

GRAS Notice (GRN) No. 518

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

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ORIGINAL SUBMISSION

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Soni & Associates Inc.

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May 7, 2014

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

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 preparation (King-Prebiotics® GOS-1000-P) 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.

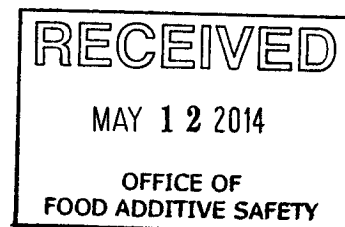
Given the compositional and manufacturing process differences, we believe that King-Prebiotics® GOS-1000-P is different as compared to the galacto-oligosaccharide products that have already been reviewed by FDA under other GRAS notices.

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.



Soni & Associates Inc

749 46th Square
Vero Beach, FL 32968
Telephone: 772-299-0746
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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), China, has determined that galacto-oligosaccharides derived from lactose is Generally Recognized As Safe, and therefore, exempt from the requirement of premarket approval, under the conditions of its intended use. This determination is based on scientific procedures as described in the following sections, under the conditions of galacto-oligosaccharides's intended use in food, among experts qualified by scientific training and expertise.

Signed,

(b) (6)



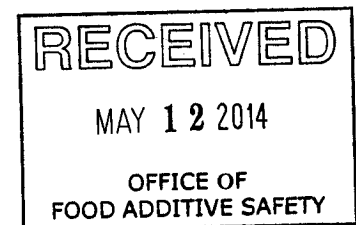
Date

May 7, 2014

Madhu G. Soni, Ph.D., FACN

Agent for:

New Francisco Biotechnology Corporation
Yunfu City, Guangdong Province,
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B. Name and Address:

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C. Common or usual name of the GRAS substance:

The common name of the substance of this GRAS assessment is galacto-oligosaccharides (GOS). GOS for food uses will be marketed as standardized powder (King-Prebiotics® GOS-1000-P).

D. Conditions of use:

Galacto-oligosaccharides (GOS) are intended for use in food categories such as Milk and Milk Products, Soups, Bakery Products, Cereals, Fruit and Vegetable Juices, Sugars and Sweets, and Non-alcoholic Beverages (all food categories mentioned in GRN 334) at use levels of 0.17 to 5.27 g/serving (reference amounts customarily consumed; 21 CFR 101.12). In addition to this, GOS will also be added to food categories mentioned in GRN 285 such as certain baby, infant, and toddler foods at levels ranging from 0.80 to 1.19 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 (all food categories mentioned in GRN 285) at a level of 1.19 g/serving. Thus, the intended use of GOS is in the same food products and at levels proportional to those mentioned in the GRN 334 (Yakult, 2010) and GRN 285 (GTC Nutrition, 2009a). The GOS content mentioned in GRN 334 and GRN 285 was 55% and 92%, respectively, while the subject of current GRAS notice contains no less than 99% GOS. The GOS that is the subject of this GRAS determination is not intended for use in meat and poultry products. Additionally, New Francisco Biotechnology Corporation (NFBC) specifically excludes food categories such as egg products and soup and soup mixes that come under USDA jurisdiction. The intended uses of GOS in the above mentioned food categories is estimated to result in the mean and 90th percentile intake for the total population of 12.2 and 25.3 g/person/day, respectively.

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 five GRAS notifications (GRN 334;

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GRN 286; GRN 285; GRN 236; and GRN 233). Among these notices, 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 safety determination of GOS for the present GRAS assessment is based on the totality of the available scientific evidence that includes human observations and a variety of preclinical and clinical studies. An Expert Panel was assembled to evaluate the health aspects of GOS. Based on the available safety-related information, the estimated daily intake, if ingested daily over a lifetime, the Expert Panel concluded that the intended uses of GOS as 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 name King-Prebiotics® GOS

B. Physical Characteristics

Off white light yellow powder

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.

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D. Chemical Formula and Molecular Weight

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.

