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GALACTOOLIGOSACCHARIDE GRAS NOTICE

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March 06, 2009

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I GRAS Exemption Claim

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997)

As defined herein, a galactooligosaccharide (GOS) product derived from lactose, has been determined by GTC Nutrition to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise to evaluate the safety of GOS under the conditions of intended use in food. Therefore, the use of GTC's GOS in food as described below is exempt from the requirement of premarket approval (Section 409 of the *Federal Food, Drug and Cosmetic Act*).

Signed,

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GTC Nutrition
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523 Park point Drive, Suite 300
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Date

March 6, 2009

B. Name and Address of Notifier

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C. Common Name of the Notified Substance

Galactooligosaccharide; GOS

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D. Conditions of Intended Use in Food

GTC Nutrition intends to market a GOS mixture, synthesized from lactose using a β -galactosidase obtained from *Bacillus circulans* (*B. circulans*) LOB 377, as a food ingredient in the United States under the proposed food uses as described in table A-1 (Appendix A), at use levels ranging from 0.86 to 1.28 g/serving (0.48 to 12.2%). GTC's GOS ingredient is not intended for use in meat or poultry-containing products.

E. Basis for the GRAS Determination

Pursuant to 21 CFR §170.30, GOS has been determined by GTC Nutrition to be GRAS on the basis of scientific procedures (U.S. FDA, 2008). This GRAS determination is based on data generally available in the public domain pertaining to the safety of GOS for use in food, as discussed herein and in the accompanying documents, and on a consensus among a panel of experts¹ who are qualified by scientific training and experience to evaluate the safety of GOS as a component of food.

F. Availability of Information

Data and information that serve as the basis for this GRAS Notice will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of GTC Nutrition located at the following address:

GTC Nutrition
523 Park Point Drive, Suite 300
Golden, CO
80401

Should the FDA have any questions or additional information requests regarding this Notice, GTC Nutrition also will supply these data and information.

¹ The Panel consisted of the below-signed qualified scientific experts: Dr. Ronald E. Kleinman MD. (Pediatric Gastroenterology and Nutrition Unit, Massachusetts General Hospital), Dr. David J. Brusick, Ph.D. (Consultant), and Dr. Ian C. Munro (Cantox Health Sciences International). A copy of the Expert Panel summary is located in appendix B and is titled "EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF GTC NUTRITION'S GALACTOOLIGOSACCHARIDES (PURIMUNE) FOR USE IN TRADITIONAL FOOD PRODUCTS, AND INFANT FORMULA". Note that GTC Nutrition's GOS ingredient - the ingredient that is the subject of this notification - is referred to as Purimune throughout the Expert Panel Statement in Appendix B. This simply refers to a potential trade name.

II. DETAILED INFORMATION REGARDING THE IDENTITY OF THE SUBSTANCE

A. Identity

The common or usual name of this product is galactooligosaccharide (GOS). GTC Nutrition's GOS ingredient is a spray-dried white powder produced from food grade lactose via a β -galactosidase obtained from *B. circulans* LOB 377. The ingredient contains at least 90% GOS (dry weight basis), with the remaining material characterized primarily by lactose, water and trace amounts of two monosaccharides, dextrose and galactose.

Common or Usual Name: Galactooligosaccharide(s), GOS
Chemical Name: Not Applicable
Chemical Abstracts Service (CAS) Number: Not Applicable
Empirical Formula: Not Applicable
Molecular Weight: Not Applicable
Structural Formula: See Table II.A-1 and Figure II.A-1 below.

GTC Nutrition's GOS is a mixture of β -linked GOS in various $\beta(1-3)$, $\beta(1-4)$, $\beta(1-6)$ configurations, and a degree of oligomerization ranging between 3 and 5. Typical distribution ranges for the resultant galactooligosaccharides produced during GTC's manufacturing process are listed in the table below. The general structure of a galactooligosaccharide is illustrated in Figure II.A-1.

Carbohydrates		Content (%DB)	Structure
Monosaccharides	Dextrose	0.0-1.0	α -D-glc
	Galactose	0.0-0.5	β -D-gal
Disaccharides	Lactose	7.0-10.0	β -D-gal-(1,4)-D-glc
	GOS	7.0-9.0	β -D-gal-(1,3)-D-glc; β -D-gal-(1,4)-D-gal
	GOS	9.0-12.0	β -D-gal-(1,6)-D-glc
Trisaccharides	GOS	16.0-20.0	β -D-gal-(1,4)- β -D-gal-(1,4)- β -D-glc
	GOS	8.0-13.0	β -D-gal-(1,6)- β -D-gal-(1,4)- β -D-glc
	GOS	14.0-19.0	β -D-gal-(1,3)- β -D-gal-(1,4)- β -D-glc
Tetrasaccharides and higher oligomers $n \geq 3$	GOS	25.0-29.0	β -D-gal-[(1,6)- β -D-gal-(1,4)] n - β -D-gal-(1,4)-D-glc
Total GOS		90.0-92.0	-

DB = Dry Basis; Gal = Galactose; Glc = Glucose

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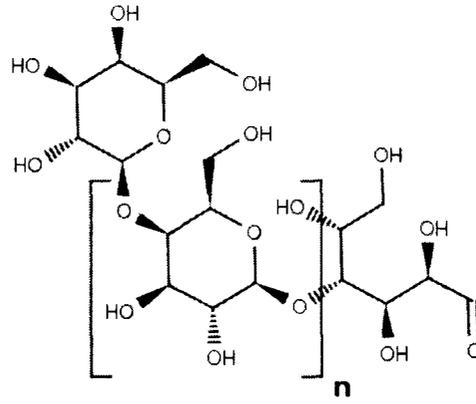


Figure II.A-1 General structure of a β -(1,4) linked galactooligosaccharide molecule

B. Method of Manufacture

GOS is manufactured from food grade lactose *via* a transgalactosylation reaction catalyzed by a β -galactosidase obtained from the non-toxicogenic non-pathogenic microorganism, *B. circulans* LOB 377. All raw materials and processing aids used in the manufacture of GOS are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations as described in Table II.B-1 below.

