ORIGINAL SUBMISSION

Spherix
CHROMADEX CONSULTING

September 20, 2016 #671

GRN 000671

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

To Whom It May Concern:

Enclosed please find three copies of the dossier entitled "Generally Recognized As Safe Determination for the Use of VITAGOS™ in Infant Formula and Selected Conventional Foods" and the GRAS Expert Panel Consensus Statement. This GRAS determination has been prepared by Spherix Consulting, Inc., on behalf of its client, Vitalus Nutrition Inc.

The data and information that serve as the basis for this GRAS determination is available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, Inc., 11821 Parklawn Drive, Suite 310, Rockville, MD 20852, Telephone: 301-230-2181; Email: clairek@chromadex.com, or will be sent to FDA upon request.

Should you have any questions or concerns, please contact me at the number listed above.

Sincerely,
(b) (6)

Claire L, Kruger, Ph.D., D.A.B.T.
President

Enclosures:

Three copies of the dossier entitled "Generally Recognized As Safe Determination for the Use of VITAGOS™ in Infant Formula and Selected Conventional Foods"

Three copies of the GRAS Panel Consensus Statement for the above-referenced GRAS Notification

11821 Parklawn Drive, Suite 310
Rockville, MD 20852
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Generally Recognized As Safe Determination for the Use of VITAGOS™ in Infant Formula and Selected Conventional Foods

Prepared for:

Vitalus Nutrition Inc.
3911 Mt. Lehman Road
Abbotsford, BC V2T 5W5
Canada

Prepared by:

Spherix Consulting, Inc.
A Division of ChromaDex, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852

August 2, 2016

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LIST OF ABBREVIATIONS

CCP: Critical Control Points

CFR: Code of Federal Regulations

DP: Degree of Polymerization

EPA: United States Environmental Protection Agency

FDA: United States Food and Drug Administration

FFDCA: Federal Food, Drug, and Cosmetic Act

FOS: Fructo-oligosaccharides

FOSHU: Food for Specified Health Uses

FSANZ: Food Standards of Australia and New Zealand

FSSC: Food Safety System Certification

GLP: Good Laboratory Practices

GMO: Genetically Modified Organisms

GOS: Galacto-oligosaccharides

GRAS: Generally Recognized As Safe

GRN: GRAS Notification

HACCP: Hazard analysis critical control point

HDPE: High Density Polyethylene

OVA: Ovalbumin

SCF: Scientific Committee on Food

USP: United Stated Pharmacopeia

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I. GRAS EXEMPTION CLAIM

A. NAME AND ADDRESS OF THE SPONSOR

Vitalus Nutrition Inc.
3911 Mt. Lehman Road
Abbotsford, BC V2T 5W5
Canada

B. COMMON OR USUAL NAME

Galacto-oligosaccharides (GOS), also known as oligogalactosyllactose, oligogalactose, oligolactose, transgalactosylated oligosaccharide, and transgalacto-oligosaccharide.

C. INTENDED USE

VITAGOS™ will be added to powdered non-exempt term infant formulas, selected conventional foods, coffees, and teas at levels not exceeding 7.2 g/L in reconstituted infant formula and 11 g/serving in selected conventional foods, coffees, and teas.

D. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of GOS for the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of VITAGOS™ has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of VITAGOS™ as an ingredient for the intended uses in foods and infant formulas has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. GOS are non-digestible oligosaccharides consisting of 1 to 7 galactose units linked via β(1→3), β(1→4), or β(1→6) glycosidic bonds to either a terminal glucose or galactose. Although tri- to hexa-saccharides with 2 to 5 galactose units (degree of polymerization (DP) of 3 to 6) tend to be the main components of GOS-containing products, disaccharides (DP2) consisting of galactose and glucose with different β-

glycoside bonds from lactose are also present and defined as GOS because they have physiological characteristics that are similar to longer GOS.

a. VITAGOS™ is a GOS-containing product manufactured using lactose and β-galactosidases derived from *Aspergillus oryzae* and *Kluyveromyces lactis* in a manner similar to other GOS-containing products that have received "no questions" letters from the United States Food and Drug Administration.

b. All processing aids used to produce VITAGOS™ comply with appropriate federal regulations.

c. A comparison of the manufacturing processes and product specifications for VITAGOS™ and other GOS-containing products shows that VITAGOS™ is essentially equivalent to the other GOS-containing products currently marketed in the United States for use in infant formulas and conventional foods.

2. GOS are transported through the upper gastrointestinal tract to the colon where they are fermented by the resident microbiota into short-chain fatty acids, carbon dioxide, methane, and hydrogen.

3. GOS present in food are either naturally occurring in human milk and colostrum, bovine colostrum, and fermented milk products or synthetic, which are then added to the food during processing and formulation.

4. Synthetic GOS have a long history of use worldwide.

a. In Japan, GOS have been commercially available since 1995 and are considered as Food for Specified Health Uses (FOSHU).

b. In the United States, the first GOS product was determined GRAS for use in term infant formula and selected conventional foods, and received a "no questions" letter from the FDA in 2008 (GRN 236). Since then, six additional GOS-containing products have been determined GRAS for use in infant formulas and selected conventional foods at levels up to 7.8 g/L and 11 g/serving, respectively, resulting in ten GRAS Notifications (GRN) to the FDA (GRN 236, 285, 286, 334, 484, 489, 495, 518, 569, and 620).

c. In the European Union, the safety of GOS was reviewed by the Scientific Committee on Food (SCF) in 2003 and is approved for use in infant and

follow-on formulas GOS in combination with fructo-oligosaccharides (FOS) at levels up to 8 g (90% GOS and 10% FOS)/L (7.2 g GOS and 0.8 g FOS/L) (Select Committee on Food EU 2016/127).

d. In Australia and New Zealand, the safety of GOS was reviewed by the Food Standards of Australia and New Zealand (FSANZ) in 2008 and is permitted in infant and follow-on formulas at levels up to 290 mg/100 kJ, or approximately 8 g/L (Australia New Zealand Food Standards Code – Standard 2.9.1 – 7).

5. GOS-containing products are not genotoxic.

6. GOS-containing products are not toxic to rats when administered for up to 90 days by gavage and the no observed adverse effect levels (NOAELs) determined in all the supporting toxicology studies were determined to be the highest dose tested (6900, 5000, 2000 mg/kg/day). The safety of VITAGOS™ was also determined in a 90-day repeat dose rat toxicology study and the NOAEL was determined to 2000 mg/kg/day.

7. GOS-containing products are well tolerated in humans and have been reported to increase the abundance of bifidobacteria and lactobacilli in the gastrointestinal tract in infants and adults, increase fecal short-chain fatty acid concentrations in infants and adults, improve stool consistency in infants, reduce the incidence of atopic dermatitis in infants, and alleviate the symptoms of irritable bowel syndrome in adults.

8. GOS is available worldwide and, although GOS-containing products have been reported to provoke allergic reactions in sensitized individuals living in Vietnam and Signapore, there have been no reported reactions to GOS-containing products outside of Vietnam and Singapore. Thus, the reported cases likely represent unique, rare, geographically localized allergic reactions to GOS-containing products.

9. The addition of VITAGOS™ to infant formula at 7.2 g GOS/L is the same use level of other GOS products in infant formula that have been determined GRAS and received "no questions" letters from the FDA (GRN 286, 334, 569) and will result in intakes of approximately 5.1 and 6.9 g GOS/day for one-month-old and six-month-old infants, respectively.

10. The addition of VITAGOS™ to selected conventional foods, coffees, and teas at levels ranging from 0.3 to 11 g GOS/serving is the same as other GOS products that

have been determined GRAS and received "no questions" letters from the FDA (GRN 285, 334, 484, and 518). Thus, the dietary exposure to VITAGOS™ from the intended uses will not increase in the GOS-consuming population in the United States. The estimated mean and 90th percentile exposure to VITAGOS™ from the intended uses in selected conventional foods are 12.2 and 25.3 g per person per day (g/p/d) and in coffees and teas of 5.0 g/p/d for adult males and 4.4 g/p/d for the total population.

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

There is no evidence in the available information on VITAGOS™ that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when VITAGOS™ is used at levels that might reasonably be expected from the proposed applications of VITAGOS™ for use in powdered non-exempt term infant formulas and selected conventional foods, teas, and coffees as proposed by Vitalus Nutrition Inc.

Therefore, VITAGOS™ is safe and GRAS at the proposed levels of addition to the intended foods. VITAGOS™ is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

E. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, PhD, DABT, President, Spherix Consulting, A Division of ChromaDex, Inc., at 11821 Parklawn Drive, Suite 310, Rockville, MD 20852. Telephone: 301-230-2180; Email: clairek@chromadex.com, or be sent to FDA upon request.

F. SIGNATURE

Pursuant to the criteria provided in proposed 21 CFR 170.36, Vitalus Nutrition Inc. hereby notifies the United States Food and Drug Administration (FDA) that the use of GOS in foods under the intended conditions of use is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Vitalus Nutrition Inc. has determined that such use is Generally Recognized As Safe through scientific procedures.

(b) (6)

Signature
Marcela Cota Rivas
Authorized Representative of Vitalus Nutrition Inc.

September 9, 2016
Date

II. DESCRIPTION OF SUBSTANCE

A. COMMON OR USUAL NAME

Galacto-oligosaccharides (GOS), also known as oligogalactosyllactose, oligogalactose, oligolactose, transgalactosylated oligosaccharide, and transgalacto-oligosaccharide.

B. TRADE NAME

VITAGOS™

C. DESCRIPTION OF GALACTO-OLIGOSACCHARIDES

Galacto-oligosaccharides are non-digestible transgalactosylated oligosaccharides consisting of 1 to 7 galactose units linked to either a terminal glucose or galactose via β(1→3), β(1→4), or β(1→6) glycosidic bonds (Figure 1). Although tri- to hexa-saccharides with 2 to 5 galactose units (degree of polymerization (DP) of 3 to 6) tend to be the main components of GOS-containing products, disaccharides (DP2) consisting of galactose and glucose with β-glycoside bonds different from lactose are also present and defined as GOS because they have physiological characteristics that are similar to longer GOS (Sangwan et al., 2011; Sako et al., 1999).

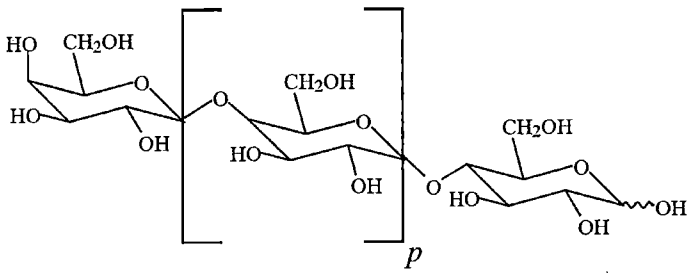

Figure 1. Structure of Galacto-oligosaccharides.
Brackets denote the repeating units, p = 0 to 6 to generate galactooligosaccharides consisting of 1 to 7 galactose units linked to a terminal glucose or galactose via β(1→3), β(1→4), or β(1→6) glycosidic bonds

VITAGOS™ is a GOS-containing syrup sythesized from lactose using β-galactosidases derived from *Aspergillus oryzae* and *Kluyveromyces lactis*. VITAGOS™ contains predominantly disaccharide and trisaccharide GOS (DP2 and DP3), which account for approximately 50% of the total saccharide content of the finished product (Table 1).