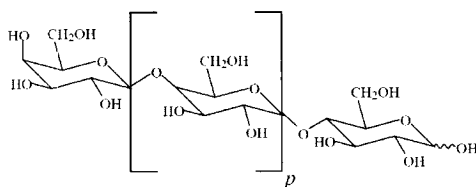


Figure 1. General Chemical Structure of GOS (p = 0 to 6)

F. Typical Specifications

Typical food grade specifications of GOS have been established by NFBC. NFBC intends to market GOS in the form of powder (GOS-1000-P). The specifications for GOS-1000-P (powder) are presented in Table 1. To demonstrate conformance with the food-grade specifications, NFBC analyzed several batches of GOS. Analytical results from five non-consecutive lots (Appendix I) 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 subject of this GRAS determination, GOS is substantially equivalent to GOS that was the subject of GRAS notified substances reviewed by the FDA without any questions [including GRN 334 (Yakult, 2010) and GRN 285 (GTC Nutrition, 2009a)].

Table 1. Physical and Chemical Specifications of GOS (GOS-1000-P)*

Parameters	Specifications	Methods**
Appearance	Off white light yellow powder	Visual
Taste	Slightly sweet taste	Sensory observation
Galacto-oligosaccharides	> 99% (DM)	Q/XJS 0001S-2011
Lactose + monosaccharide	< 1% (DM)	Q/XJS 0001S-2011
pH (10%)	3.0-6.0	pH meter
Moisture	≤ 3.5%	GB/T 20885-2007
Sulphated ash	≤ 0.3%	GB/T 20885-2007
Heavy metals		
Lead	≤ 0.02 ppm	GB 5009.12-2010
Arsenic	≤ 0.05 ppm	GB/T 5009.11-2003
Cadmium	≤ 0.1 ppm	GB/T 5009.15-2003
Total mercury	≤ 0.01 ppm	GB/T 5009.17-2003
Microbiological		
Total plate count	< 50 cfu/g	GB 4789.2-2010
Yeast	< 20 cfu/g	GB 4789.15-2010
Molds	< 20 cfu/g	GB 4789.15-2010
<i>Escherichia coli</i> and <i>Salmonella</i>	Negative/g	GB 4789.38-2012
<i>Salmonella</i>	Negative/g	GB 4789.4-2010
<i>Shigella</i>	Negative/g	GB 4789.5-2012
<i>Staphylococcus aureus</i>	Negative/g	GB 4789.10-2010
Enterobacteriaceae	Negative/g	ISO21528-1:2004
<i>Listeria</i>	Negative/50 g	GB 4789.30-2010
<i>Bacillus cereus</i>	< 10 cfu/g	GB/T 4789.14-2003

*Based on information provided by NFBC; DM = dry matter; ** All analytical methods used are in full compliance with Chinese Regulations; For methods, GB means "National Standard" in China, whereas Q/XJS is the Enterprise Standard, which has been filed with Ministry of Health, China.

G. 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→4 Gal, though other β -glycosidic linkages may also be present. Typical oligosaccharide distribution found in GOS is summarized in Table 2.

Table 2. Typical distribution or fractions of GOS (King-Prebiotics® GOS-1000-P)*

Component name	Amount/Percentage
Galacto Oligosaccharides	99.0% (DM)
-Trisaccharides	39.0%
-Tetrasaccharides	27.0%
-Pentasaccharides	18.0%
-Hexa, hapta- and Octo-saccharides	15.0%
Lactose + monosaccharide	1% (DM)
Total	100%

*Based on information provided by NFBC; DM = Dry matter

H. 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. 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- β 1-4 glucose bond to release glucose and galactose, transgalactosylation for converting lactose into galacto-oligosaccharides, and isomerization for the formation of galactose- β 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.

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. Following the termination of enzymatic reaction, GOS concentration of the syrup is further increased through fermentation by addition of yeast extract and sodium carbonate. The yeast extract is derived from a non-toxicogenic non-pathogenic microorganism, *Kluyveromyces lactis* strain², commonly used in food processing. Citric acid is added to inactivate 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 King-prebiotics® GOS-1000-P. A flow diagram of the manufacturing process is shown in Figure 2.

