Conducted Using GOS/FOS					
Veereman- Wauters et	110 infants	Treatment:	28 days	Growth	NSD in length and body weight gain

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Reference and Study Design	Subjects ^a	Dose	Duration	Results ^b	
				Adverse event reporting	No serious adverse events reported NSD in number of withdrawn subjects NSD in formula intake NSD in crying behavior, regurgitation, or vomiting
				Fecal microflora	↑ <i>Bifidobacteria</i> in GOS/FOS and breast-fed groups NSD in <i>lactobacilli</i>
				Stool effects	NSD in stool frequency Softer stools in GOS/FOS and breast-fed groups
Vaisman et al., 2010 Prospective, randomized, double-blind, placebo-controlled	42 children (9 to 24 months old; body weight NR; M and F; acute diarrhea)	Treatment: AOS with GOS/FOS (4.8 g/d; 9:1) taken with food Control: Maltodextrin with food	12 days	Immune cell effects	NSD in absolute number or relative numbers of cytokines ↓ TNF-α when comparing subgroups of no relative change in cytokines vs. relative increase or decrease in cytokines
				Stool effects	NSD in stool cultures NSD in number of stools ↑ Stool consistency
Westerbeek et al., 2011a,b Randomized, double-blind, controlled trial	113 infants (pre-term; average 2.1 days old; gestational age <32 weeks; birth weight <1,500 g; 67 M, 46 F; healthy)	Treatment: AOS with GOS/FOS (Danone Research) (1.5 g/kg/d; 9:1) Control: Pre-term formula with maltodextrin	30 days	Intestinal permeability	NSD in lactulose or mannitol concentrations NSD between different host- and treatment-related factors on intestinal permeability
				Stool effects	NSD in stool frequency ↓ Stool viscosity at Day 30 ↓ Stool pH at Day 30 NSD in incidence of necrotizing enterocolitis
van der Aa et al., 2010, 2011, 2012; de Kivit et al., 2012 Double-blind, placebo-controlled, multi-center trial	90 infants (term; <7 months old; birth weight NR; 59 M, 31 F; atopic dermatitis with SCORAD ≥15) (82 infants completed, 75 infants completed the 1-year follow-up; 53 M, 22 F)	Treatment: <i>B. breve</i> M-16V and GOS/FOS (Immunofortis®) (8 g/L; 9:1) Control: Standard formula	12 weeks 1-year follow-up for asthma-like symptoms and immune effects	Growth	NSD in growth or weight
				Adverse event reporting	NSD in adverse events NSD in renal and liver function NSD in diarrhea or gastroenteritis NSD in parent-reported bowel cramps, flatulence, and regurgitation
				Fecal microflora	↑ Percentage of <i>Bifidobacteria</i> Week 1 and 12 ↓ <i>Lactobacilli/enterococci</i> Week 1 ↓ Percentages of <i>C. lituseburens</i> / <i>C. histolyticum</i> and

Reference and Study Design	Subjects ^a	Dose	Duration	Results ^b	
					<i>E. rectal/C. coccoides</i> at 12 weeks
				Effects on atopic dermatitis	<p>NSD in decrease of atopic dermatitis severity as determined by SCORAD score</p> <p>NSD in frequency of use of topical corticosteroids</p> <p>NSD in increase in total serum IgE</p> <p>NSD in decrease in serum eosinophilic granulocytes</p> <p><u>Subgroup with IgE-associated atopic dermatitis:</u></p> <p>NSD in improvement of atopic dermatitis at Week 4 and 8 Improvement in atopic dermatitis at Week 12</p>
				Effects on asthma-like symptoms	<p>Less frequent wheezing (≥ 3 episodes) and wheezing and/or noisy/rattly breathing apart from colds</p> <p>NSD in wheezing apart from colds</p> <p>↓ Use of asthma medication</p> <p>↓ New users of asthma medication NSD in change in IgE</p> <p>↓ Percentage of children with elevated specific IgE against cat from baseline to Week 12</p> <p>NSD in percentage of children with elevated specific IgE against dog or house dust mite from baseline to Week 12</p> <p><u>Subgroup with IgE-negative atopic dermatitis:</u></p> <p>↓ IgE at 1 year</p> <p>↓ Change in IgE</p>
				Immune effects	<p>NSD in plasma IL-5, IgG1, IgG4, CTAK and TARC</p> <p>↓ IL-4 production in unstimulated PBMCs at Week 12</p> <p>NSD in production of IL-5, IL-6, IL-10, IL12p40/p70, IL- 12p70, IL-13, IL-17, IFN-γ and TGF-β in unstimulated PBMCs</p> <p>NSD in cytokine production in allergen and anti-</p>