Table 1. Total Sugar Composition of VITAGOS™			
Saccharide (DP)	**Carbohydrate**	**Chemical Name**	**Total Amount (%)**
Monosaccharides (DP1)	Galactose		3
	Glucose		16
Disaccharides (DP2)	Lactose	(Gal-β(1→4)-Glc)	15
	GOS	(Gal-β(1→6)-Glc)	19
		(Gal-β(1→6)-Gal)	
		(Gal-β(1→4)-Gal)	
		(Gal-β(1→3)-Gal)	
Trisaccharides (DP3)	GOS	(Gal-β(1→6)-Gal-β(1→4)-Glc	32
		(Gal-β(1→4)-Gal-β(1→4)-Glc	
		(Gal-β(1→3)-Gal-β(1→4)-Glc	
Tetrasaccharides (DP4)	GOS	(Gal-β(1→6)-Gal-β(1→6)-Gal-β(1→4)-Glc	11
Pentasaccharides and higher oligomers (DP≥5)	GOS	ND	4
DP = Degree of Polymerization; ND = not determined; GOS = Galactooligosaccharides			

D. PRODUCTION PROCESS

VITAGOS™ is manufactured by Vitalus Nutrition Inc., located at 3911 Mt. Lehman Rd. Abbotsford, British Columbia, V2T 5W5, Canada under food grade conditions. Vitalus Nutrition Inc. has a hazard analysis critical control point (HACCP) management system in place and their manufacturing facility has been audited by a third party and determined to be compliant with the Food Safety System Certification (FSSC) 22000 standards. Importantly, all food contact surfaces used in manufacturing VITAGOS™ are either stainless steel, aluminum or suitable for use in the production of food ingredients. The whey used to produce the lactose is free of antibiotics and all raw materials and processing aids are either Food Chemical Codex grade, comply with conditions of use stipulated in Parts 168, 173, 177, 182 and 184 of Title 21 of the United States Code of Federal Regulations or have been determined GRAS (GRN 90). The ingredients and processing aids also comply with European Union and Codex requirements, and, because current Canadian legislation prohibits the use of bovine growth hormones in dairy cattle, free of recombinant bovine somatotropic and growth hormones. In addition, VITAGOS™ does not contain genetically modified organisms (GMOs) or ingredients derived from GMO-derived products and has been certified as meeting kosher and halal specifications.

1. Production Process

Similar to GOS that has been previously determined GRAS and received "no questions" letters from the United States Food and Drug Administration (FDA; GRN 236, 285, 286, 334, 484, 489, 495, 518, 569), VITAGOS™ is produced using β-galactosidases derived from *A.*

oryzae and *K. lactis*, which hydrolyze the β1-4 glycosidic bond between the galactose and glucose moieties of lactose and transgalactosylate the residual galactose with additional galactose moieties.

To produce VITAGOS™, food-grade lactose is dissolved in municipal drinking water and heated to a temperature greater than 80°C under agitation (Figure 2). The temperature and pH of the solution are then adjusted to optimum conditions for transgalactosylation. β-Galactosidase is added and the solution is agitated for a set period of time to convert the lactose to GOS. The temperature and pH are then readjusted and a second β-galactosidase is added to achieve the desired final GOS purity. The enzymes are deactivated using heat (80°C) and the enzyme residues and other impurities are removed by filtration and adsorption processes using several resins. The resulting material is concentrated by evaporation and then further heat-treated prior to packaging. Packaging occurs under hygienic conditions using containers lined with ultra-low density polyethylene food-grade bags. Packaged VITAGOS™ is stored under ambient conditions and when compliance with the product specifications is met, cleared for distribution.

Two critical control points (CCPs) in the production process control the quality of the finished product. The first, CCP1, occurs at the concentration step where Vitalus Nutrition Inc. controls the temperature and duration of heating of the product to prevent microbial growth during production. The second, CCP2, occurs at the packaging step where Vitalus Nutrition Inc. passes the material through a screen to ensure a homogenous syrup. The quality of the product is also monitored during processing with in-line testing for solids, conductivity, pH, color, and sugar profile.

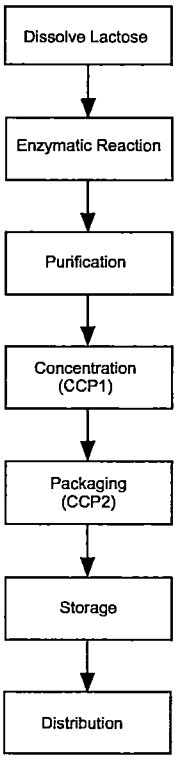

Figure 2. Production Process for VITAGOS™

Lactose is dissolved in water and mixed with β-galactosidases. The enzymes are then deactivated and GOS are purified by filtration and adsorption with resins. VITAGOS™ is concentrated by evaporation, heat-treated, packaged in containers lined with food grade bags, and stored under ambient conditions. When compliance with the product specifications is met, VITAGOS™ is distributed to customers.

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Product Specifications

To ensure a consistent food-grade product, each batch of VITAGOS™ is evaluated against an established set of product specifications (Table 2). Data from five pilot batches demonstrate control of the production process and compliance with the product specifications.

Table 2. VITAGOS™ Product Specifications and Batch Data

Parameter	Specification	Method	Batch Number				
			(b)(4)	(b)(4)	(b)(4)	(b)(4)	(b)(4)
Physical Characteristics							
Appearance	Clear to slight yellow	Visual	Clear to slight yellow	Clear to slight yellow	Clear to slight yellow	Clear to slight yellow	Clear to slight yellow
pH	2.7 - 3.7	TMS-QC 772*	2.9	3.1	2.7	2.9	3.0
Viscosity (cPs @ 26°C)	1000 - 5000 cPs	Brookfield (TMS-QC2562)*	2000	2000	1800	1950	2000
Dry Matter (Total %)	74-76	SMEDP 15.10B*	74.7	74.3	74.8	74.3	74.3
Chemical Composition							
Galacto-oligosaccharides (% DM)	≥ 62	HPLC (TMS-QC 2535)*	65.9	65.8	65.7	65.3	65.4
Lactose (% DM)	≤ 16	HPLC (TMS -QC 2561)*	15.0	14.4	15.0	15.3	14.7
Glucose (% DM)	≤ 22	HPLC (TMS -QC 2535)*	17.1	17.2	17.7	17.5	18.0
Galactose (% DM)	≥ 1	HPLC (TMS -QC 2535)*	2.0	2.5	1.6	1.8	1.9
Sulfated Ash (% DM)	≤ 0.3	USP / NF Current Version	0.01	0.01	0.01	0.03	0.03
Protein (% DM)	≤ 0.4	AOAC 991.20.I	0.2	0.2	0.2	0.2	0.2
Microbiological Parameters							
Standard Plate Count (cfu/g)	< 3000	MFHPB-33*	ND	ND	ND	ND	ND
Enterobacteriaceae (cfu/ g)	< 10	MFLP-43*	ND	ND	ND	ND	ND
Escherichia coli (cfu/g)	< 10	MFHPB-34*	ND	ND	ND	ND	ND
Yeast and Mold (cfu/g)	< 100	MFHPB-22*	ND	ND	ND	ND	ND
Staphylococcus aureus (cfu/g)	< 10	MFHPB-21*	ND	ND	ND	ND	ND
Salmonella (per 25g)	Negative	MFLP-29*	Neg.	Neg.	Neg.	Neg.	Neg.
Heavy Metals							
Arsenic (ppm; w/w)[1]	< 0.4	EPA 3050/6020 USP <730>	ND	ND	ND	ND	ND
Lead (ppm; w/w)[1]	< 0.2	EPA 3050/6020 USP <730>	0.02	0.04	0.03	0.03	0.06
Cadmium (ppm; w/w)[1]	< 0.06	EPA 3050/6020 USP <730>	0.001	0.001	ND	ND	ND
Mercury (ppm; w/w)[1]	< 0.005	EPA 3050/6020 USP <730>	ND	ND	ND	ND	ND

cPs = centipoises; cfu = colony forming units; USP = United Stated Pharmacopeia; EPA = United States Environmental Protection Agency; ppm = parts per million; w/w = weight/weight; g = gram; DM = dry matter; ND = not determined; Neg. = negative

[1]Limit of detection: Arsenic = 0.01 ppm; lead = 0.01 ppm; cadmium = 0.001 ppm; mercury = 0.005 ppm.

*Internal method at Vitalus Nutrition, Inc.

2. Other Quality Attributes

a. Degree of Polymerization

To demonstrate control of the production process, Vitalus Nutrition Inc. analyzed the GOS DP content of five batches of VITAGOS™ by HPLC (Table 3). Each batch had similar amounts of the different GOS DP fractions, indicating that the manufacturing process produces a consistent product. Importantly, Vitalus Nutrition Inc. monitors the GOS DP content of the finished product on a quarterly basis.

Table 3. GOS Content of VITAGOS™

Saccharide (DP)[1]	Batch Number					Average +/- St. Dev. (%)
	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	
DP2	16.7	16.5	17.1	17.1	17.6	17.0 +/- 0.42
DP3	33.8	33.8	33.2	32.8	32.9	33.3 +/- 0.48
DP4	11.6	11.7	11.5	11.4	11.2	11.5 +/- 0.20
DP≥5	3.8	3.8	3.9	4.0	3.7	3.8 +/- 0.11
Total GOS (% DM)[2]	65.9	65.8	65.7	65.3	65.4	65.6 +/- 0.25

DP = Degree of Polymerization; ND = not determined; GOS = Galactooligosaccharides; DM = dry matter
[1]Does not include lactose. Determined by HPLC (TMC-QC 2535).
[2]Corresponds to the data presented for the GOS specification in Table 3.

b. Pathogenic Bacteria

To confirm the absence of *Cronobacter sakazaki* and *Bacillus cereus*, Vitalus Nutrition Inc. analyzed five batches of VITAGOS™ using the appropriate microbiological techniques (Table 4). *C. sakazaki* and *B. cereus* were undetectable in each batch. Importantly, Vitalus Nutrition Inc. monitors VITAGOS™ for the presence of these pathogenic bacteria on a quarterly basis.

Table 4. Pathogenic Bacteria in VITAGOS™

Bacteria	Method	LOD	Batch Number				
			(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Cronobacter sakazaki	MFLP-42	<10 cfu/g	UD	UD	UD	UD	UD
Bacillus cereus	MFLP-27	Neg./25 g	Neg.	Neg.	Neg.	Neg.	Neg.

UD = undetectable; GOS = Galactooligosaccharides; LOD = limit of detection; Neg = negative

c. Protein Allergens

VITAGOS™ is manufactured on a production line that processes only milk products. No other potentially allergenic substances are used. For due diligence purposes, Vitalus Nutrition Inc. determined the amount of casein, a major milk allergen, in VITAGOS™ by SDS-PAGE. No casein was detected (Limit of Detection <2.6 ppm).