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

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

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

premium quality of a series of international-grade oligosaccharide (King-Prebiotics®) products that are manufactured from food grade lactose.

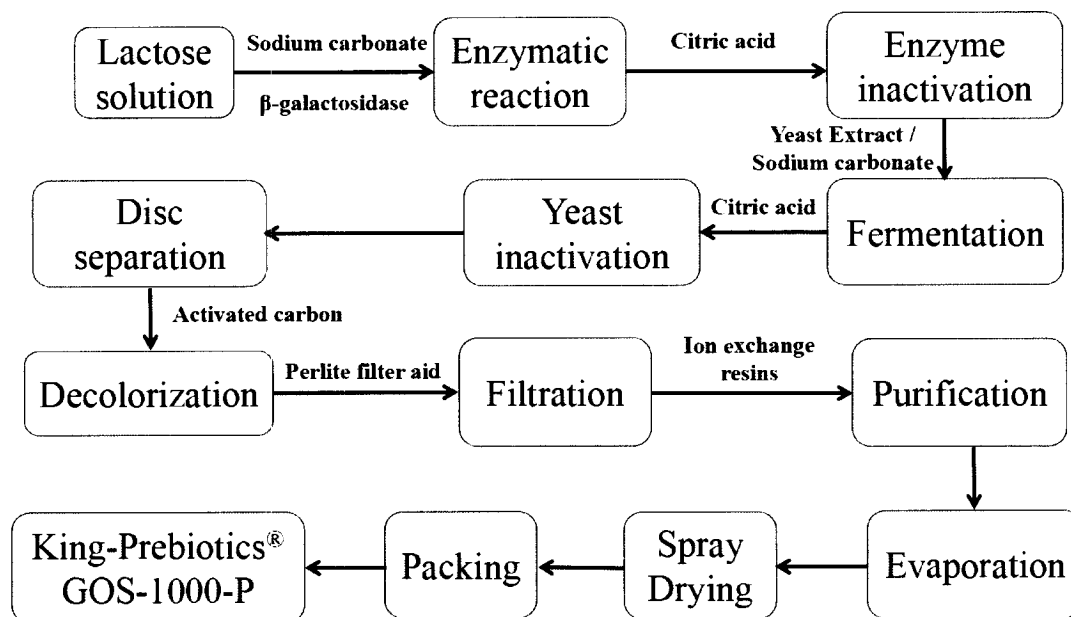


Figure 2. Manufacturing Process of GOS

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); Professor Douglas L. Archer, Ph.D. (University of Florida); and Madhusudan G. Soni, PhD, FACN, FATS (Food Ingredient Safety Consultant). Given Dr. Archer's background in microbiology, he was also assigned to specifically review the safety aspects related to the use of microorganisms, *Bacillus circulans* and *Kluyveromyces lactis*, strains.

A comprehensive search of the scientific databases for safety and toxicity information on GOS was conducted through March 2014. 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.

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 the foods [Milk and Milk Products,

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Soups, Bakery Products, Cereals, Fruit and Vegetable Juices, Sugars and Sweets, and Non-alcoholic Beverages at use levels of 0.17 to 5.27 g/serving (all food categories mentioned in GRN 334) and also in certain baby, infant, and toddler foods at levels ranging from 0.80 to 1.19 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 1.19 g/serving (all food categories mentioned in GRN 285)], when not otherwise precluded by a Standard of Identity, meeting the specification cited above and manufactured in accordance with 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 five GRAS notifications (GRN 234; GRN 286; GRN 285; GRN 236; and GRN 233) to the FDA for use as a food ingredient. 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. 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 March 2014 by Soni & Associates Inc., to determine the Generally Recognized As Safe (GRAS) status of GOS as a food ingredient. NFBC intends to use GOS as a food ingredient in Milk and Milk Products, Soups, Bakery Products, Cereals, Fruit and Vegetable Juices, Sugars and Sweets, and Non-alcoholic Beverages (all food categories mentioned in GRN 334) at use levels of 0.17 to 5.27 g/serving, and in certain baby, infant, and toddler foods at levels ranging from 0.80 to 1.19 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 (all food categories mentioned in GRN 285) at a level of 1.19 g/serving (reference amounts customarily consumed, 21CFR 101.12). The exposure from added GOS in the proposed food uses in the total U.S. population is estimated to result in the mean and 90th percentile intake of 12.2 and 25.3 g GOS/person/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 diet.