Table II.B-1 Raw Materials and Processing Aids Used in the Manufacture of GTC Nutrition's GOS		
Material	Use	Regulatory Status
Raw Materials		
Lactose	Substrate for GOS synthesis	Permitted food ingredient (FCC, 2008)
Water	Solvent	N/A
Processing-Aids		
Sodium Hydroxide	pH adjustment	In accordance with 21 CFR § 184.1763, sodium hydroxide is permitted for use in food with no limitations other than cGMP
Hydrochloric Acid	pH adjustment ion-exchange eluent	Under 21 CFR §182.1057 hydrochloric acid is GRAS when used as a buffer and neutralizing agent in accordance with cGMP

Table II.B-1 Raw Materials and Processing Aids Used in the Manufacture of GTC Nutrition's GOS		
Material	Use	Regulatory Status
Celite (plankton diatomite)	Purification-aid	Resin-bonded filters containing diatomaceous earth can be safely used in producing, manufacturing, processing, and preparing food, subject to the provisions of 21 CFR § 177.2260 Cleared under 27 CFR § 24.243 (FILTERING AIDS) for use in the cellar treatment and finishing of wine
Activated Carbon (granular)	Purification-aid	No federal regulations specific to the intended use were identified Similar uses of activated carbon are considered GRAS for purification and clarification of wine as per 27 CFR §24.246 (U.S. ATTTB, 2008)
<u>Cation Column</u> <ul style="list-style-type: none"> • Strongly acidic cation exchange resin • Cross-linked polystyrene matrix • Sulphonate functional group • Na⁺ counter-ion 	Purification-aid	Used in accordance with 21 CFR §173.25
<u>Anion Column</u> <ul style="list-style-type: none"> • Weakly basic anion exchange resin • Cross-linked polystyrene matrix • Dimethylammonium functional group • OH counter ion 	Purification-aid	Used in accordance with 21 CFR §173.25
<u>Mixed Bed Column</u> A) Strongly acidic cation exchange resin <ul style="list-style-type: none"> • Cross-linked polystyrene matrix • Sulphonate functional group • Na⁺ counter-ion B) Strongly basic anion exchange resin <ul style="list-style-type: none"> • Cross-linked polystyrene matrix • Dimethylethanolammonium functional group • Cl⁻ counter ion 	Purification-aid	Used in accordance with 21 CFR §173.25
<u>Chromatographic column</u> <ul style="list-style-type: none"> • Strongly acidic cation exchange resin • Cross-linked polystyrene matrix • Sulphonate functional group • K⁺ counter-ion 	Purification-aid	Used in accordance with 21 CFR §173.25
β-Galactosidase derived from <i>Bacillus circulans</i> LOB 377	Catalyze transgalactosylation of lactose to produce GOS mixture.	GRAS (GRN000236) See section IV.J for data supporting safe use of the enzyme

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GTC Nutrition's GOS is produced through a multi-step process that begins with the dissolution of lactose powder in heated water (~ 90°C) in the saccharification tank. Once the desired concentration is obtained, the temperature of the solution is reduced (55 to 70°C), and the pH is adjusted to mildly acid conditions (pH between 5.0 to 6.5) using sodium hydroxide (NaOH) or hydrochloric acid solutions as required. The galactosidase is then added to the solution where it reacts with lactose to produce GOS². Saccharification occurs in the stirred solution tank over a two-day period until the desired oligosaccharide content (≥ 50% w/v) is achieved.

The hydrolysate formed during saccharification is then pumped through a heat exchanger where the solution is heated to 80°C, resulting in inactivation of the β-galactosidase, and optimization of the decolorization step. The inactivated enzyme is removed *via* a Celite filter (plankton diatomite), and decolorization occurs using a fixed-bed continuous decolorization system where the syrup is introduced to the top of an adsorption column packed with active carbon, and is passed through from top to bottom. The organic impurities are adsorbed by the active carbon granules, which are discharged, replaced by a fresh carbon layer, and regenerated in the furnace for later use. The decolorized syrup is then cooled *via* a heat exchanger and then proceeds through a 3 column ion exchange process [*i.e.*, cation column with strongly acidic cation exchange resin; anion column with intermediate basic anion exchange resin; and a mixed bed column that has a combination of both strongly acidic and strongly basic resins] removing any ionic impurities [*e.g.*, calcium, chlorides, sulfates, phosphates, and other ionic components including amino acids, peptides and proteins].