F. STABILITY OF VITAGOS™

The intended shelf-life of VITAGOS™ is at least 12 months. To support this, one lot of VITAGOS™ was stored in high density polyethylene (HDPE) bottles under ambient conditions (18-25°C). Oligosaccharide content, microbiological content, and pH were determined at various time points and compared to the acceptance limits stipulated in the product specifications. Over the course of 18 months, GOS, galactose, glucose, and lactose content were similar to freshly made VITAGOS™ and at all time points complied with the product specifications (Table 5). The distribution of GOS in DP2, DP3, DP4, and DP5 or greater was similar to VITAGOS™ at the beginning of the testing period. Microbiological content and pH were determined over the course of 12 months and, although not all parameters were determined at each time point, all complied with the product specifications over the course of the testing period (Table 6). Importantly, determining the stability of VITAGOS™ is an ongoing process and will continue to be monitored to support the intended shelf-life of the finished product.

Table 5. Oligosaccharide Stability of VITAGOS™

Parameter	Specification[1]	Time (Months)			
		0	3	12	18
Galacto-oligosaccharides (% DM)	≥ 62	65.1	65.1	64.7	65.2
Galactose (% DM)	≥ 1	2.9	3.3	3.3	3.3
Glucose (% DM)	≤ 22	16.2	16.3	16.6	16.3
Lactose (% DM)	≤ 16	15.7	15.3	15.4	15.2
DP2 (% GOS)	ns	18.4	18	18.1	18.4
DP3 (% GOS)	ns	32.5	32.7	32.5	32.5
DP4 (% GOS)	ns	11.1	10.9	10.7	10.9
≥DP5 (% GOS)	ns	3.1	3.5	3.4	3.35

DM = dry matter; DP = degree of polymerization; ns = no specification
[1]Methods cited in Table 3

Table 6. Microbiological Stability of VITAGOS™

Parameter	Specification[1]	Time (Months)											
		1	2	3	4	5	6	7	8	9	10	11	12
Standard Plate Count	< 3000 (cfu/g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriaceae	< 10 (cfu/g)	ND	ND	ND	ND	-	-	-	-	-	ND	ND	ND
Escherichia coli	< 10 (cfu/g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yeast	< 100 (cfu/g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mold	< 100 (cfu/g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Staphylococcus aureus	< 10 (cfu/g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella	Neg./25 g	Neg.	Neg.	Neg.	Neg.	-	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
pH	2.7 - 3.7	3.2	3	2.8	-	2.8	-	2.7	3.1	2.7	3.4	-	3.2

cfu = colony forming units; "-" = not tested; ND = not determined; Neg. = negative
[1]Methods cited in Table 3

III. INTENDED EFFECT

The intended effect of adding synthetic GOS to infant formula and selected conventional foods is to increase oligosaccharide intake in formula-fed infants and the general population and promote the growth of beneficial bacteria, including, but not limited to bifidobacteria and lactobacillus.

IV. HISTORY OF USE, INTENDED USE, AND ESTIMATED DAILY INTAKE

A. HISTORY OF USE

GOS present in food are either naturally occurring or synthetic forms added to the food during processing and formulation. Naturally occurring GOS are present in human milk and colostrum, bovine colostrum, and fermented milk products (Kunz et al., 2000; Coppa et al., 1991; Coppa et al., 1997; Toba et al., 1982; Saito et al., 1987) whereas synthetic GOS are found in a wide variety of products (Table 6). The levels of naturally occurring GOS range from 5 – 15 g/L, 8.5 g/L, and 0.03 – 0.09% in human milk, bovine colostrum, and fermented milk products, respectively (Kunz et al., 2000; Coppa et al., 1991; Coppa et al., 1997; Saito et al., 1987; Toba et al., 1982). It is important to note that, although synthetic GOS are structurally and compositionally less diverse than naturally occurring GOS, both types contain the same glycosidic bonds that render them resistant to the digestive enzymes in the stomach and small intestine and fermentable by the gastrointestinal microbiota present in the small intesting and colon (Wisker et al., 1985; Ohtsuka et al., 1990; Chonan et al., 2004).

Synthetic GOS have a long history of use worldwide. In Japan, GOS have been commercially available since 1995 and are considered as Food for Specified Health Uses (FOSHU).

In the United States, the first GOS product was determined GRAS for use in term infant formula and selected conventional foods, and received a "no questions" letter from the FDA in 2008 (GRN 236). Since then, six additional GOS-containing products have been determined GRAS for use in infant formulas and selected conventional foods at levels up to 7.8 g/L and 11 g/serving, respectively, resulting in ten GRAS Notifications (GRN) to the FDA (GRN 236, 285, 286, 334, 484, 489, 495, 518, 569, and 620). Except for GRN 620, which proposes to increase the use levels in infant formulas from 7.2 to 7.8 g/day and is still under review, all GRNs received "no questions" letters from FDA.

In the European Union, the safety of GOS was reviewed by the Scientific Committee on Food (SCF) in 2003. The ingredient is currently approved for use in infant and follow-on formulas GOS in combination with fructo-oligosaccharides (FOS) at levels up to 8 g (90% GOS and 10% FOS)/L (7.2 g GOS and 0.8 g FOS/L) (Scientific Committee on Food, EU 2016/127).

In Australia and New Zealand, the safety of GOS was reviewed by the Food Standards of Australia and New Zealand (FSANZ) in 2008 and, similar to the EU, GOS is currently permitted

in infant and follow-on formulas at levels up to 290 mg/100 kJ, or approximately 8 g/L (Australia New Zealand Food Standards Code – Standard 2.9.1 – 7).

B. INTENDED USE

Vitalus Nutrition Inc. intends to use VITAGOS™ as a substitute for other GOS preparations that are currently used in powdered non-exempt term infant formulas, selected foods, coffees, and teas at levels not exceeding 7.2 g/L in reconstituted infant formula and 11 g/serving in selected conventional foods, coffees, and teas (Table 7).

Table 7. Intended Uses of VITAGOS™ in Selected Conventional Foods

Food group	Proposed Food Uses	Approximate serving size	Maximum GOS per serving (g)[2]
Milk and milk products	Milk, milk substitute such as soy milk	250 g (240 ml)	5
	Milk drink	250 g (240 ml)	9.5
	Yogurt	225 g	7.5
	Milk-based meal replacement	250 g (240 ml)	5
	White sauces, milk gravies and cheese sauces	80 g (1/4 cup or 60 ml)	1
	Milk desserts, frozen like ice creams	75 g (1/2 cup)	1.5
	Pudding and custards including baby foods	108 g (1/2 cup)	1.5
	Cheese Soups	245 g	1.5
	Powdered non-exempt infant formula	7.2 g/L	NA[1]
Frozen dairy desserts and mixes	Frozen dairy desserts	125 g	3
	Ice cream	125 g	1.28
Soups	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 g	1.5
Nut beverages	Coconut beverages	250 g (240 ml)	4
Bakery products	Bread	50 g	0.5
	Brownies	40 g	0.4
	Cakes, heavy weight	125 g	1.25
	Cakes, medium weight	80 g (1/4 cup or 60 ml)	0.8
	Cakes, light weight	55 g	0.55
	Coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads, muffins, toaster Pastries	55 g	0.55
	Cookies	30 g	0.3
	Crackers that are usually used as snacks	30 g	0.3
	French toast, pancakes	110 g	1.1

Table 7. Intended Uses of VITAGOS™ in Selected Conventional Foods

Food group	Proposed Food Uses	Approximate serving size	Maximum GOS per serving (g)[2]
	Pies, cobblers, fruit crisps, turnovers, other pastries	125 g	1.25
	Waffles	85 g	0.85
	Grain-based bars with or without filling or coating, e.g., breakfast bars, granola bars, rice cereal bars	40 g	0.4
Cereals	Ready-to-eat cereals	35 g	0.7
	Ready-to-eat cereals (dry) for baby food	15 g	0.6
	Ready-to-serve cereals for baby food	110 g	0.6
	Pasta	55	1.28
Fruit and vegetable juices	Fruit juices (including citrus fruit juices) and nectars	250 g (240 ml)	4
	Vegetable juices	250 g (240 ml)	4
	Fruit juices, vegetable juices and juice mixtures baby food	125 g (120 ml)	2
Sugars and sweets	Jellies, jams, preserves	20 g (1 tbsp)	5
Non-alcoholic beverages	Fruit drinks such as fruit juice drinks, fruit flavored drinks, sports drinks, etc.	250 g (240 ml)	5
	Non fruit beverages including energy drinks	250 g (240 ml)	11
	Beverage concentrate (powder)	250 g (240 ml)	5
Snacks	Novelty snacks	30	1.28
Sweet Sauces	Syrup flavorings	30	3
Coffee and Tea	Coffee and Tea	240	1.5
Dairy Product Analogs	Coffee creamers and whiteners (liquid)	15	0.8
	Coffee creamers and whiteners (Powder)	2	0.8
	Soy beverages	240 ml	3

NA = not applicable
[1]Maximum amount of GOS ingested is based on the caloric need of the infant (see Chapter IV, Section C.1.)
[2]Use levels are consistent with those stipulated in GRN 334, 484, and 518.

C. ESTIMATED DAILY INTAKE

1. Infant Formula

Powdered non-exempt term infant formulas will contain 7.2 g GOS/L as consumed. Infant formulas in the US market provide approximately 670 kcal/L (20 kcal/fl oz) (Martinez and Ballew, 2011). Assuming infant formulas are the sole source of nutrition, reconstituted at 141 g/L, or a caloric density of 670 kcal/L, and the caloric requirements of a one month-old and six month-old infants are 472 kcal/day and 645 kcal/day, respectively (Institute of Medicine (US) Panel on Macronutrients and Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005), 1 and 6 month-old infants consume approximately 0.704 and 0.963 L formula/day. The addition of 7.2 g GOS/L in infant formula will therefore result in a GOS intake of approximately 5.1 and 6.9 g/day for one-month-old and six-month-old infants, respectively. Importantly, the use of other GOS products in infant formula at this intended use levels has been determined GRAS and received "no questions" letters from the FDA (GRN 286, 334, 569).

2. Selected Conventional Food Uses

For the selected conventional food uses, Vitalus Nutrition Inc. intends to use VITAGOS™ as a substitute for other GOS preparations that are currently used in foods, coffees, and teas. The intended use levels will range from 0.3 to 11 g/serving (Table 7). Importantly, both the intended uses and use levels for VITAGOS™ are the same as those for other GOS products that have been determined GRAS and received "no questions" letters from the FDA (GRN 285, 334, 484, and 518). Thus, the dietary exposure to VITAGOS™ from the intended uses will not increase in the GOS-consuming population in the United States. Currently, the estimated mean and 90th percentile exposure to VITAGOS™ from the intended uses excluding coffees and teas are 12.2 and 25.3 g per person per day (g/person/d), which were determined GRN 285, 334, 484, and 518. For the intended uses of VITAGOS™ in coffees and teas, the daily exposure to GOS from the intended uses in coffee and tea is 5.0 g/p/d for adult males and 4.4 g/p/d for the total population (users only), which was determined GRN 484.