Reference and Study Design	Subjects ^a	Dose	Duration	Results ^b	
					CD3/anti- CD28 stimulated PBMCs NSD in percentages of CD3+, CD4+CD8- , FoxP3+CD25+, or T regulatory cells ↑ Serum galectin-9 levels at 12 weeks <u>Subgroup with IgE-associated atopic dermatitis:</u> NSD in plasma IL-5, IgG1, IgG4, CTACK and TARC NSD in ex vivo cytokine production by unstimulated and anti-CD3/anti-CD28 stimulated PBMCs NSD in circulating percentage of T regulatory cells NSD in PBMCs stimulated with peanut and cow's milk allergen ↑ Change in IL-12p40/p70 in egg-stimulated PBMCs
				Stool effects	↓ Fecal pH ↑ L-lactate and D-lactate concentrations ↓ Percentages of butyric, isobutyric, and isovaleric acid NSD in percentages of acetic and propionic acid NSD in fecal frequency Softer fecal consistency ↓ Parent-reported constipation ↓ Episodes of dry stools ↓ Diaper dermatitis
Vivatvakin et al., 2010 Single-center, prospective, double-blind, randomized, parallel-group, controlled	224 infants (term; < 30 days old; birth weight 2,500 g to 4,500 g; 68 M, 76 F; healthy) (169 infants completed)	Treatment: LC-PUFA with GOS/FOS (4 g/L; 9:1) Control: Standard formula (Breast-fed reference)	~3 months 18-month follow-up	Growth	NSD in mean body weights NSD in body length or head circumference
				Adverse event reporting	NSD in acceptability of the formula NSD in adverse events
				Fecal microflora	NSD in total bacterial count ↑ <i>Bifidobacteria</i> (significance NR)
				GI effects	NSD in gastric emptying time
				Stool effects	↑ Soft stools NSD in stool frequency

Reference and Study Design	Subjects ^a	Dose	Duration	Results ^b	
					NSD in stool color and odor
Raes et al., 2010 Randomized, double-blind, placebo-controlled, parallel-arm Note: results on serum lipids and fecal microflora available from previous kin publications before 2010 (Alliet et al., 2007; Scholtens et al., 2008)	215 infants (term; 0 days old; birth weight >p5 and <p95 ^d ; 119 M, 96 F; healthy) (187 infants completed)	Treatment: GOS/FOS (Immunofortis®) (6 g/L; 9:1) Control: Standard formula (Breast-fed reference)	6 months	Adverse event reporting	NSD in adverse events
				Immune cell effects	NSD in WBC count, lymphocyte numbers and CRP ↑ WBC count in breast-fed group at Week 26 NSD in total IgE, IgG, IgA or IgM ↑ IgG compared to standard formula only ↑ secretory IgA concentration in feces at Week 26 NSD in percentages of lymphocyte subsets between formula-fed groups ↑ Activated (CD38+) CD4+ T cells at Week 8 ↑ Activated (CD23+) B cells in breast-fed group compared to GOS/FOS or control at Week 8 ↑ Activated (CD25+) CD8+ T cells in breast-fed group compared to GOS/FOS ↑ Activated (CD25+) CD4+ T cells and NK cells in breast-fed compared to control ↓ Percentage of CD2+ cells, (CD38+) CD4+ and (CD38+) CD8+ T cells in the breast-fed group at Week 26 ↓ TNF-α in breast-fed compared to GOS/FOS at Week 8 ↓ IL5 in breast-fed compared to GOS/FOS at Week 26 NSD in cytokines between formula-fed groups
Holscher et al., 2012 Multi-centre, randomized, double-blind, placebo-controlled, parallel-arm	123 infants (term; 2 to 8 weeks old; birth and body weight NR; M and F; healthy)	Treatment: GOS/FOS (Vivinal® GOS + Beneo P95 FOS) (4 g/L; 9:1) Control: Standard formula (Breast-fed reference)	6 months	Growth	NSD in body weight
				Adverse event reporting	NSD in tolerance or occurrence of adverse events NSD in caregiver-perceived incidence of crying, fussing, parent-perceived "colic"/cramps, spitting up, vomiting, or flatulence frequency
				Fecal microflora	↑ <i>Bifidobacteria</i> in GOS/FOS and breast-fed groups NSD in <i>Bacteriodes/Prevotella</i> , <i>C. difficile</i> , or <i>Lactobacilli</i>
				Stool effects	NSD in stool frequency, consistency, color, or odor ↓ Fecal pH in GOS/FOS and breast-fed groups
Salvini et al., 2011 Double-blind, randomized, placebo-controlled,	20 infants (term; average birth weight 3,320 g; 7 M, 13 F; healthy)	Treatment: GOS/FOS (source NR) (8 g/L; 9:1) Control:	6 months 12-month follow-up	Growth	NSD in weight gain, length growth, or head circumference