Following active carbon ion exchange purification, the GOS solution is concentrated using an evaporator to produce a syrup (~50 to 60% w/v). The concentrated GOS syrup then proceeds through a chromatographic separation process where glucose, galactose, and lactose are separated from the GOS mixture. The separated products are recovered from the adsorbent bed through displacement with sterilized/purified water. Following chromatographic purification, the oligosaccharide fraction is composed of greater than 90% GOS, while the secondary fraction is composed of approximately 3 to 7% lactose, 10 to 15% oligosaccharide, 20 to 25% galactose, and 60 to 65% dextrose. The oligosaccharide fraction continues onto further processing, while the monosaccharide fraction is recycled back to glucose syrup. The oligosaccharide fraction is then further refined through a second round of ion exchange, activated carbon and evaporative concentration treatments. The final purified and concentrated syrup is then retained in a storage tank where the composition of the GOS syrup is approximately 90 to 92% oligosaccharide, 7 to 10% lactose, 0 to 1.0% dextrose, and 0 to 0.5% galactose. The syrup is then pumped through a heat exchanger to increase the temperature of the syrup prior to hot air (~200°C) spray drying, where the final product, a purified white GOS

² β-galactosidases are generally known as enzymes that catalyze the hydrolysis of β-D-galactopyranosides such as lactose, however, the enzyme also catalyzes transgalactosylation of these sugars, and when lactose is present at high concentrations, the transgalactosylation reaction predominates (Playne and Crittenden, 1996).

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powder (97% dry solids) is produced. The final product is packaged in poly-lined craft paper bags and stored at room temperature.

C. Product Specifications

GOS is produced in accordance with cGMP, and in order to ensure consistent, safe products, GTC Nutrition has established food-grade specification parameters for the final ingredient. These parameters comprise specifications for the physical appearance, purity, and GOS distribution, as well as specifications for potential chemical and microbiological impurities and contaminants. The chemical and microbiological specifications for GOS are presented in Table II.C-1. Analyses of three commercial batches of GOS confirm that the material produced by the manufacturing process is consistent and complies with these product specifications. The analytical data also demonstrate the absence of contaminating protein, lead, and pathogenic microbiological organisms. The complete analyses of these batches are presented in Table C-1 (Appendix C). All analytical procedures are conducted using standard validated internal methodologies of Corn Products International as developed by the Corn Refiners Association, and where applicable have been developed from established methodologies [*i.e.*, Association of Official Analytical Chemists (AOAC), United States Pharmacopeia (USP), Food Chemicals Codex (FCC)].

Table II.C-1 Product Specifications for GOS		
Specification Parameter	Specification	Method (Reference)
Physical/Chemical		
GOS Contents (%DB)	90.0 to 92.0	CPSMA S30 (internal validated) Based on AOAC 979.23
Moisture (%)	Maximum 10	CPSMA M50 (internal validated) (AOAC)
Carbohydrate Profile		CPSMA S30 (internal validated) Based on AOAC 979.23
Dextrose (%DB)	0 to 1.0	
Galactose (%DB)	0 to 0.5	
Lactose (%DB)	7.0 to 10.0	
Galactooligosaccharides (%DB)	90.0 to 92.0	
Disaccharides	16.0 to 21.0	
Trisaccharides $\beta(1,3)$ (1,4)	14.0 to 19.0	
Trisaccharides $\beta(1,4)$ (1,4)	16.0 to 20.0	
Trisaccharides $\beta(1,6)$ (1,3)	8.0 to 13.0	
Tetrasaccharides and greater	25.0 to 29.0	
Granular size (% through 40 mesh)	100	-
Ash (% w/w)	0.05	CPSMA A90 (Internal validated) (Based on AOAC method)

Table II.C-1 Product Specifications for GOS		
Specification Parameter	Specification	Method (Reference)
Protein (%)	Negative (LOD = 10 ppm)	CPSMA P60 (internal validated) Based on Kjeldhal method (USP "chemical tests:<461> Nitrogen Determination". "Maltodextrin NF Monograph")
pH (in 10% solution)	4.0 to 7.0	CPSMA P40 (internal validated) (Based on USP method)
Lead (ppm)	<0.01	CPSMA L40 (internal validated*)
Arsenic (as AS ₂ O ₃)	≤1	CPSMA A80 (internal validated) (Based on FCC method)
Foreign substance	Negative	-
Appearance	White powder	Visual inspection
Foreign taste and odor	Negative	Sensory test
Microbiological (counts/g)		
Mesophilic bacteria	300	CPSMA Microbiological Methods (internal validated)
Mold and yeast	20	
Coliforms	Negative	Based on Microbiological Methods of the Member Companies of the Corn Refiners Association
Anaerobic thermophilic spores	<10	
Aerobic thermophilic spores	<10	
Anaerobic mesophilic spores	<10	
Aerobic mesophilic spores	<10	
<i>Salmonella</i>	Negative	
<i>Staphylococcus aureus</i>	Negative	
<i>Escherichia coli</i>	Negative	
Listeria (tested in 50 g)	Negative	Food Codex of the Korean Food and Drug Administration

CPSMA = Corn products of America Standard Methods of Analyses; CRA = Corn Refiners Association; Association of Official Analytical Chemists (AOAC), United States Pharmacopeia (USP), Food Chemicals Codex (FCC); LOD = limit of detection.

*Methodology for lead analyses based on the following references: (1) Corn Industries Research Foundation Division of Corn Refiners Association, Inc.: Standard Analytical Methods. Sixth Edition. Washington; (2) CPC International: Moffet Center - Analytical Methods L20. Determination of Lead by Dithizone: Spectrophotometric Procedure. 1961; (3) Food Chemicals Codex. Current Edition. Appendix III. Lead Limit Test; (4) The United States Pharmacopeia. Current Edition. "Chemical Tests: <251> Lead".

D. Stability of GOS

The results of bulk stability studies are shown below in Figure II.D-1. GTC Nutrition's GOS was stored in polypropylene bags at 30°C and 80% Relative Humidity (RH) over a 6-month period. Moisture content, GOS purity, pH in solution (10%), and microbial contamination were determined at time 0, and at 1 and 6 months using validated assays as per Table II.C-1. The moisture content and percent galactooligosaccharide, pH in solution (10%), and microbial

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content were observed to be within product specifications for at least 6 months (Table II.D-1). These data support a shelf-life of at least 6 months for the bulk material.