V. SAFETY

Numerous metabolic (*in vitro* and *in vivo*), short-term toxicity, longer-term toxicity, and infant and adult clinical studies are publicly available and support the safe use of GOS in infant formulas and selected conventional foods. Moreover, the use of GOS in infant formulas and selected conventional foods has been determined safe in the United States, European Union, Australia and New Zealand (GRN 236, 2008; GRN 285, 2009; GRN 286, 2009; GRN 334, 2010; GRN 484, 2014; GRN 489, 2014; GRN 495, 2014; GRN 518, 2014; GRN 569, 2015; GRN 620, 2016; Scientific Committee on Food, 2001; Scientific Committee on Food, 2001; FSANZ, 2008). In the United States, seven GOS-containing products have been determined GRAS for use in infant formulas and selected conventional foods at levels up to 7.8 g/L and 11 g/serving, respectively, resulting in ten GRAS Notifications (GRN 236, 285, 286, 334, 484, 489, 495, 518, 569, and 620). Except for GRN 620, which is currently under review and proposes to increase in the use level in infant formula from 7.2 to 7.8 g/L, all GRNs received "no questions" letters from the FDA. In the European Union, GOS is approved for use in infant and follow-on formulas GOS in combination with fructo-oligosaccharides (FOS) at levels up to 8 g (90% GOS and 10% FOS)/L (7.2 g GOS and 0.8 g FOS/L) (Scientific Committee on Food, EU 2016/127). In Australia and New Zealand, GOS is permitted in infant and follow-on formulas at levels up to 290 mg/100 kJ, or approximately 8 g/L (Australia New Zealand Food Standards Code – Standard 2.9.1 – 7). The essential equivalence of the different GOS products that have been determined safe for use in infant formulas and conventional foods in the United States, and the results of the metabolic, short-term toxicity, longer-term toxicity, and clinical studies that support the safe use of GOS in infant formulas and selected conventional foods are summarized below.

A. ESSENTIAL EQUIVALENCE OF VITAGOS™ AND OTHER GOS PRODUCTS IN THE US MARKET

To understand the equivalence of VITAGOS™ and the other GOS products that have been determined GRAS, the VITAGOS™ manufacturing process and product specifications were compared to the GOS products that have been determined GRAS based on their intended use in infant formula and conventional foods (Table 8 and 9). Although VITAGOS™ is manufactured using β-galactosidases derived from *K. lactis* and *A. oryzae*, β-galactosidases derived from these strains have either been used alone or in combination with β-galactosidases derived from other microbial species to produce other GOS formulations. Moreover, the raw material is consistent what is used for the production of other GOS products that have been determined GRAS, and the method for purifying VITAGOS™ (ion exchange and evaporation) and the VITAGOS™ product specifications are similar to those for GOS products that are GRAS for use in infant formulas and selected conventional foods. Based on these criteria, VITAGOS™ is therefore essentially equivalent to the GOS products currently marketed in the United States for use in infant formulas and conventional foods.

Table 8. Comparison of Product Specifications between VITAGOS™ and other GOS Preparations Intended for Infant Formula

		GRAS Notification Number[1]										
			569									
Parameter	**VITAGOS™**	**620**	**GOS-1000-P***	**GOS-900y-P***	**GOS-700-P***	**GOS-570-S***	**GOS-270-P***	**495**	**489**	**334**	**286****	**236*****
Raw Materials	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose and sweet whey ultra-filtration permeate
Enzyme	β-galactosidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galact-osidase
Enzyme Source	*Aspergillus oryzae & Kluyveromyces lactis*	*Aspergillus oryzae*	*Bacillus circulans*	*Bacillus circulans*	*Bacillus circulans*	*Bacillus circulans*	*Bacillus circulans*	*Bifidobacterium bifidum*	*Aspergillus oryzae*	*Sporobolomyces singularis & Kluyveromyces lactis*	*Bacillus circulans & Aspergillus oryzae*	*Bacillus circulans*
Purification Method	Ion exchange and evaporation	Filtration and evaporation	Ion exchange, yeast fermentation, and evaporation	Ion exchange, yeast fermentation, and evaporation	Ion exchange, yeast fermentation, and evaporation	Ion exchange	Ion exchange and dilution with maltodextrin	Evaporation	Filtration and evaporation	Ion exchange and evaporation	Ion exchange and evaporation	Ion exchange
Physical State	Syrup	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Syrup
Physical Characteristics												
Appearance	Clear to slight yellow	-	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder	-	-	-	White Powder	-
pH	2.7 - 3.7	5 - 6	3.0 – 6.0	3.0 – 6.0	3.0 – 6.0	3.0 – 6.0	3.0 – 6.0	-	-	3.0 - 5.5	4.0 – 7.0	2.8 – 3.8
Viscosity	1000-5000 cPs	-	-	-	-	-	-	-	-	-	-	1000-5000 cPs
Chemical Composition												
Dry Matter	74 - 76 %	≥ 96 %	-	-	-	≥ 74%	-	≥ 70%	≥ 73.5%	75 - 76 %	≥ 74.5%	74 - 76 %
Galacto-oligosaccharides	≥ 62%	≥ 46%	> 99%	≥ 90%	≥ 70%	≥ 57%	≥ 27%	≥ 57%	≥ 26%	≥ 55%	≥ 57%	≥ 57%
Lactose	≤ 16%	20 – 40 %	≤ 1% (Lactose and mono-saccharides)	≤ 10% (Lactose and mono-saccharides)	≤ 30% (Lactose and mono-saccharides)	≤ 23%	≤ 12%	≤ 26%	≤ 18%			≤ 23%
Glucose	≤ 22%	≤ 10 %				≤ 22%	≤ 12%	≤ 23 %	≤ 22%			≤ 22%
Galactose	≥ 1%	≤ 5 %				≥ 0.8%	≥ 0.8%	> 0.8%	≥ 8%	-	-	≥ 0.8%
Ash	≤ 0.3%	≤ 4%	≤ 0.3%	≤ 0.3%	≤ 0.3%	≤ 0.3%	≤ 0.3%	< 1.5 %	< 0.5%	≤ 0.1%	≤ 0.05%	≤ 0.3%
Protein	≤ 0.4%	≤ 4.47 (N x 6.38)	-	-	-	-	-	< 0.3 %	≤ 0.2 %	≤ 0.1/100g	Neg (LOD = 10 mg/kg)	-

Table 8. Comparison of Product Specifications between VITAGOS™ and other GOS Preparations Intended for Infant Formula

Parameter	VITAGOS™	GRAS Notification Number[1]									
		620	569					495	489	334	286**	236***
			GOS-1000-P*	GOS-900y-P*	GOS-700-P*	GOS-570-S*	GOS-270-P*					
Microbiologicals												
Standard Plate Count	< 3000 cfu/g	<10000 cfu/g	≤ 50 cfu/g	≤ 50 cfu/g	≤ 50 cfu/g	≤ 50 cfu/g	≤ 50 cfu/g	< 3500 cfu/g	≤ 100 cfu/g	≤ 300 cfu/ml	300 cfu/g	≤ 3000 cfu/g
Enterobacteriaceae	< 10 cfu/g	< 10 cfu/g	< 0.3 MPN/g	< 0.3 MPN/g	< 0.3 MPN/g	< 0.3 MPN/g	< 0.3 MPN/g	-	< 10 cfu/g	Neg. (coliforms)	Neg./g (coliforms)	Neg./g
Escherichia coli	< 10 cfu/g	-	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 10 cfu/g	Neg./5g	-	Neg./g	Neg./5 g
Yeast and Mold	< 100 cfu/g	-	≤ 20 cfu/g	≤ 20 cfu/g	≤ 20 cfu/g	≤ 20 cfu/g	≤ 20 cfu/g	< 10 cfu/g	≤ 50 cfu/g	≤ 50 cfu /ml	20 cfu/g	≤ 50 cfu/g
Staphylococcus aureus	< 10 cfu/g	-	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	< 10 cfu/g	Neg./1 g	-	Neg./g	Neg./g
Salmonella	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25g	-	Neg./g	Neg./25g
Heavy Metals												
Arsenic (ppm)	< 0.4	-	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	-	≤ 0.4	≤ 1.0	≤ 1.0	< 0.4
Lead (ppm)	< 0.2	-	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.01	≤ 0.2	≤ 1.0	≤ 0.01	<0.2
Cadmium (ppm)	< 0.06	-	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.01	≤ 0.06	-	-	<0.06
Mercury (ppm)	< 0.005	-	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	≤ 0.5	-	-	< 0.0005

Neg. = negative; MPN = most probably number; cfu = colony forming units; g = grams; ppm = parts per million; "-" = nonspecified.

[1]Specifications presented include only those that are specific to VITAGOS™ and the GRAS Notifications list may have additional specifications.

*GOS-570-S is the base product containing ≥ 57% GOS. GOS-270-P is produced by blending GOS-570-S with maltodextrin. GOS-1000-P, GOS-900-P, and GOS-700-P are produced by fermenting GOS-570-S with yeast and yeast extract derived from *Kluyveromyces lactis*.

**Amended on June 16, 2014. The new product is manufactured with β-galactosidase produced by either *B. circulans* or *A. oryzae*. The new product specifications no longer included the percent ranges for the various oligosaccharides.

***Amended on April 4, 2013. The product specifications were modified as follows: the acceptable pH range was reduced from 3.2 – 3.8 to 2.8 to 3.8; the limit for nitrogen content was increased to from ≤ 0.016% to ≤ 0.032%.

Table 9. Comparison of Production Strain and Product Specifications between VITAGOS™ and other GOS Preparations Intended for Selected Conventional Foods

		GRAS Notification Number[1]											
		569							484				
Parameter	**VITA-GOS™**	**GOS-1000-P***	**GOS-900-P***	**GOS-700-P***	**GOS-570-S***	**GOS-270-P***	**518**	**489**	**Syrup**	**Powder**	**334**	**285****	**236*****
Raw Material	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose and sweet whey ultra-filtration permeate
Enzyme	β-galacto-sidase	β-galactosidase	β-galactosidase	β-galactosidase	β-galactosidase	β-galactosidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase	β-galacto-sidase
Enzyme Source	*Aspergillus oryzae & Kluyveromyces lactis*	*Bacillus circulans*	*Bacillus circulans*	*Bacillus circulans*	*Bacillus circulans*	*Bacillus circulans*	*Bacillus circulans*	*Aspergillus oryzae*	*Bifido-bacterium bifidum*	*Bifido-bacterium bifidum*	*Sporobolo-myces singularis & Kluyvero-myces lactis*	*Bacillus circulans & Aspergillus oryzae*	*Bacillus circulans*
Purification Method	Ion exchange and evaporation	Ion exchange, yeast fermentation, and evaporation	Ion exchange, yeast fermentation, and evaporation	Ion exchange, yeast fermentation, and evaporation	Ion exchange	Ion exchange and dilution with maltodextrin	Ion exchange and evaporation	Filtration and evaporation	Evapor-ation	Evapor-ation	Ion exchange and evaporation	Ion exchange and evaporation	Ion exchange
Physical State	Syrup	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Syrup
Physical Characteristics													
Appearance	Clear to slight yellow	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder	Off white light yellow powder	-	-	-	-	White Powder	-
pH	2.7 - 3.7	3.0 – 6.0	3.0 – 6.0	3.0 – 6.0	3.0 – 6.0	3.0 – 6.0	3.0 – 6.0	-	-	-	3.0 - 5.5	4.0 – 7.0	2.8 – 3.8
Viscosity	1000-5000 cPs	-	-	-	-	-	-	-	-	-	-	-	1000-5000 cPs
Chemical Composition													
Dry Matter	74 - 76 %	-	-	-	≥ 74%	-	-	≥ 73.5%	≥ 70%	≥ 90%	75 - 76 %	≥ 74%	74 - 76 %
Galacto-oligosaccharides	≥ 62%	> 99%	≥ 90%	≥ 70%	≥ 57%	≥ 27%	> 99%	≥ 26%	46 – 60%	55 – 80%	≥ 55%	≥ 57%	≥ 57%
Lactose	≤ 16%	≤ 1% (Lactose and mono-saccharides)	≤ 10% (Lactose and mono-saccharides)	≤ 30% (Lactose and mono-saccharides)	≤ 23%	≤ 12%	≤ 1% (Lactose and mono-saccharides)	≤ 18%	<19%	<25%	-	*7 – 10%*	≤ 23%
Glucose	≤ 22%				≤ 22%	≤ 12%		≤ 22%	>35%	<15.3%	-	-	≤ 22%
Galactose	≥ 1%				≥ 0.8%	≥ 0.8%		≥ 8%			-	*0 - 0.5%*	≥ 0.8%
Sulfated Ash	≤ 0.3%	≤ 0.3%	≤ 0.3%	≤ 0.3%	≤ 0.3%	≤ 0.3%	≤ 0.3%	< 0.5%	<1.5%	1-1.5%	≤ 0.1%	≤ 0.05%	≤ 0.3%
Protein	≤ 0.4%	-	-	-	-	-	-	≤ 0.2 %	<0.3%	1-1.5%	≤ 0.1/100g	Neg. (LOD = 10 mg/kg)	-