Reference and Study Design	Subjects ^a	Dose	Duration	Results ^b	
explorative study		Standard formula with maltodextrin			
Modi et al., 2010 Multi-center, double-blind, prospective, randomized, controlled	154 infants (pre-term; gestational age <33 weeks; average birth weight 1,540 g; 98 M; 56 F; healthy)	Treatment: GOS/FOS (source NR) (8 g/L; 9:1) Control: Standard pre-term formula	40 weeks or discharge, whichever occurred	Growth	NSD in weight, length, or head circumference gain
				Adverse event reporting	NSD in mortalities 6 adverse events (5 not test article-related, 1 caused abdominal distension and tenderness) NSD in GI signs or indices of fluid balance
				Fecal microflora	NSD in microflora
				Stool effects	NSD in daily number of stools and stool characteristics
van Stuijvenberg et al., 2011 Randomized, double-blind, placebo-controlled, multi-center	830 infants (term; median age 37 days; median birth weight 3,345 g; 420 M, 410 F; healthy) (592 analyzed)	Treatment: AOS with GOS/FOS (Immunofortis®) (6.8 g/L; 9:1) Control: Standard formula (Breast-fed reference)	1 year	Adverse event reporting	No serious adverse events
				Effects on fever	NSD in fever episodes at 1 year NSD in suspected causes of fever and symptoms (<i>i.e.</i> infections)
Bocquet et al., 2013 Multi-center, prospective, randomized, controlled, double-blind, parallel-group	439 infants (term; <42 days old; birth weight 2,500 g to 4,200 g; M and F; healthy) (321 infants analyzed)	Treatment: <i>B. lactis</i> and GOS/FOS (source NR) (4 g/L; 9:1) Control: Formula with <i>B. lactis</i>	1 year (4 to 6 months starter formula, then follow-on formula)	Growth	NSD in daily volume of formula intake NSD in growth, except for length-for-age at 4 and 6 months and BMI-for-age at 12 months NSD in mean daily weight gain, or length and head circumference gain
				Adverse event reporting	No adverse events or serious adverse events attributed to the test article
				Infections	NSD in type of infection or annual rates of infection NSD in total number of infections or GI infections NSD in frequency of antibiotic use
				Stool effects	NSD in mean daily stool frequency ↑ Liquid stools before 10 months (except at 6 months) NSD in number of hard stools
Piemontese et al., 2011 Multi-center,	1130 infants (term; <8 weeks old; birth weight	Treatment: GOS/FOS (Immunofortis®) (8 g/L;	1 year (6 months starter formula, 6	Growth	NSD in mean body weight, length, or head circumference (lower compared to breast-fed) NSD in skin fold thicknesses and arm circumference (larger compared to breast-fed Week 8, smaller Week