The stability of a 5% solution of GOS at increasing temperature and pH is depicted in Figure II.D-2 below. At ambient and elevated temperatures (temperature = 30, 50, 75, 100°C; time = 60 min), GOS was observed to be stable as a 5% solution over a range of pH values (3 to 7). The results of this information indicate that GOS will be stable under the proposed food uses.

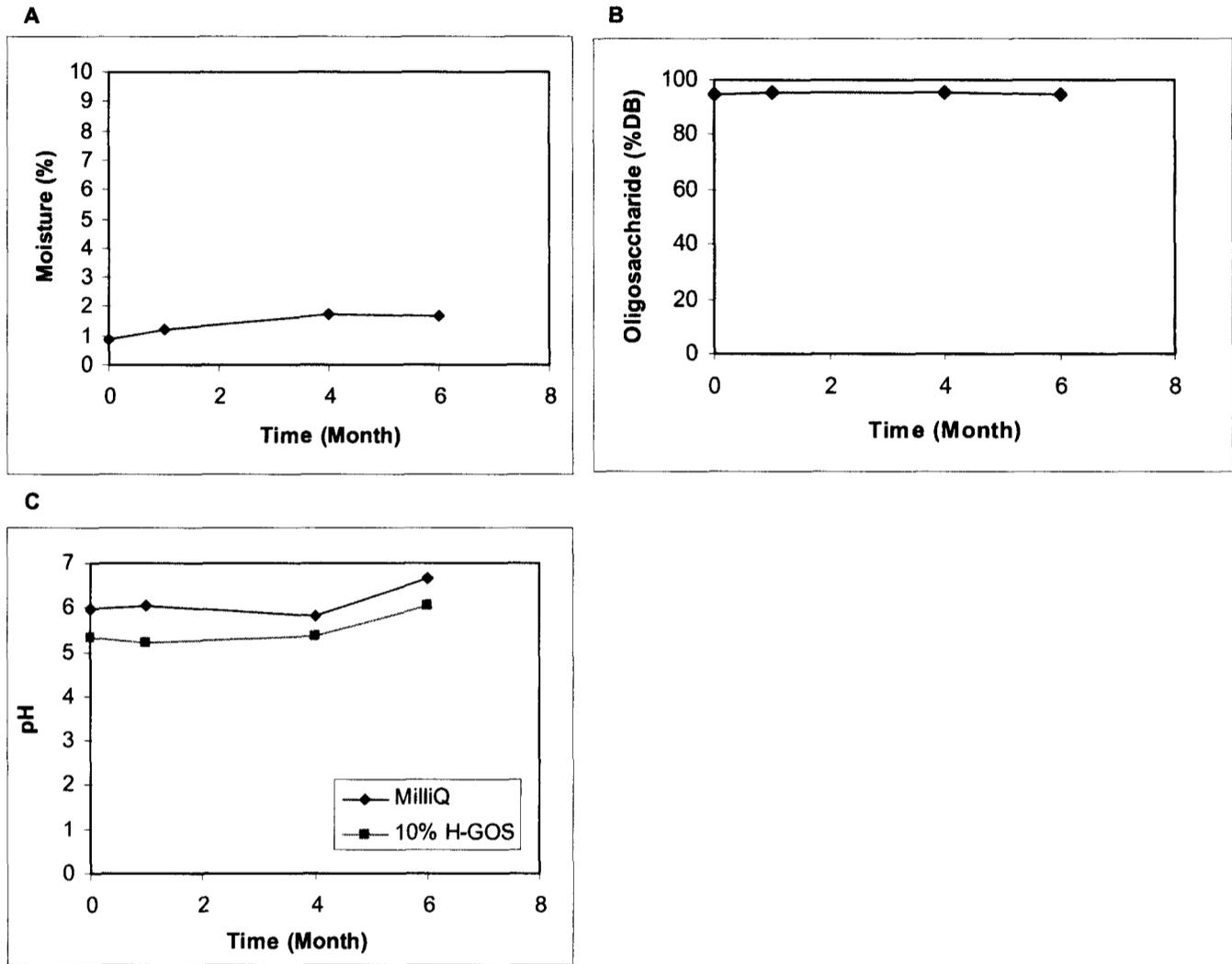


Figure II.D-1 GOS Bulk Stability. GTC Nutrition's GOS ingredient was stored in polypropylene bags at 30°C and 80% RH. Moisture content (A), GOS concentration (B), and pH (10% solution prepared from bulk sample) (C) were determined at times t=0, 1 month and 6 months. Analytical procedures are validated methods as per those outlined in the product specifications.

Table II.D-1 Microbial Stability of GOS under Storage Conditions (stored in polypropylene bags at 30°C and 80% RH)					
Parameter	Specification	t = 0	t = 1 month	t = 4 months	t = 6 months
Mesophilic bacteria	300	40	55	100	150
Mold & Yeast	20	5	8	10	13
Coliforms	Negative	Negative	Negative	Negative	Negative
<i>Salmonella</i> (Tested in 25g)	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative	Negative	Negative	Negative	Negative
<i>E. coli</i>	Negative	Negative	Negative	Negative	Negative
<i>Listeria</i> (tested in 50g)	Negative	Negative	Negative	Negative	Negative