Table 9. Comparison of Production Strain and Product Specifications between VITAGOS™ and other GOS Preparations Intended for Selected Conventional Foods

Parameter	VITA-GOS™	GRAS Notification Number[1]											
		569					518	489	484		334	285**	236***
		GOS-1000-P*	GOS-900-P*	GOS-700-P*	GOS-570-S*	GOS-270-P*			Syrup	Powder			
Microbiologicals													
Standard Plate Count	< 3000 cfu/g	≤ 50 cfu/g	≤ 50 cfu/g	≤ 50 cfu/g	≤ 50 cfu/g	≤ 50 cfu/g	< 50 cfu/g	≤ 100 cfu/g	< 3500 cfu/g	< 1000 cfu/g	≤ 300 cfu/ml	≤ 300 cfu/g	≤ 3000 cfu/g
Entero-bacteriaceae	< 10 cfu/g	< 0.3 MPN/g	< 0.3 MPN/g	< 0.3 MPN/g	< 0.3 MPN/g	< 0.3 MPN/g	Neg./g	< 10 cfu/g	-	-	Negative (coliforms)	Neg./g (coliforms)	Neg./g
Escherichia coli	< 10 cfu/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	< 3.0 MPN/g	Neg./g	Neg./5g	< 10 cfu/g	< 10 cfu/g	-	Neg./g	Neg./5 g
Yeast and Mold	< 100 cfu/g	≤ 20 cfu/g	≤ 20 cfu/g	≤ 20 cfu/g	≤ 20 cfu/g	≤ 20 cfu/g	< 20 cfu/g	≤ 50 cfu/g	< 10 cfu/g	< 100 cfu/g	≤ 50 cfu /ml	≤ 20 cfu/g	≤ 50 cfu/g
Staphylococcus aureus	< 10 cfu/g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./g	Neg./1 g	< 10 cfu/g	< 10 cfu/g	-	Neg./g	Neg./g
Salmonella	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./25 g	Neg./g	Neg./25g	Neg./25 g	Neg./25 g	-	Neg./g	Neg./25g
Heavy Metals													
Arsenic (ppm)	< 0.4	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	≤ 0.4			≤ 1.0	≤ 1.0	< 0.4
Lead (ppm)	< 0.2	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	≤ 0.2	<0.1	<0.1	≤ 1.0	≤ 0.01	<0.2
Cadmium (ppm)	< 0.06	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	≤ 0.06	<0.1	<0.1	-	-	<0.06
Mercury (ppm)	< 0.005	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	≤ 0.5	<0.1	<0.1	-	-	< 0.0005

Neg. = negative; MPN = most probably number; cfu = colony forming units; g = grams; ppm = parts per million; "-" = not specified.

[1]Specifications presented include only those that are specific to VITAGOS™ and the GRAS Notifications list may have additional specifications.

*GOS-570-S is the base product containing ≥ 57% GOS. GOS-270-P is produced by blending GOS-570-S with maltodextrin. GOS-1000-P, GOS-900-P, and GOS-700-P are produced by fermenting GOS-570-S with yeast and yeast extract derived from *Kluyveromyces lactis*.

**Amended on June 16, 2014. The new product is manufactured with β-galactosidase produced by either *B. circulans* or *A. oryzae*. The new product specifications no longer included the percent ranges for the various oligosaccharides.

***Amended on April 4, 2013 and the product specifications were modified. The acceptable pH range was reduced from 3.2 – 3.8 to 2.8 to 3.8 and the limit for nitrogen content was increased to from ≤ 0.016% to ≤ 0.032%.

B. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The absorption, distribution, metabolism and excretion of GOS and their metabolites have been extensively reviewed in GRNs 236, 286, and 334, and by the Scientific Committee on Food (2001) and FSANZ (2008). In general, GOS are resistant to lactases present in the small intestine and are not absorbed (Ohtsuka et al., 1990; Wisker et al., 1985; Chonan et al., 2004). Instead, they move through the upper gastrointestinal tract to the colon where they are metabolized by the resident microbiota into short-chain fatty acids, carbon dioxide, methane, and hydrogen (Ohtsuka et al., 1991; Suarez et al., 1999; Smiricky-Tjardes et al., 2003). Importantly, short-chain fatty acids, carbon dioxide, methane, and hydrogen, are the same metabolites as those produced by the microbiota following the ingestion of other foods and are either absorbed, exhaled, or excreted (reviewed in Slavin, 2013).

C. GENOTOXICITY STUDIES

The genotoxicity of GOS-containing products have been extensively reviewed in GRNs 334 and 620. As summarized in GRN 334, Kobayashi et al. (2009) showed that GOS are not mutagenic, genotoxic, or clastogenic using a bacterial reverse mutation, a chromosomal aberration assay, and an *in vivo* micronucleus study. As summarized in GRN 620, Narumi et al. (2014) showed that GOS are not genotoxic using an *in vivo* comet assay. In addition, two non-publicly available bacterial reverse mutation assay and an *in vitro* micronucleus assay are reviewed in GRN 620 and, importantly, corroborate the lack of genotoxicity reported by Kobayashi et al. (2009) and Narumi et al (2014).

D. TOXICOLOGY STUDIES

1. Subchronic Toxicology Studies

The subchronic toxicology studies that support the current uses of GOS in infant formulas and conventional foods include those conducted with other GOS preparations, which have been extensively reviewed in GRNs 236, 286, and 620, and a corroborative OECD-compliant 90-day repeat dose rodent toxicology study conducted by Vitalus Nutrition Inc. with VITAGOS™.

a. Subchronic Toxicology Studies Reviewed in GRNs 236, 286, 620

The studies reviewed in GRN 236, 286, and 620 include an unpublished 90-day study conducted in adult rats, two published 90-day repeat dose toxicology studies conducted in adult rats (Anthony et al., 2006; Kobayashi et al., 2009), an unpublished 30-day study conducted in

adult rats, and a published 42-day study conducted in juvenile rats (Kobayashi et al., 2014a). In general, GOS preparations were not toxic to rats when administered for up to 90 days by gavage and the NOAELs in these studies were determined to be the highest dose tested, which were 6900, 5000, 2000, 2000, and 2000 mg/kg/day, respectively.

In GRN 236, an unpublished 90-day study and the 90-day study conducted by Anthony et al. (2006) were summarized. In the unpublished 90-day study, male and female Wistar rats were treated with feed containing 0, 5, 10, or 20 % GOS for 90 day, corresponding to approximately 1600, 3200, or 6100 mg GOS/kg body weight/day in male rats and 1800, 3600, or 6900 mg GOS/kg body weight/day in female rats. Due to the lack of signs of toxicity, the NOAEL was set at 6900 mg/kg/day. In the study conducted by Anthony et al. (2006), male and female Sprague Dawley rats were treated with either water, 2500, or 5000 mg/kg/day of a syrup containing approximately 45% GOS by gavage. Although there was a significant decrease in feed intake in the rats that had been treated with 5000 mg GOS/kg/day compared to the group receiving water, the NOAEL was set at 5000 mg/kg/day due to the lack of toxicologically relevant effects on other parameters such as clinical observations, gross necropsies, organ weights, and histological examinations.

In GRN 286, a 90-day study conducted by Kobayashi et al. 2009 (cited as Kobayashi et al., 2003 in GRN 286) was summarized. Male and female Sprague Dawley rats were treated with water, 500, 1000, or 2000 mg/kg/day of a syrup containing approximately 55% GOS by gastric feeding tube. There were no GOS-related changes in clinical signs, body weight, water intake, feed intake, urinalysis, opthamology, hematology, blood chemistry organ weights, gross pathology, or histopathology. The NOAEL was set to 2000 mg/kg/day for the GOS-containing product.

In GRN 620, an unpublished 30-day study in adult rats and 42-day study in juvenile rats conducted by Kobayashi et al. (2014a) were summarized. In the unpublished 30-day study, male and female rats were treated with 0, 500, 1000, or 2000 mg/kg/day of a GOS-containing product (46% oligosaccharides) by gavage. There were no deaths, relevant clinical signs, or GOS-related ophthalmological findings reported during the study. There were also no differences in body weight, food consumption, organ weight, macroscopic, histopathological changes, hematology, coagulation, serum clinical chemistry, or urine parameters between groups. The NOAEL was determined to be 2000 mg/kg/day. In the study conducted by Kobayashi et al. (2014a), 4-day old rat pups were treated with daily doses of 0, 500, 1000, or 2000 mg/kg/day of a product containing 56.9% GOS by gavage for 42 days. There were no deaths and no differences in feed and water consumption, and body weight gain between the groups. The reported adverse effects included a significant reduction in the grip strength of the hind limbs in the females treated with

500 mg/kg, a significant increase in hematocrit and eosinophil count and a significant decrease in platelet count and urine chloride levels in the females treated with 1000 mg/kg, a significant reduction in the absolute weights of the epididymis in the males treated with 500 mg/kg, and a significant reduction in the urea nitrogen in males treated with 2000 mg/kg. However, because these effects were not dose-dependent and/or observed in the other sex, the authors deemed to have no toxicological relevance. The NOAEL was determined to be 2000 mg/kg/day for the GOS-containing product.

b. Subchronic study conducted with VITAGOS™

To corroborate published findings from toxicology studies of GOS, the subchronic toxicity of VITAGOS™ GOS was assessed in Sprague-Dawley rats (10 per sex per group) for 90 consecutive days by oral gavage at 0, 500, 1,000, or 2,000 mg GOS product/kg/day. The study was performed in accordance with cGLP [OECD c(97)/186Final and US FDA (21 CFR Part 58)] and as per OECD guideline No. 408.