1.1. Background

GOS is a collective term for a group of carbohydrates composed of oligo-galactose with small amounts of lactose and glucose. Recently, this group of carbohydrates 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 fermentable. Because of these properties, 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, 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 and compositional distribution of GOS are presented in Table 1 and 2, respectively. The manufacturing process is summarized in Figure 2.

1.3. Natural Occurrence

As a complex mixture, GOS occur naturally in human breast milk and colostrums, as well as in bovine milk. McVaugh and Miller (1997) reported that GOS in human milk exhibit a complex and diverse chemical profile of over 130 different compounds. 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

GOS and other prebiotic ingredients are increasingly being recognized as useful dietary tools for the modulation of the colonic microflora toward a healthy balance. GOS compares well to other oligosaccharides in terms of their prebiotic and functional properties in foods. It 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 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.

For at least 25 years, GOS have been used as food ingredients in Europe and Japan. 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% 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

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

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 October 25, 2013, the agency has received five notices on GOS and provided "no questions" letters to all of the notifiers. In September 2007, Mead Johnson & Company submitted GRAS notification (GRN 233) for combination of GOS and polydextrose to FDA (Mead Johnson, 2007). On September 04, 2009, FDA issued "no questions" letter for this GRAS notice (FDA, 2009a). Subsequently, four GRAS notifications specifically on GOS were submitted to FDA by the following companies: Friesland Foods Domo (Friesland, 2008; GRN 236); GTC Nutrition (2009b; GRN 285), GTC Nutrition (2009c; GRN 286); and the most recent notice by Yakult Pharmaceutical Industry Co., Ltd. (Yakult, 2010; GRN 334). Each of these firms received a "no questions" letter from FDA (FDA, 2008, 2009b, 2009c, 2010). 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 and Food Categories

GOS is intended for use in the same foods and at levels proportional to those mentioned in the GRN 334 and GRN 286. As both notices were reviewed by the FDA and GRN 334 appeared subsequent to GRN 286, it is likely that the FDA considered cumulative intake from both notices. There are no new food uses proposed by NFBC for GOS. The substance mentioned in GRN 334 (Yakult, 2010) and GRN 286 (GTC, 2009a) has been reported to contain $\geq 55\%$ and 92% GOS, respectively, while the subject of present GRAS determination contains $\geq 99\%$ GOS. Thus the levels of GOS in NFBC GOS powder (99%) and those in GRN 334 (55%) and GRN 286 (92%) are different. Given these differences, NFBC intends to proportionately reduce the use levels so that it is identical to the levels mentioned in GRN 334 and GRN 286.

NFBC intends to use GOS as a food ingredient in Milk and Milk Products, Soups, Bakery Products, Cereals, Fruit and Vegetable Juices, Sugars and Sweets, and Non-alcoholic Beverages (all food categories mentioned in GRN 334) and, in certain baby, infant, and toddler foods at proportional use levels mentioned in GRN 334. Additionally, NFBC intends to use GOS in 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 (all food categories mentioned in GRN 285). The food categories and the intended use levels of GOS described in GRN 334 are summarized in Table 3.