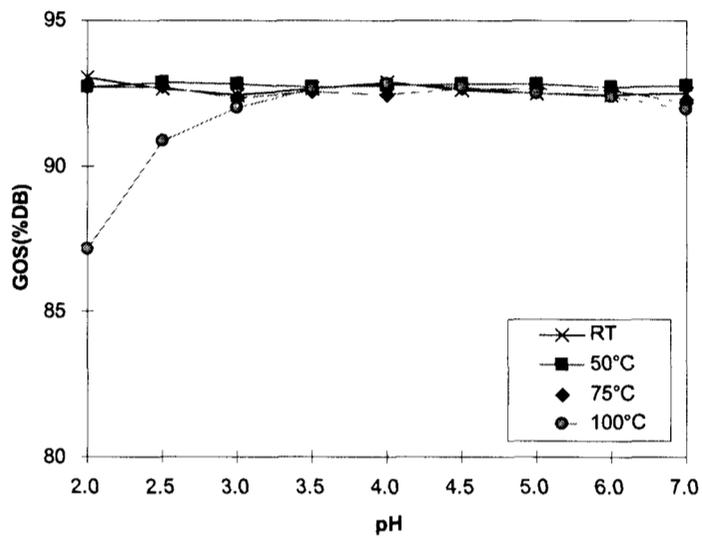


Figure II.D-2 Stability of GOS in Solution. 5% solutions were prepared from a bulk sample meeting product specifications, and were heated for 60 minutes at the indicated pH values (HCl and NaOH used for altering pH of solutions). The stability of GOS under these conditions is expressed as % GOS, as determined via HPLC, and expressed as %GOS DB.

III. SELF-LIMITING LEVELS OF USE

Under the intended conditions of use of GOS, no self-limiting use levels are expected.

IV. BASIS FOR GRAS DETERMINATION

In the absence of product specific safety studies supporting the GRAS use of GTC Nutrition's GOS, published studies using GOS products determined to be comparable to GTC Nutrition's ingredient were used to support the safety of the ingredient for its intended uses. Currently, all GOS products are manufactured in a similar manner using lactose as a starting material followed by GOS synthesis via a beta-galactosidase(s) obtained from various non-toxicogenic

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strains of bacteria. Based on the widespread use of a standard manufacturing process for GOS ingredients currently on the market, GTC Nutrition concluded that differences between various GOS products produced using these manufacturing processes, in accordance with cGMP, would be limited to slight variations in the compositional distribution of the GOS oligomers, and to differences in the residual levels of lactose and water in the final product. Thus, it was concluded that provided GOS is manufactured using a beta-galactosidase obtained from a non-toxicogenic organism using standard manufacturing techniques, and in accordance with cGMP, the safety information obtained from peer reviewed scientific literature on other GOS products would support the safety of GTC Nutrition's ingredient. As such, product specific safety studies were not considered necessary to determine that the uses of GTC Nutrition's ingredient are GRAS. This conclusion was supported by the Expert Panel, and is consistent with the European Food Safety Authority (EFSA) and Food Standards Australia New Zealand's (FSANZ) regulatory opinions of the use of GOS in traditional food products and infant formula. These agencies have determined that it was not practical to develop specifications for the use of these products in traditional food products or infant formula, and a generic³ approval of the use of these products has been granted (SCF 2001a,b; FSANZ, 2008). Pursuant to the regulations in these countries, Novel Food applications for the use of GOS in traditional food products and infant formula would not be required, provided the GOS ingredient is not manufactured using novel processes.

Therefore, the data supporting the GRAS status GTC Nutrition's GOS ingredient for the intended uses in various traditional food products described herein, was obtained from a series of published studies conducted in animals, and in humans, using sources of GOSs that were considered comparable to GTC Nutrition's ingredient. These studies, summarized below, consist of information characterizing background exposure to GOS and similar beta-linked carbohydrates in the diet, studies investigating the metabolic fate, microbial fermentation and toxicity of galactooligosaccharides. GTC Nutrition also reviewed several studies conducted in healthy adults, and safety studies conducted with infants administered GOS supplemented infant formulas⁴. Information pertaining to allergenicity also was reviewed. Finally, generally available information supporting the suitability of the β -galactosidase (source organism: *B. circulans* Jordan LOB 377) as a processing aid for use in the manufacture of GOS was obtained; unpublished studies provided by the manufacture of the β -galactosidase were included as corroborating evidence.

³ In Australia and New Zealand, GOS used in traditional food products or infant formula must meet the general requirements of standard 1.3.4 pertaining to identity and purity whereby GOS is defined as "a mixture of those substances produced from lactose by enzymatic action, comprised of between two and eight saccharide units, with one of these units being a terminal glucose and the remaining saccharide units being galactose, and disaccharides comprised of two units of galactose."

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Moreover, these data were reviewed by a Panel of Experts, qualified by scientific training and experience to evaluate the safety of GOS as a food ingredient, who concluded that the aforementioned proposed uses of GOS are safe and suitable and would be GRAS based on scientific procedures (see Appendix B for copy of the Expert Panel Statement). A summary of the data reviewed by GTC Nutrition is presented herein.

A. Current Uses of GOS World-Wide

GOS ingredients have been used as food ingredients in Japan and Europe for at least 25 years. Worldwide 15,000 metric tons of GOS are produced annually, and in Japan alone demand for the production of GOS is estimated to be 6500 metric tons/year (Crittenden and Playne, 1996; Sako *et al.*, 1999; Nakakuki, 2003). The European Union (EU) Scientific Committee on Food (SCF) reviewed the use of GOS as an ingredient for addition to infant formula, and concluded (SCF/CS/NUT/IF/65) that the inclusion of up to 8 g/L of a combination of 90% oligogalactosyl-lactose (*i.e.*, GOS) and 10% high molecular weight oligofructosyl-saccharose (*i.e.*, inulin-derived substances) to infant formula and follow-on formula is safe (SCF, 2003). FSANZ also examined the safety of the addition of GOS and inulin-derived substances to traditional foods, including infant formula and follow-on formula; the agency concluded that the addition of GOS to infant formula up to a concentration of 8 g/L was safe (FSANZ, 2008). Recently, the GRAS use of GOS produced from lactose using a β -galactosidase derived from *B. circulans* in various foods and in infant formula has been notified to the U.S FDA without objection from the agency; the proposed uses and resultant GOS exposures are comparable to those described herein for GTC Nutrition's GOS (GRN000236).