General clinical observations were performed once daily and morbidity/mortality was performed twice daily. Detailed clinical examination was done on Day 1 prior to treatment of test article and weekly thereafter. Ophthalmological examination was carried out prior to treatment and prior to being euthanized. Individual body weight was recorded on Day 1 prior to test article administration and at weekly intervals. Feed consumption was measured at weekly intervals. On Day 91, blood was collected for hematology, coagulation, and clinical chemistry evaluations. All animals were then euthanized by exsanguination and were subjected to detailed necropsy and gross pathological examination. Selected tissues and organs were collected, weighed, and preserved from all animals. In accordance with the OECD No. 408 guidelines, histopathological examination was carried out on all the preserved organs and tissue of control and high-dose (2,000 mg GOS product/kg/day) group animals. Additionally, the caecum was examined in the respective lower-groups (500 and 1,000 mg GOS product/kg/day) as test article-related histopathological change was observed in the high-dose group.

There were no deaths, relevant clinical signs, or abnormal ophthalmological findings reported at any dose levels in this study. Body weight and feed consumption were unaffected at 500 mg/kg/day dose in males and at all the doses in females. The body weight of male animals was reduced by 7 to 8% at 1000 mg/kg/day and 6 to 9% at 2000 mg GOS product/kg/day dose throughout the treatment period. However, the reduction was not considered clinically adverse since the reduction was less than 10%. In addition, no adverse effect on feed efficiency was seen in any of the dose groups.

With the exception of changes in cholesterol, triglycerides and blood urea nitrogen, described below, there were no test article-related changes reported in hematology, coagulation, serum clinical chemistry, or urine parameters in either gender in test groups compared with controls. A reduction of cholesterol and triglyceride in males at the 2,000 mg GOS product/kg/day dose was considered to be a secondary effect related to the test article-related reduction in feed consumption and terminal fasting body weight. An increased concentration of blood urea nitrogen in males at 1,000 and 2,000 mg GOS product/kg/day, and in females at 1,000 mg GOS product/kg/day was considered a test article-related non-adverse effect because it was not associated with any microscopic changes in kidneys.

No adverse effects were seen on absolute or relative organ weights and no changes in gross or histopathology were seen with the exception of increased absolute and relative weights of the caecum with and without contents at 2,000 mg GOS product (1,240 GOS) /kg/day in males and females. This change was considered test article-related as it was associated with mucosal hypertrophy/hyperplasia. However, there were no polyps observed in the caecum.

The histological change seen in the caecum of high dose animals, although related to test article administration, is considered an adaptive rather than toxic response. In corroboration of the effect of GOS on caecum, Kobayashi et al. (2009) reported relative and absolute higher caecum weight, without corresponding histopathological changes, in males at a dose of 2,000 (825 GOS) mg/kg/day. The observed mucosal hypertrophy/hyperplasia in the current study may be due to the use of much higher level of GOS than that in Kobayashi's study (Table 10).

Cecal enlargement along with mucosal hypertrophy and hyperplasia has been observed as a response in several rodent species to other food ingredients such as modified starches, polyols, some fibers, and lactose; these ingredients share the feature of being poorly absorbed and osmotically active (Haschek et al., 2010). Studies have demonstrated that consumption of pectin (Adam et al., 2015) and nondigestible oligosaccharides (Oku, 1997) can cause histological changes of caecum in rats. Because the caecum is an area of significant bacterial fermentation, cecal hypertrophy/hyperplasia is thought to occur as a result of the increased amounts of short chain fatty acids that are produced by bacterial fermentation after large amounts of non-adsorbed carbohydrate and dietary fiber enter the caecum and colon (Levine, 1991). It has also been reported that mucosal hypertrophy in rodents represents a physiological adaptation to increased osmotic forces when high doses of undigestible substances are consumed; the effect is reversible after test article is withdrawn from the diet (Greaves, 2012; Haschek et al., 2010; Newberne et al., 1988).

Therefore, hypertrophy/hyperplasia without atypical cellular features represents a compensatory and adaptive response to a large amount of GOS, consistent with the effects seen with other poorly absorbable carbohydrates (Greaves, 2012). Thus, the observed cecal hypertrophy/hyperplasia, without evidence of polyps, is considered compensatory and not preneoplastic and, although test article-related, is not considered to be a toxic response.

In conclusion, the NOAEL for VITAGOS™ following oral gavage is 2,000 mg/kg body weight/day under the test conditions employed.

Table 10. Comparison of Results From the 90-day Rodent Toxicology Studies Conducted with GOS-Containing Products

Study Parameters	90-day Rodent Toxicology Study							
	Anthony et al., 2006		Kobayashi et al., 2009			VITAGOS™		
Strain of Rats	Crl:CD(SD)IGS BR (Sprague Dawley); from Charles River		Crj:CD(SD) rats (Sprague-Dawley); from Charles River Japan			Sprague-Dawley; from Envigo (Harlan Laboratories) California		
Dose of Product (mg/kg/day)	2,500	5,000	500	1,000	2,000	500	1,000	2,000
GOS (%)	45%	45%	41%	41%	41%	62%	62%	62%
Dose of GOS (mg/kg/day)	1,125	2,250	206.25	412.5	825	310	620	1,240
Observations	No abnormalities		Relative and absolute higher caecum weight in the 2,000 mg/kg/day male group; no histopathological findings			Increased absolute and relative caecum weight and hypertrophy/ hyperplasia at 2,000 mg/kg/day in both males and females		
Conclusions	NOAEL = 5000 mg/kg/day based on the lack of toxicologically relevant effects on clinical observations, gross necropsies, organ weights, and histological examinations.		NOAEL = 2000 mg/kg/day based on no GOS-related changes in clinical signs, body weight, water intake, feed intake, urinalysis, ophthamology, hematology, blood chemistry organ weights, gross pathology, or histopathology.			NOAEL = 2000 mg/kg/day no consistent GOS-related changes in clinical signs, body weight, water intake, feed intake, urinalysis, ophthamology, hematology, blood chemistry organ weights, gross pathology, or histopathology and the adaptive response of the cecum to a diet containing increased amounts of fiber.		

NOAEL = No Observed Adverse Effect Level; GOS = galactooligosaccharides

2. Developmental and Reproductive Toxicology Studies

The developmental and reproductive toxicology studies that support the current uses of GOS in infant formulas and conventional foods have been summarized in GRNs 484, 495, 518, 569, and 620. These studies include two published studies, Desbuards et al. (2012) and Kobayashi et al. (2014b).

As summarized in GRNs 484, 495, 518, and 569, Desbuards et al. (2012) evaluated the effects of perinatal GOS in pregnant mice and their offspring. Pregnant BALB/cj mice were fed a control diet or a diet supplemented with a prebiotic mixture containing approximately 1602 mg GOS and 400 mg inulin/kg body weight/day, which was calculated based on reported maternal feed intake, body weight values, and GOS content of the diet (approximately 45%), during gestation and lactation. Although the study was not conducted in accordance with good laboratory practices (GLP) and deviated from internationally accepted guidelines, there were no significant differences in maternal body weight gain or feed intake during pregnancy between the two groups. There were also no differences in the number of offspring per dam between the two groups. In the pups, the body weights of the GOS/inulin-treated male pups were significantly higher at weaning and at days 2, 40, and 48 post weaning compared to the male pups receiving the control diet. Body length, colon length, and relative thigh muscle weight were also significantly higher in the GOS/inulin-treated male pups. No other developmental or reproductive toxicological endpoints were examined.

As summarized in GRN 620, Kobayashi et al. (2014b) evaluated the reproductive effects of GOS in parenteral male and female rats, pregnant females, and their offspring by administering 0, 500, 1000, and 2000 mg/kg of product containing 56.9% GOS by gavage to the parental males 10 weeks prior to and 3 weeks after mating, and to the parental females 2 weeks prior to mating, through pregnancy, and up to day 20 of lactation. No deaths occurred in any group, there were no abnormal clinical signs in the any of the parental males and females, and although intermittent, non-dose-dependent adverse events occurred, such as significantly low feed consumption in the 2000 mg/kg treated group on days 1, 4, and 11 of gestation and a significant reduction on the number of estrouses in the 1000 mg/kg group, GOS did not produce any toxicological effects or affect the reproductive functions of the parental rats. In the offspring, with the exception of a significant reduction in pinna detachment on day 4 after birth and a significant delay in incisor eruption on day 11 after birth in the 1000 mg/kg group, and a significant delay in eyelid opening on day 14 after birth in the 1000 and 2000 mg/kg groups that were determined to be irrelevant due to the lack of a dose response, there were no significant differences between any of the groups in the number of male and female live born pups, sex

ratio, body weights or body weight gain during lactation. The NOAEL was set at 2000 mg/kg/day of the GOS-containing product.

3. Other Studies

Three additional published animal studies were summarized in GRN 620 and corroborate the safe ingestion of GOS, Verheijden et al. (2015), Hogenkamp et al. (2015), and Morel et al. (2015).

Verheijden et al. (2015) investigated the effect of GOS on the development of allergy in adult mice. Mice were maintained on a control diet or a diet containing 1% GOS and after two weeks all mice were sensitized to house dust mite with an intranasal administration of 1 μg house dust mite. Seven to 10 days later the mice were then challenged intranasally with either phosphate buffered saline or 10 μg house dust mite, and airway hyperresponsiveness was quantified using EMKA invasive measurement of dynamic resistance (EMKA Technologies), and bronchoalveolar lavage fluid cell counts and cytokine and chemokine levels. Compared to control diet, the GOS-containing diet reduced airway hyperresponsiveness, bronchoalveolar fluid proinflammatory cytokine levels, and eosinophil cell counts, indicating that GOS may reduce the risk of developing allergy.

Hogenkamp et al. (2015) mated parental female mice that had been maintained on either a control diet or 3% GOS/FOS-supplemented diet (9 parts GOS, 1 part FOS) with male mice fed the control diet and continued the dietary intervention during gestation. At 6 weeks post-delivery, the male offspring were then sensitized to ovalbumin (OVA)-induced allergy with an intraperitoneal injection of 10 μg ovalbumin adsorbed into 22.5% aluminum hydroxide. The allergic response was then provoked with a subcutaneous injection of 12.5 μg OVA in the pinnae of one ear. The other ear was injected with saline. The reaction was quantified by measuring ear swelling. One week later, the offspring were challenged 3 times with 10 g/L of nebulized OVA and airway responsiveness (lung resistance) was quantified. Importantly, the offspring of the GOS/FOS-treated dams showed significantly reduced increases in ear swelling compared to the offspring derived from the females that received the control diet. In addition, although there were not enough control mice in the study to determine the significance of the GOS/FOS diet on airway responsiveness, the airway hyperresponsiveness was less in the offspring derived from the GOS/FOS-treated dams.

Morel et al. (2015) characterized the cecal microbiota of suckling rats that had been gavaged with vehicle or a GOS/FOS mixture (9:1 ratio), which delivered approximately 2.25 g GOS/kg/day, from post-natal day 5 to 14. All rats were then weaned to normal chow on day 21.