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

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Table 3. Intended Uses of Galacto-oligosaccharides*

Food Category	Approximate serving size (g)	Maximum g GOS per serving	NFBC proposed GOS use level
Beverage concentrated (powder)	250	5.0	2.87
Bread	50	0.5	0.29
Brownies	40	0.4	0.22
Cakes, heavy weight	125	1.25	0.69
Cakes, light weight	55	0.55	0.31
Cakes, medium weight	80	0.8	0.44
Cheese soups	245	1.5	0.83
Coconut beverages	250	4	2.22
Coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads, muffins, toaster pastries	55	0.55	0.31
Cookies	30	0.3	0.17
Crackers that are usually used as snacks	30	0.3	0.17
Egg soups; soups with legumes as major ingredient; soups with grain products as major ingredient; potato soups; deep-yellow vegetable soups; tomato soups; other vegetable soups	245	1.5	0.83
French toast, pancakes	110	1.1	0.61
Fruit drinks such as fruit juice drinks, fruit flavored drinks, sports drinks, etc	250	5.0	2.87
Fruit juices (including citrus fruit juices) and nectars	250	4	2.22
Fruit juices, vegetable juices and juice mixtures baby food	125	2	1.11
Grain-based bars with or without filling or coating, e.g., breakfast bars, granola bars, rice cereal bars	40	0.4	0.22
Jellies, jams, preserves	20	5.0	2.87
Milk based meal replacement	250	5.0	2.87
Milk desserts, frozen like ice creams	75	1.5	0.83
Milk drink	250	9.5	5.27
Milk, milk substitute such as soy milk	250	5.0	2.87
Non fruit beverages, including energy drinks	250	11	6.10
Pies, cobblers, fruit crisps, turnovers, other pastries	125	1.25	0.69
Pudding and custards including baby foods	108	1.5	0.83
Ready-to-eat cereals	35	0.7	0.39
Ready-to-eat cereals (dry) for baby food	15	0.6	0.33
Ready-to-serve cereals for baby food	110	0.6	0.33
Vegetable juices	250	4	2.22
Waffles	85	0.85	0.47
White sauces, milk gravies and cheese sauces	80	1	0.55
Yogurt	225	7.5	4.16

*Adapted from FDA response letter to GRN 334

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1.5.1. Estimated Daily Intake from the Intended Uses

As indicated above, GOS is intended for use in the same foods, and at proportional levels of addition, as notified by Yakult Pharmaceutical Industry Co., Ltd. in GRN 334. The intended food use categories and use levels of GOS from GRN 334 are presented in Table 3. The intended use of GOS in the same foods and at proportional use levels as those in GRN 334 is not expected to noticeably affect the intake of GOS in the overall diet of the public from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. Based on a statistical analysis of potential dietary intake, in the GRN 334 notice it was estimated that the mean consumption of GOS for the total population would be 12.2 g/person/day (0.28 g/kg bw/day) and the 90th percentile consumption would be 25.3 g/person/day (0.70 g/kg bw/day). The dietary analysis summarized below in Table 4 was presented in GRN 334 and was not questioned by FDA in its response letter of October 27, 2010 (FDA, 2010). NFBC specifically excludes food categories such as egg products and soup and soup mixes that come under USDA jurisdiction.

Table 4. Estimated Two-day Average Daily Intake of GOS from All Proposed Uses in Food*

Population Group	Age (Month/Years)	Percent Users	2-Day Average GOS Intakes per User			
			g GOS/day		g GOS/kg bw/day	
			Mean	90 th Percentile	Mean	90 th Percentile
Infant	0-5 months	100	6.9	11.3	1.16	1.82
	6-11 months	99.6	7.9	11.8	0.90	1.40
	12-23 months	100	19.4	27.8	1.73	2.60
All Infants	0-1 years	99.9	14.7	26.8	1.44	2.42
Children	2-11 years	100	18.1	30.4	0.77	1.42
Teen Females	12-19 years	99.1	12.4	24.2	0.22	0.47
Teen Males	12-19 years	99.4	17.4	33.0	0.28	0.57
Adult Female	20+ years	99.8	9.5	19.4	0.14	0.29
Adult Male	20+ years	99.7	11.7	24.8	0.14	0.30
Total Population		99.7	12.2	25.3	0.28	0.70

*Adapted from GRN 334

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 ingredient in food. 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. 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 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. FDA did not question the acceptability and suitability of the available evidence to support the proposed uses described in five 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. 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.