B. Background Exposure to GOS in the Diet

The intake of oligosaccharides in the human diet is difficult to estimate, and only small amounts of GOS are expected to occur in the diet from the consumption of traditional foods; for example trace amounts of GOS are expected in specialty dairy products, where β -galactosidases are used to reduce the lactose content of the product to make it suitable for lactose sensitive individuals. Although GOS as produced by GTC Nutrition are not present in the diet in significant quantities, various oligosaccharides (*e.g.*, inulin and fructooligosaccharides) are present in the diet through their natural occurrence in fruits, vegetables, and grains, as well as through the addition of oligosaccharides - produced *via* biosynthetic processes from natural sugars or polysaccharides - to food products for their nutritional properties or organoleptic characteristics. Delzenne (2003) has reported that the intake of fructooligosaccharides alone may range from 3 to 13 g/person/day depending on the population. The intake of oligosaccharides in children was estimated by the Food Standards Australia New Zealand (FSANZ) during their evaluation of the proposed use of GOS:FOS mixtures in infant formula and various traditional foods (Proposal P306). The agency estimated baseline intakes of inulin-derived substances and GOS based on natural sources and added sources according to market

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use of the substances in processed foods; intakes of 17 and 42 g per person per day were calculated for mean and 95th percentile dietary intakes in New Zealand children 1 to 3 years of age (FSANZ, 2008). Estimates for older users were not reported by the agency.

Human milk is rich in oligosaccharides that contain a similar core molecular structures to GOS, the compositional identities of which are represented by a highly complex mixture of oligosaccharides that differ in chain length, charge, sequence, and relative abundance. The structural identities for a number of neutral, acid and sialylated oligosaccharides have been identified, and it has been reported that as many as 1,000 different oligosaccharide molecules may be present in human milk (Stahl *et al.*, 1994). The concentrations of oligosaccharides in human milk have been reported to be between 8 to 12 g/L in mature human milk, and as high as 25 g/L in colostrum (Kunz *et al.*, 1999, 2000). However, although infants are exposed to high concentrations of oligosaccharides at birth *via* the consumption of breast milk, breast milk only contains trace quantities of oligogalactose molecules. Due to the complexity of human milk oligosaccharides, economical sources of human milk oligosaccharides are not available. Therefore, oligosaccharide preparations (*e.g.*, GOS) produced from various microbial derived β -galactosidases, and/or fructooligosaccharides obtained from chicory roots are currently the most comparable commercially available alternatives for use as human milk oligosaccharide substitutes. The estimated consumption of GOS from the use of GOS under the proposed food uses are described below.

C. Estimated Intake of GOS

GTC Nutrition intends to market GOS as a food ingredient in the United States in a variety of food products including baby, infant and toddler foods (excluding infant formula), beverages and beverage bases, dairy product analogs, milk products, bakery products, beverages, cereal and other grain products, desserts, fruit and fruit juices, snacks, soups, and soft and hard candy, at use levels of 0.86 to 1.28 g per serving⁵. On a percent basis, the intended food uses of GOS result in use levels of between 0.48 to 12.21%. The individual proposed food uses and use levels for GOS are located in Appendix A in Table A-1. Food codes representative of each proposed food use were chosen from the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Survey (NHANES) (CDC, 2006; USDA, 2008) and were grouped in food use categories according to Title 21, Section §170.3 of the *Code of Federal Regulations* (U.S. FDA, 2008).

Approximately 100% of the total U.S. population was identified as consumers of GOS from the proposed food-uses (8,168 actual users identified). Consumption of these types of foods by the total U.S. population resulted in estimated mean and 90th percentile all-user intakes of GOS of 9.3 g/person/day (172 mg/kg body weight/day) and 15.4 g/person/day (351 mg/kg body

⁵ GOS is not intended for use in meat and poultry containing products

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weight/day), respectively (Tables IV.C-1 and IV.C-2). On an individual population basis, the greatest mean and 90th percentile all-user exposures were estimated to occur in male teenagers (aged 12 to 19 years), at 12.1 g/person/day (192 mg/kg body weight/day) and 20.2 g/person/day (335 mg/kg body weight/day), respectively. On a body weight basis, mean and 90th percentile all-user intakes of GOS were highest in infants, ages 0 to 2 years, with intakes of 499 and 815 mg/kg body weight/day, respectively.

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (g)	90 th Percentile (g)	Mean (g)	90 th Percentile (g)
Infant	0-2	90.1	838	5.4	9.7	5.7	9.8
Child	3-11	100	1,287	9.5	14.2	9.5	14.2
Female Teenager	12-19	99.9	991	9.4	14.6	9.4	14.6
Male Teenager	12-19	100	999	12.1	20.2	12.1	20.2
Female Adult	20 and Up	99.8	2,125	8.1	14.0	8.1	14.0
Male Adult	20 and Up	99.9	1,928	10.4	17.8	10.4	17.8
Total Population	All Ages	98.8	8,168	9.2	15.4	9.3	15.4

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (mg/kg)	90 th Percentile (mg/kg)	Mean (mg/kg)	90 th Percentile (mg/kg)
Infant	0-2	90.1	838	467	805	499	815
Child	3-11	100	1,287	363	617	363	617
Female Teenager	12-19	99.9	991	164	275	164	275
Male Teenager	12-19	100	999	192	335	192	335
Female Adult	20 and Up	99.8	2,125	114	197	114	197
Male Adult	20 and Up	99.9	1,928	123	207	123	207
Total Population	All Ages	98.8	8,168	171	351	172	351

GTC nutrition has noted that the methodology used to estimate the consumption of GOS described above is generally considered to result in 'worst case' estimates of exposure as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the

maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently. Thus, the estimated intakes reported in tables IV.C-1 and IV.C-2 above are considered to be a gross over-estimates of the actual expected intake of GOS in the U.S population.