Although no safety or tolerance endpoints were assessed, the GOS/FOS mixture increased Bifidobacteria and decreased Firmicutes counts at day 14. By day 131 the distribution of the microbiota in the GOS/FOS group resembled that of the suckling rats that received the vehicle control.

E. HUMAN STUDIES

Numerous clinical studies have been conducted in infants and adults to support the safe use of GOS in infant formulas and conventional foods. Importantly, these studies included endpoints that evaluated the effects of GOS on fecal microflora, gastrointestinal physiology, the immune system, and tolerance. Although some of the studies are unpublished, all have been extensively summarized in GRAS Notifications 236, 285, 286, 334, 484, 489, 495, 518, and 620. In general, GOS are well tolerated, and have been reported to increase the abundance of bifidobacteria and lactobacilli in the gastrointestinal tract in infants and adults, increase fecal short-chain fatty acid concentrations in infants and adults, improve stool consistency in infants, reduce the incidence of atopic dermatitis in infants, and alleviate the symptoms of irritable bowel syndrome in adults (Silk et al., 2009; Vulevic et al., 2008; Depeint et al., 2008; Bouhnik et al., 2004; Ito et al., 1990; Gopal et al., 2003; Fanaro et al., 2009; Walton et al., 2012; Moro et al., 2006; Schmelzle et al., 2003).

1. Studies Conducted in Infants

The use level of 7.2 g GOS/L in infant formulas was proposed by GTC Nutrition in GRN 286, which received a "no questions" letter from the FDA in 2009. To support the safety of GOS for the intended use, GTC summarized published clinical studies conducted in infants and showed the 7.2 g/L GOS in combination with 0.8g/L FOS had no adverse effects. Since then, the use level of GOS in infant formulas has remained the same in GRNs 334, 489, 495, and 569, all of which provided updates of the published studies that support the intended use of GOS in infant formulas at 7.2 g/L and received "no questions" letters from FDA. The most recent GRAS Notification for the use of GOS in infant formula, GRN 620 was filed on February 8, 2016, is still under review, and proposes to increase the use level in infant formulas to 7.8 g/L. In GRN 620, the published and unpublished clinical studies conducted with infant and follow-on formulas containing the Nestle's GOS and the other clinical trials conducted with other types of GOS that were not included in GRN 569 are summarized. In these studies, infant and follow-on formulas containing up to 20 g GOS/L with and without other ingredients (probiotics, FOS, and/or long chain polyunsaturated fatty acids) were administered to preterm and term infants, and children for periods of time ranging from three months to one year (GRN 620, 2016; Underwood et al., 2014; Armanian et al., 2014; Chatchatee et al., 2014; da Costa Ribeiro et al.,

2015; Dasopoulou et al., 2015; Giovannini et al., 2014; Williams et al., 2014; Meli et al., 2014; Simeoni et al., 2015; Lee et al., 2015). Although two preterm infants that received a GOS-containing formula in the study conducted by Underwood et al. (2014) developed feeding intolerance, GOS was generally well tolerated.

Since GRN 620 was filed with the FDA, two additional studies in healthy term infants, one study in term infants with a positive history of allergy, and one study in children have been published (Matsuki et al., 2016; Civardi et al., 2015; Boženský et al., 2015; Pontes et al., 2016) The experimental details and results from these newly published studies are summarized in Table 11. Consistent with the results reported in the clinical trials summarized in previous GRAS Notifications, GOS-containing infant and follow-on formulas are well-tolerated.

Table 11. Recent Studies of GOS Ingestion in Infants

Reference	Study Design and Population	Treatments (Numbers of Subjects)	Duration	Safety Parameters
Civardi et al., 2015	Randomized, double-blind, placebo-controlled trial in healthy full-term infants (<12 months of age)	Group 1 (Control): Formula; n=59 Group 2: Formula enriched with 7g GOS/L, β-palmitate, and acidified milk; n=51	135 days	Withdrawals: • 62 subjects were enrolled in Group 1 • 55 subjects were enrolled in Group 2 • Compliance was similar for both groups • Three subjects were lost to follow-up in Group 1 • Four subjects were lost to follow-up in Group 2 Adverse Events: • Only gastrointestinal adverse events were evaluated; those that were reported were mild and there were no differences between the two groups. • No drop-outs due to adverse events occurred. • No severe adverse events were reported. Tolerance: • Mean number of stools/day was similar between the two groups. • Frequency of intestinal gas and bowel cramps were similar in both groups. Growth Parameters: • Weight change was similar in the two groups. • Length change was similar in the two groups. • Head circumference was similar in the two groups. Other Parameters: • Clostridia counts (determined by quantitative PCR) were similar in the two groups. • Bifidobacteria counts (determined by quantitative PCR) were significantly increased ($p < 0.05$) in Group 2.
Matsuki et al., 2016	Randomized, double-blind, placebo-controlled trial in healthy full-term infants (<12 months of age)	Group 1 (Control): Formula with dextrins; n=14 Group 2: Formula with 3 g GOS/L; n=16 Note: Supplementation with up to 20% breast milk was permitted	14 days	Withdrawals: • Eighteen subjects were enrolled Group 1 • Seventeen were enrolled in Group 2 • Compliance was similar for both groups. • Four subjects withdrew in Group 1. • One subject withdrew in Group 2. • Withdrawals were due to introduction of probiotics, change in feeding, or a parental decision. Adverse Events: • Adverse side effects were monitored and none were reported.

Table 11. Recent Studies of GOS Ingestion in Infants

Reference	Study Design and Population	Treatments (Numbers of Subjects)	Duration	Safety Parameters
				Tolerance: • There were no significant differences between the groups in fecal short-chain fatty acid levels, pH, or stool frequency. Other Parameters: • Abundance of bifidobacteria significantly increased ($p<0.05$) in the GOS-treated group compared to the control group. • At the species level, there were no significant chances in the bifidobacteria.
Bozensky et al., 2015	Randomized, placebo-controlled trial in term infants with a positive history of allergy (atopic eczema, allergic rhinitis, and/or asthma) in their parents or siblings (<12 months of age)	Group 1 (Control): Formula with hydrolyzed protein; n= 51 Group 2: Formula with hydrolyzed protein supplemented with 5g GOS/L; n=52	6 months	Withdrawals: • 60 subjects were enrolled in each group. • Compliance was similar for both groups. • Nine subjects withdrew in Group 1 (four did not adhere to the protocol; five discontinued due to intolerance. • Eight subjects withdrew in Group 2 (four did not adhere to the protocol; one was excluded due to another serious illness; three did not tolerate the product. • Withdrawals were due to introduction of probiotics, change in feeding, or a parental decision. Adverse Events: • None were reported. Tolerance: • There were no significant differences between the groups in stool, vomiting, or crying frequency. • GOS significantly reduced ($p<0.05$) stool consistency (watery, runny, and mushy). Other Parameters: • There was no significant difference in atopic symptoms as judged by the SCORAD (Scoring Atopic Dermatitis) values.

Table 11. Recent Studies of GOS Ingestion in Infants

Reference	Study Design and Population	Treatments (Numbers of Subjects)	Duration	Safety Parameters
Pontes et al., 2016	Randomized, double-blind, placebo-controlled study in healthy children (1 to 4 years old)	Group 1 (Control): Cow's milk-based beverage; n=131 Group 2: Cow's milk-based beverage containing 30 mg of docosahexanoic acid, 1.2 blend of polydextrose and GOS (1:1) ratio, and 8.7 mg of yeast β-glucan; n=125 Children were to consume the products 3x/day; GOS intake = 1.8 g/day	28 days	Withdrawals: • Two subjects withdrew in Group 1 • Five subjects withdrew in Group 2 • Reasons for withdrawal were not reported. Adverse Events: • Ninety-nine types of adverse events were reported; only the occurrence of thrush was significantly increased in Group 2. • Ten subjects in the Group 1 experienced at least on serious adverse event • Two subjects in Group 2 reported at least one serious adverse event Tolerance: • Average daily intake of the products during the study was not significantly different between the groups. • There was no difference in the incidence of acute respiratory infections or diarrheal disease (≥ liquid or semi-liquid stools in 24 hr with fever and/or vomiting and/or dehydration and compromised general status) between the groups. • Group 2 had significantly less allergic manifestations (allergic rhinitis or conjunctivitis, wheezing, allergic cough, eczema, and urticaria) than Group 1. • Group 2 had significantly softer stools ($p<0.05$) compared to Group 1 in the first 3 months of the study. • Eight of the 98 subjects that were 12- 24 month were constipated; all were in the control group; five remained constipated at the end of the trial. • In the children 25 to 48 months old, there was no significant difference in the percentage of children who remained constipated at the end of the study. Other Parameters: • In both groups there was significant increase from baseline to end of study in weight- and length/height-for-age z scores; there was no difference between the groups. • There were no differences between the two groups in fecal IgA, serum IL-10, TGF-β1, TGF- β2, IL-4, and IFNγ, and stool parasites. • There were no significant differences in serum zinc and ferritin, hemoglobin, hematocrit, red blood cells, white blood cells, and platelets.

2. Studies Conducted in Adults

New Francisco Biotechnology Corporation proposed increasing the use level to 11 g/serving in conventional foods in GRN 518. GRN 518 subsequently received a "no questions" letter from FDA in 2014. To support the safety of GOS for the intended use and at the intended use level, New Francisco Biotechnology Corporation incorporated by reference the information summarized in GRNs 236, 285, 286, and 334, and summarized the clinical studies conducted in adults that were published from 2010 to 2014. Since GRN 518, GRN 569 and 620 were filed with the FDA. Both GRN 569 and 620 provided updates of published studies. GRN 569 received a "no questions" letter in 2015 whereas GRN 620 is still in review. Importantly, no clinical studies involving adults have been published since the filing of GRN 620.

F. ALLERGENICITY

The allergenicity of GOS has been summarized in an amendment to GRN 236 and GRN 620. Two case series documenting 21 cases of allergic reactions associated with the ingestion of GOS are publicly available, Vo et al. (2012) and Chiang et al. (2012), and were reviewed in an amendment to GRN 236, which received a "no questions" letter from FDA on April 24, 2014. All cases reported by Vo et al. (2012) and Chiang et al. (2012) were localized to Southeast Asia and, although these reports suggest that GOS can provoke an allergic reaction, there is no direct evidence indicating that GOS directly sensitizes consumers to an allergic reaction to GOS. More recently two additional studies have been published and are summarized in GRN 620, Kaneko et al. (2014) and Soh et al. (2015).

Vo et al. (2012) identified 17 cases of allergic reactions to ingested milk products in Vietnam from October 9 to October 28, 2009. All subjects were Vietnamese and 16 of the 17 cases had consumed milk containing GOS. The median age of the 17 subjects was 10 years old (range 2 to 15 years old). Four subjects were allergic to various foods, but not milk. Twelve subjects developed symptoms within 20 minutes and 3 subjects developed symptoms 1, 6, and 49 hr after consuming the milk product. The most frequent symptom was an itchy maculo-papular skin rash (94%). Three subjects experienced difficult breathing. The source of the GOS in the supplemented milk was not disclosed. Importantly, a case-by-case description of the reactions was not provided, making it difficult to interpret the results. Moreover, in a case-control study of 50 neighborhood controls, the methods were not reported and the specific allergens and/or provoking substances were not identified.