2.2. GRAS Notices on GOS

2.2.1. GRN 233- September 04, 2009

This GRAS notice by Mead Johnson 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, 2009). 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.

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2.2.2. GRN 236- July 28, 2008

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).

2.2.3. GRN 285- September 4, 2009

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 $\beta(1-3)$, $\beta(1-4)$, $\beta(1-6)$ configurations, having a degree of oligomerization ranging between 3 and 5.

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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 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).

2.2.4. GRN 286- September 4, 2009

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.

2.2.5. GRN 334- October 27, 2010

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

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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).

2.3. 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

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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 three years since the submission of the GRAS notice GRN 334 (Yakult, 2010; FDA, 2010). 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 GRNs 334, 286, 285, 236, and 233, and the data made available to EFSA and FSANZ. Some of the pertinent information related to the safety, primarily from recent publications is summarized in the following sections.

2.5.1. Summary of Recent Safety Studies

2.5.1.1. Metabolism

As described in the above GRAS notices and regulatory assessments, pharmacokinetic studies of GOS 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 recent 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 a recent *in-vitro* study conducted in a fermentation screening-platform, the impact of GOS on adult gut microbiota composition and activity upon treatment with four antibiotics at two doses (Ladirat et al., 2013) was compared. 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.

In another *in vitro* study, fermentation of several purified GOS, specifically the trisaccharides 4'-galactosyl-lactose and 6'-galactosyl-lactose and a mixture of the disaccharides 6-galactobiose and allolactose, was investigated (Rodriguez-Colinas et al., 2013). In this study,

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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.

2.5.1.2. 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.

In another similar study from the above 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, the safety of GOS, produced from lactose by a two-step enzymatic process, was investigated (Kobayashi et al., 2009). 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

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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).

2.5.1.3. Human Studies

The available human studies with GOS are described in the FDA GRAS notices. Additionally, these studies are also summarized in the EFSA and FSANZ reports. In a recent double-blind cross-over trial, 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 adolescent girls aged 10-13 years consumed smoothie drinks twice daily with 0 (control), 2.5 or 5.0 g GOS for three 3-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 control, 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.

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 (Osborne and Sinn, 2013).

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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 (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.

In a randomized, double-blind, placebo-controlled crossover trial, 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 study, 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.

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

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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.

2.6. Safety of Bacterial Enzyme

GOS, the subject of this GRAS determination, 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 (Schallmeyer 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 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 glycosyltransferase 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 use in food and beverages. The products will be marketed under the trade name King-Prebiotics® GOS. 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-toxicogenic non-pathogenic microorganism. 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. 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.

Currently, GOS are used in a variety of foods [Milk and Milk Products, Soups, Bakery Products, Cereals, Fruit and Vegetable Juices, Sugars and Sweets, and Non-alcoholic Beverages (all food categories mentioned in GRN 334) at use levels of 0.3 to 9.5 g/serving and, in certain baby, infant, and toddler foods at levels ranging from 0.86 to 1.28 g/serving and in 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 (all food categories mentioned in GRN 285) at a level of 1.28 g/serving (reference amounts customarily consumed, 21 CFR 101.12)] as described in GRAS notices by different notifiers to FDA. NFBC intends to use GOS in the same foods and at levels proportional to those mentioned in the GRN 286 and GRN 334. Based on the GOS content given the two FDA GRAS notices, the proportional use levels will be 0.17 to 5.27 g/serving as compared to GRN 334 and 0.80 to 1.19 g/serving as compared to GRN 285. The intended uses of GOS in the above mentioned food categories, including the existing uses, is estimated to result at mean and 90th percentile cumulative intakes of 12.2 g/person/day (0.28 g/kg bw/day) and 25.3 g/person/day (0.70 g/kg bw/day), respectively.