Reference and Study Design	Subjects ^a	Dose	Duration	Results ^b	
randomized, double-blind, placebo-controlled	>p10 and <p90 ^e ; 554 M, 576 F; healthy) (858 infants completed)	9:1) Control: Standard formula (Breast-fed reference)	months follow-on formula)		52)
				Adverse event reporting	NSD in incidence of adverse events or serious adverse events NSD In occurrence of nappy rash NSD in number of spitting episodes within the first 24 weeks of age (lower compared to breast-fed) NSD in frequency of vomiting Occurrence of colic at 8 weeks higher than breast-fed NSD in incidence of any GI symptom between formula groups All significance NR
				Stool effects	NSD in stool frequency Stool consistency lower Weeks 8, 16, and 24 (similar to breastfed)
Scalabrini et al., 2010 Double-blind, Randomized	289 infants (term; 21 to 30 days old; average body weight 4275 g; 138 M, 151 F; healthy)	Treatment: PDX/GOS (Litesse Two PDX + Vivinal GOS) (4 g/L; 1:1) Control: Standard formula (Breast-fed reference)	60 days	Growth	Higher mean formula intake Day 16 to 30 NSD in weight growth rate from baseline to 30 or 60 days Mean weight growth rate at 60 days lower in breastfed group NSD in length or head circumference growth rates or achieved weight, length, or head circumference
				Adverse event reporting	NSD in number or type of adverse events NSD in gassiness (lower for breast-fed) or fussiness
				Fecal microflora	↓ <i>Bifidobacteria</i> in the breast-fed group at baseline NSD in bacterial counts at 30 days Trend towards higher counts in PDX/GOS group
				Stool effects	↑ Stool frequency in breast-fed group NSD in stool frequency between formula groups ↑ Stool consistency scores in breast-fed, and PDX/GOS groups ↑ Levels of fecal secretory IgA in breast-fed group
Ashley et al., 2012 Multi-center double-blind, parallel-design.	287 infants (term; 12 to 16 days old; birth weight ≥2,500 g; 167 M, 120 F; healthy)	Treatment: 1. PDX/GOS (Litesse Two PDX + Vivinal GOS)	106 days	Growth	↓ Growth rate Day 14 to 30 for M in PDX/GOS and F in GOS groups NSD in mean achieved weight, length, or head circumference

Reference and Study Design	Subjects ^a	Dose	Duration	Results ^b	
gender-stratified		(4 g/L; 1:1) 2. GOS (4 g/L) Control: Standard formula			
				Adverse event reporting	NSD in adverse events or reasons for study discontinuation
				GI effects	NSD in formula intake NSD in infant fussiness and gassiness
				Stool effects	↑ Stool frequency in PDX/GOS and GOS groups (Week 1 and 2, and at 60 days of age) NSD in stool frequency at 90 days ↑ Stool consistency (softer and formed stools) in PDX/GOS and GOS groups (Week 1 and 2) ↑ Stool consistency in PDX/GOS group from Week 2 to 120 days
Ribeiro et al., 2012 Randomized, double-blind, prospective	133 children (9 to 48 months old; body weight NR; 74 M, 59 F; healthy) (129 children completed)	Treatment: PDX/GOS (source NR) (~4.08 g/L; 1:1) Control: Follow-on formula	108 days	Growth	NSD in formula intake NSD in body-weight-for-length/height measurements
				Adverse event reporting	NSD in number of episodes of diarrheal disease, defecation, ARI, or systemic antibiotic use Both formulas well tolerated NSD in incidence of adverse events
				Stool effects	Higher odds of having increased defecation

*Adapted from GRN 495

ARI = acute respiratory infection; ALRI = acute lower respiratory infection; AOS = acidic oligosaccharides; BMI = body mass index; CTAK = cutaneous T cell attracting chemokine; F = female; FOS = fructo-oligosaccharides; GI = gastrointestinal; GOS = galacto-oligosaccharides; IFN- γ = interferon- γ ; LC-PUFA = long- chain-poly-unsaturated fatty acids; M = male; NR = not reported; NSD = no significant difference; PBMC = peripheral blood mononuclear cells; PDX = polydextrose; SCFA = short-chain fatty acids; SCORAD = SCORing Atopic Dermatitis; TARC = thymus activation-regulated chemokine; TGF- β = tumor growth factor- β ; TNF- α = tumor necrosis factor- α ; URTI = upper respiratory tract infections; UTI = urinary tract infections

^aunless stated otherwise, number of subjects are reported on a per-protocol basis; ^bunless stated otherwise, all reported effects are statistically significantly different relative to control group(s)

G = gestational age of 37 to 42 weeks; ^dnormal birth weight between the 5th and 95th percentile; ^enormal birth weight between the 10th and 90th percentile

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SUBMISSION END