Chiang et al. (2012) identified five cases (four subjects were Chinese and one was Malaysian) in Singapore from December 2007 to January 2012. The median age of the subjects

was 6 years old. All the subjects were tolerant to cow's milk, had no known previous exposure to GOS, and reacted to a cow's milk product supplemented with GOS. There were no deaths and all subjects were treated with different combinations of antihistamines, steroids, β2-agonists, adrenaline, and/or oxygen. Subsequent testing of the five cases showed that they were all reactive to fractions of the GOS product, which contained the lactose core and one or more additional galactose molecules, via skin prick testing. *In vitro* studies also revealed that basophils harvested from each of the subjects increased cell surface expression of the activation marker CD203c in a GOS-dependent manner and sera from two of three subjects was able to confer GOS-reactivity to basophils that were otherwise GOS-tolerant. Although serum GOS-specific IgE levels were not determined in any of the subjects, the results suggest that a blood component, such as IgE, may have mediated the acute reactions.

To identify the allergenic epitopes in GOS and a potentially hypoallergenic form of GOS, Kaneko et al. (2014) compared the allergenicity of six different GOS preparations using blood harvested from three subjects known to have anaphylactic reactions to GOS and histamine-release assays. The products used by Kaneko et al. included, one manufactured using a combination of β-galactosidases derived from *A. oryzae* and *Streptococcus thermophilus*, three manufactured using a β-galactosidase derived from *Bacillus circulans,* one manufactured with a β-galactosidase derived from *Sporobolomyces singularis*, and one product manufactured with a combination of β-galactosidases derived from *S. singularis* and *K. lactis*. Previous compositional analyses of GOS products have shown that GOS manufactured with β-galactosidases derived from *B. circulans*, *S. singularis*, or a combination of β-galactosidases derived from *S. singularis* and *K. lactis* contain sugar chains bound predominantly with β 1-4 bonds whereas GOS manufactured with a combination β-galactosidases derived from *A. oryzae* and *S. thermophilus* contain sugar chains bound predominantly with β 1-6 bonds (reviewed in Sako et al., 1999). Importantly, Kaneko et al. proposed that linear and branched tetrasaccharides were more potent at releasing histamine than linear and branched trisaccharides, and, moreover, GOS manufactured using either a β-galactosidase derived from *Sporobolomyces singularis* or a combination of β-galactosidases derived from *A. oryzae* and *S. thermophilus* or *S. singularis* and *K. lactis* were less potent at releasing histamine than GOS manufactured using β-galactosidase derived from *B. circulans.* Subsequent compositional analyses conducted by Kaneko et al., revealed lower levels of branched tetrasaccharides in the GOS product manufactured with the β-galactosidases derived from *S. singularis* and *K. lactis*. The authors therefore suggested that GOS manufactured with the β-galactosidases derived from *S. singularis* and *K. lactis* may be less allergenic due to reduced levels of branched tetrasaccharides.

Soh et al. (2015) conducted a clinical study to evaluate the prevalence of allergy to two different formulations of GOS, Vivinal GOS, which is manufactured by Friesland Foods using a β-galactosidases derived from *B. circulans* (GRN 236), and Oligomate GOS, which manufactured by Yakult using β-galactosidases derived from *Sporobolomyces singularis* and *Kluyveromyces lactis* (GRN 334). 487 individuals with eczema, asthma, allergic rhinitis, and food allergies were enrolled and reactivity to GOS was determined using skin prick tests, basophil activation assays, and oral challenges. Skin prick testing identified thirty subjects that were reactive to Vivinal GOS. In subsequent basophil activation testing, 15 of the 30 had positive skin prick tests to Vivinal GOS. Reactivity to Oligomate via skin prick and basophil activation testing was not determined. Thirteen of the 30 subjects that had positive skin prick tests also consented to oral challenges with Vivinal GOS and Oligomate GOS. Six of these 13 subjects reacted to Vivinal GOS in oral challenges (five in the BAT positive group and one in the BAT negative group) whereas none reacted to Oligomate. As a result, Soh et al. estimated the prevalence of Vivinal GOS allergy to be as great as 3.5 % in the Singapore atopic population. Moreover, Soh et al. speculated that the variable reactivity to GOS products may be due to structural differences in GOS products, which is consistent with the results reported by Kaneko et al. (2014).

IgE-mediated allergic reactions occur when individuals re-encounter a sensitizing antigen. Furthermore, it is currently thought that environmental stimuli and genetics contribute to the development of allergy (reviewed in Wang and Sampson, 2011). Because all 21 cases reported by Vo et al. (2009) and Chiang et al. (2012) were in children and adults in Southeast Asia, and the five reactions reported by Chiang et al. were after the subjects' first known exposure to GOS, it is possible that GOS-reactivity may be acquired through environmental exposures. Similar scenarios have been proposed for the peculiar GOS-reactivity of oyster shuckers in Japan and the cetuximab-reactivity of cancer patients in the southeastern United States (as cited by Chiang et al., 2012; and Chung et al., 2008; Commins et al., 2011). In both of these scenarios, the allergic reactions were geographically localized and occurred in subjects without known prior exposure to the provoking substance.

Importantly, GOS-containing products are available worldwide and there have been no reported reactions to GOS-supplemented milk or GOS-containing products outside of Southeast Asia. Moreover, the sensitizing agent in the 21 cases reported by Vo et al. (2009) and Chiang et al. (2012) is still unknown. Thus, despite millions of consumers worldwide, the reported cases likely represent unique, rare, geographically-localized allergic reactions to GOS-containing milk beverages.

VI. REFERENCES

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GENERALLY RECOGNIZED AS SAFE DETERMINATION FOR THE USE OF VITAGOS™ IN INFANT FORMULA AND SELECTED CONVENTIONAL FOODS

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of the use of VITAGOS™ as an ingredient in powdered non-exempt term infant formulas, selected conventional foods, coffees, and teas. This GRAS determination for the use of VITAGOS™ for the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of VITAGOS™ has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of VITAGOS™ as an ingredient for the intended uses in foods and infant formulas has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. GOS are non-digestible oligosaccharides consisting of 1 to 7 galactose units linked via β(1→3), β(1→4), or β(1→6) glycosidic bonds to either a terminal glucose or galactose. Although tri- to hexa-saccharides with 2 to 5 galactose units (degree of polymerization (DP) of 3 to 6) tend to be the main components of GOS-containing products, disaccharides (DP2) consisting of galactose and glucose with different β-glycoside bonds from lactose are also present and defined as GOS because they have physiological characteristics that are similar to longer GOS.

    a. VITAGOS™ is a GOS-containing product manufactured using lactose and β-galactosidases derived from *Aspergillus oryzae* and *Kluyveromyces lactis* in a manner similar to other GOS-containing products that have received "no questions" letters from the United States Food and Drug Administration.

    b. All processing aids used to produce VITAGOS™ comply with appropriate federal regulations.

    c. A comparison of the manufacturing processes and product specifications for VITAGOS™ and other GOS-containing products shows that VITAGOS™ is essentially equivalent to the other GOS-containing products currently marketed in the United States for use in infant formulas and conventional foods.

2. GOS are transported through the upper gastrointestinal tract to the colon where they are fermented by the resident microbiota into short-chain fatty acids, carbon dioxide, methane, and hydrogen.

3. GOS present in food are either naturally occurring in human milk and colostrum, bovine colostrum, and fermented milk products or synthetic, which are then added to the food during processing and formulation.

4. Synthetic GOS have a long history of use worldwide.

    a. In Japan, GOS have been commercially available since 1995 and are considered as Food for Specified Health Uses (FOSHU).

    b. In the United States, the first GOS product was determined GRAS for use in term infant formula and selected conventional foods, and received a "no questions" letter from the FDA in 2008 (GRN 236). Since then, six additional GOS-containing products have been determined GRAS for use in infant formulas and selected conventional foods at levels up to 7.8 g/L and 11 g/serving, respectively, resulting in ten GRAS Notifications (GRN) to the FDA (GRN 236, 285, 286, 334, 484, 489, 495, 518, 569, and 620).

    c. In the European Union, the safety of GOS was reviewed by the Scientific Committee on Food (SCF) in 2003 and is approved for use in infant and follow-on formulas GOS in combination with fructo-oligosaccharides (FOS) at levels up to 8 g (90% GOS and 10% FOS)/L (7.2 g GOS and 0.8 g FOS/L) (Select Committee on Food EU 2016/127).

    d. In Australia and New Zealand, the safety of GOS was reviewed by the Food Standards of Australia and New Zealand (FSANZ) in 2008 and is permitted in infant and follow-on formulas at levels up to 290 mg/100 kJ, or approximately 8 g/L (Australia New Zealand Food Standards Code – Standard 2.9.1 – 7).

5. GOS-containing products are not genotoxic.

6. GOS-containing products are not toxic to rats when administered for up to 90 days by gavage and the no observed adverse effect levels (NOAELs) determined in all the supporting toxicology studies were determined to be the highest dose tested (6900, 5000, 2000 mg/kg/day). The safety of VITAGOS™ was also determined in a 90-day repeat dose rat toxicology study and the NOAEL was determined to 2000 mg/kg/day.

7. GOS-containing products are well tolerated in humans and have been reported to increase the abundance of bifidobacteria and lactobacilli in the gastrointestinal tract in infants and adults, increase fecal short-chain fatty acid concentrations in infants and adults, improve stool consistency in infants, reduce the incidence of atopic dermatitis in infants, and alleviate the symptoms of irritable bowel syndrome in adults.

8. GOS is available worldwide and, although GOS-containing products have been reported to provoke allergic reactions in sensitized individuals living in Vietnam and Singapore, there have been no reported reactions to GOS-containing products outside of Vietnam and Singapore. Thus, the reported cases likely represent unique, rare, geographically localized allergic reactions to GOS-containing products.

9. The addition of VITAGOS™ to infant formula at 7.2 g GOS/L is the same use level of other GOS products in infant formula that have been determined GRAS and received "no questions" letters from the FDA (GRN 286, 334, 569) and will result in intakes of approximately 5.1 and 6.9 g GOS/day for one-month-old and six-month-old infants, respectively.

10. The addition of VITAGOS™ to selected conventional foods, coffees, and teas at levels ranging from 0.3 to 11 g GOS/serving is the same as other GOS products that have been determined GRAS and received "no questions" letters from the FDA (GRN 285, 334, 484, and 518). Thus, the dietary exposure to VITAGOS™ from the intended uses will not increase in the GOS-consuming population in the United States. The estimated mean and 90th percentile exposure to VITAGOS™ from the intended uses in selected conventional foods are 12.2 and 25.3 g per person per day (g/p/d) and in coffees and teas of 5.0 g/p/d for adult males and 4.4 g/p/d for the total population.

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

> *There is no evidence in the available information on VITAGOS™ that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when VITAGOS™ is used at levels that might reasonably be expected from the proposed applications of VITAGOS™ for use in powdered non-exempt term infant formulas and selected conventional foods, teas, and coffees as proposed by Vitalus Nutrition Inc.*

Therefore, VITAGOS™ is safe and GRAS at the proposed levels of addition to the intended foods. VITAGOS™ is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

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GRAS Expert Panel Member
School of Pharmacy
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Date: August 19, 2016

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SUBMISSION END