D. Metabolic Fate and Kinetics

The human digestive tract can efficiently hydrolyze glucose polymers linked by alpha-glycosidic linkages, like those present in starch and glycogen (Wisker *et al.*, 1985); however, with the exception of brush border beta-galactosidases (lactases), which are responsible for the degradation of lactose, luminal digestion of dietary sugars linked *via* beta-glycosidic linkages does not occur to any significant degree (Wisker *et al.*, 1985). All undigested, and therefore unabsorbed, dietary fibre passes into the colon where it is metabolized by colonic microflora to normal metabolites of fermentation (short-chain fatty acids, and CO₂, methane and hydrogen gases). Studies supporting the non-digestibility of GOS are reviewed below.

(i) In vitro

The digestibility of a GOS mixture prepared from lactose *via* the transgalactosylation reaction of two β -galactosidase expressing microorganisms (*Sporobolomyces singularis* and *Kluyveromyces lactis*) was evaluated by Chonan *et al.* (2004). The GOS product was separated into di-, tri-, and tetrasaccharides using a Bio-Gel P-2 column and subjected to various digestive enzyme preparations *in vitro*. The samples containing tri- and tetrasaccharides were not digested by any of the preparations, which consisted of α -amylase from human saliva, artificial gastric juice, α -amylase from hog pancreas, and rat intestinal acetone powder. Disaccharides were partially digested by the rat intestinal acetone powder, but not by the other preparations (Chonan *et al.*, 2004).

The indigestibility of β -linked oligosaccharides also was demonstrated by Ohtsuka *et al.* (1990), who performed a series of tests on a $\beta(1,4)$ linked GOS trisaccharide synthesized by *Cryptococcus laurentii* OKN-4 from lactose. Human salivary and hog pancreatic α -amylase were unable to hydrolyze GOS *in vitro*, and a homogenate produced from rat small intestinal mucosa digested only a small amount of GOS. In addition, the authors also show that GOS is highly resistant to acid hydrolysis, as 3 different preparations of artificial gastric juice with pH values of 1.0, 1.5, and 2.0 were unable to hydrolyze the molecule after incubation for 6 hours at 37°C.

The digestibility of human milk oligosaccharides was investigated by Engfer *et al.* (2000). The authors isolated fractions of neutral and acidic oligosaccharide fractions from samples of human breast milk using gel permeation chromatography. These fractions were then incubated for up

to 20 hours with human pancreatic juice and brush border membranes from human or porcine intestinal samples. Evidence of hydrolysis was measured using enzymatic detection of liberated monosaccharides, and matrix-assisted laser desorption ionization mass spectrometry. No evidence of oligosaccharide hydrolysis was observed. When the oligosaccharide fraction was incubated in the presence of porcine pancreatic tissue homogenates containing intracellular zymogens and lysosomal enzymes, extensive hydrolysis of both oligosaccharide fractions was observed.

(ii) Animal Studies

The metabolic fate of GOS administration in male Sprague-Dawley rats was investigated by Ohtsuka *et al.* (1991). Radiolabeled GOS ([U-¹⁴C]GOS) was prepared from [U-¹⁴C]lactose using *C. laurentii* OKN-4, and purified using a charcoal celite column. Male Sprague-Dawley (SD) rats treated with penicillin and chloramphenicol for 1 week, and germ-free male Wistar rats were administered 0.5 mL of [U-¹⁴C]GOS in saline solution *via* gastric intubation. Radioactive expired air was collected for 24 hours, and samples were taken out at 2, 4, 6, 8, 10, 12, and 24 hours after administration to determine the time course of excretion. In rats not treated with antibiotics, excretion of ¹⁴CO₂ was very slow in the first 2 hours, increased at 4 hours and showed maximum excretion rates between 6 and 8 hours after dosing. Excretion rates slowed after 8 hours and remained slow for the remainder of the 24-hour time period, and 49% of the administered radioactivity was recovered during this 24-hour interval. In rats administered antibiotics, recovery of ¹⁴CO₂ was delayed by approximately 2 hours in comparison with conventional rats not treated with antibiotics. Excretion rates were slow for the first 4 hours, increased to 6 hours, and showed maximum excretion of ¹⁴CO₂ from 6 to 10 hours. Total recovery of ¹⁴CO₂ also was 25% less than that reported in rats not treated with antibiotics. Excretion of ¹⁴CO₂ from germ-free rats administered [U-¹⁴C]GOS was delayed even greater than in antibiotic treated rats and displayed a very slow excretion rate with only 18% of the administered radioactivity recovered in the 24-hour time period. The authors concluded that their results suggested that GOS was not absorbed from the upper gastrointestinal tract and had reached the colon where it was fermented by the intestinal microflora.