There is sufficient qualitative and quantitative scientific evidence to determine the safety-in-use of GOS in the above mentioned applications. GOS occurs naturally in human breast milk and colostrums, as well as in bovine milk. Its natural presence in the diet supports the safety-in-use. In addition, manufactured GOS products have been used in food for over 25 years with no evidence of adverse effects related to the safety of its use. GOS has been the subject of five GRAS notices to FDA. In these submissions, use of GOS in specified food categories was estimated (90th percentile) to result in levels of up to 25.3 g/person/day. In response to five separate GRAS notifications on GOS (GRN 334; GRN 286; GRN 285; GRN 236; and GRN 233), FDA did not question the safety of GOS for the specified food uses. The subject of this present GRAS determination is substantially equivalent to GOS that has been the subject of FDA GRAS notified substances. 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 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.

The FDA responses to GRAS notifications on GOS indicate that the agency is satisfied with the safety-in-use of GOS at use levels up to 25.3 g/person/day. Additionally, EFSA and FSANZ also completed safety evaluation of GOS and did not raise any safety concerns for the uses of GOS in infant formula and traditional foods. 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 procedures⁵, exposure from diet and current uses, the consumption of GOS derived from lactose as a food ingredient at use levels ranging from 0.17 to 5.27 g/serving (reference amounts customarily consumed, 21 CFR 101.12) in certain specified foods resulting in a 90th percentile intake of 25.3 g/person/day is considered safe. The proposed uses are compatible with current regulations, *i.e.*, GOS (King-Prebiotics®) is used in Milk and Milk Products, Soups, Bakery Products, Cereals, Fruit and Vegetable Juices, Sugars and Sweets, and Non-alcoholic Beverages (all food categories mentioned in GRN 334) at use levels of 0.17 to 5.27 g/serving and, in certain baby, infant, and toddler foods at levels ranging from 0.80 to 1.19 g/serving and in 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 (all food categories mentioned in GRN 285) at a level of 1.19 g/serving (reference amounts customarily consumed, 21 CFR 101.12) when not otherwise precluded by a Standard of Identity, and is produced according to current good manufacturing practices (cGMP).

⁵ 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.

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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 specifications cited above, and when used as a food ingredient in selected food products [Milk and Milk Products, Soups, Bakery Products, Cereals, Fruit and Vegetable Juices, Sugars and Sweets, and Non-alcoholic Beverages at use levels ranging from 0.17 to 5.27 g/serving; in certain baby, infant, and toddler foods at levels ranging from 0.80 to 1.19 g/serving; and in 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 1.19 g/serving (reference amounts customarily consumed, 21 CFR 101.12)], when not otherwise precluded by a Standard of Identity as described in this dossier and resulting in the 90th percentile all users estimated intake of 25.3 g/person/day is Generally Recognized As Safe (GRAS).

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.

April 30, 2014
Date

(b) (6)

Robert L. Martin, Ph.D.

April 28, 2014
Date

(b) (6)

Douglas L. Archer, Ph.D.

May 5, 2014
Date

(b) (6)

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

May 7, 2014
Date

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6. APPENDIX I

Analytical data from five non-consecutive manufacturing lots of GOS preparation (GOS-1000-P)

Parameters	Standard Specifications	Batch #1303	Batch#1305	Batch #1306	Batch #1308	Batch #1401
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%	0.5%	0.4%	0.6%	0.5%	0.8%
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
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
<i>Enterobacteriaceae</i>	Negative/g	Negative	Negative	Negative	Negative	Negative
<i>Listeria</i>	Negative/50 g	Negative	Negative	Negative	Negative	Negative
<i>Cronobacter Sakazakii</i>	Negative/g	Negative	Negative	Negative	Negative	Negative
<i>Sulphite-reducing clostridium</i>	≤10 cfu/g	<10	<10	<10	<10	<10

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SUBMISSION END

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