Ohtsuka *et al.* (1991) also examined the recovery of radioactivity from [U-¹⁴C]GOS in the intestine, liver, serum, carcass, and urinary and fecal excretions. In conventional rats, 8.7, 4.1, and 4.8% of the ingested radioactivity was recovered from the total intestine, fecal excretions, and urinary excretions, respectively. In contrast, in germ-free rats, the recovery rates were 58.3, 0.5, and 5.5% of the administered radioactivity in the respective organs. Recovery of radioactivity from the liver, serum, and carcass was decreased in germ-free rats as compared to conventional rats, with 0.6, 0.4, and 7.7% recovered *versus* 1.4, 0.6, and 12.8% recovered, respectively.

In conjunction with their studies on absorption, Ohtsuka *et al.* (1991) also examined the excretion of GOS (β -1,4 linked). Male SD rats (\geq 230 g; n=5) were fed a diet containing 5%

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GOS (~2,500 mg/kg body weight/day, U.S. FDA, 1993) for 2 weeks and feces were collected on the final day. High-performance liquid chromatography (HPLC) was performed to determine the GOS content of the feces; however, no GOS was recovered. The authors concluded that the lack of GOS in the feces indicates that GOS was almost completely fermented by the microflora of the large intestine.

(iii) Human Studies

There are limited human studies investigating the absorption, metabolism, and excretion of GOS. Moro *et al.* (2005) have shown the dietary oligosaccharides are detectable in the feces of formula-fed infants. The authors administered a standard infant formula or infant formula supplemented with GOS:FOS (8 g/L; 9:1 ratio) to groups of 16 infants during the first 2 weeks of life for a period of 28 days. In each of the infants fed the GOS:FOS supplemented formula, GOS and FOS could be detected in the stool samples (quantitative measurements were not conducted). No GOS or FOS was detected in the control subjects. The authors concluded that “dietary galactooligosaccharides and long-chain fructooligosaccharides remain during the whole passage in the lumen of the gastrointestinal tract, similarly to human milk oligosaccharides.”

(iv) Summary

GOS are not hydrolyzed by human salivary amylase, hog pancreatic α -amylase, or artificial gastric juice (Ohtsuka *et al.*, 1990; Chonan *et al.*, 2004). Incubation of GOS in human pancreatic juice and brush border membranes also did not result in hydrolysis of GOS (Engfer *et al.*, 2000), observations that are consistent with those reported by Moro *et al.*, (2005) where intact GOS was detected in the feces of infants consuming GOS supplemented infant formula (8 g/L; ~5.0 g/kg body weight)

Although extensive comparative data is not available, the metabolic fate of GOS in rodents appears similar to humans, and studies conducted in rodents are expected to be relevant to humans.

In conclusion, following consumption of GOS, the compounds are expected to travel intact through the gastrointestinal tract, where fermentation by endogenous microflora will take place, unfermented dietary GOS would be excreted in the feces.

E. Effects on Gastrointestinal Microflora, and Fecal Characteristics

(i) In vitro

As reviewed above, dietary galactooligosaccharides enter the colon intact, and the material becomes a substrate for bacterial fermentation. The metabolic products of GOS fermentation are typical of that observed with most indigestible carbohydrates, where short chain fatty acids

(SCFA), including butyrate, propionate, and acetate, are produced. The production of small amounts of methane, hydrogen, and carbon dioxide also are expected.

Tanaka *et al.* (1983) tested the ability of various facultative anaerobes to grow in the presence of a GOS preparation synthesized from lactose *via* a β -galactosidase derived from *Aspergillus oryzae*. The GOS was composed of tri- (55%), tetra- (33%), and penta- and hexa-(12%) saccharides connected *via* β (1,3), β (1,4), and β (1,6) linkages. All species of *Bifidobacterium* and *Bacteroides* tested, and most *Lactobacillus* and *Enterobacteriaceae* species tested were able to grow using GOS as a substrate; *Bifidobacterium* species demonstrated the most vigorous growth (Tanaka *et al.*, 1983).

Smiricky-Tjardes *et al.* (2003a) determined the fermentation characteristics of granular and liquid GOS preparations (Friesland Foods, Netherlands) using fecal microflora obtained from 3 healthy pigs. The preparations were fermented *in vitro* and samples were taken at 0, 2, 4, 8, and 12 hours for determination of gas production, pH, and SCFA production. Fermentation of the GOS preparations resulted in the production of acetate, propionate, and butyrate, and at 12 hours the SCFA composition was dominated by acetate and propionate. Gas production between granular and liquid GOS was similar at all time points and increased over time. After 12 hours of incubation, fermentation of the granular and liquid GOS preparations reduced the pH of the medium from 6.3 to 5.3 and 5.6 respectively.

Under *in vitro* conditions, the ability of various strains of gastrointestinal microflora to metabolize a mixture of β (1-4) linked di-, tri-, and tetra-GOS was investigated by Ishikawa *et al.* (1995). All strains of *Bifidobacteria* tested were able to use the mixture of oligosaccharides and the purified di- and trisaccharides for growth, while 8 of 12 strains were able to utilize the tetrasaccharides. Of the lactobacilli strains tested, most were able to ferment the GOS mixture and the disaccharides, 3 of 9 could ferment the trisaccharides, and none were capable of using the tetrasaccharides as an energy source. Of the 5 *Bacteroides* species tested moderate fermentation to all GOS substrates was reported. Fermentation of GOS by *Clostridium* species were limited to the GOS disaccharides, with increasingly poorer capacity for fermentation observed with the tri- and tetra-saccharides. Of the remaining bacteria tested (*Fusobacterium*, *Eubacterium*, *Peptostreptococcus*, *Veillonella*, *Megasphaera*, *Mitsuokella*, *Propionibacterium*, *Streptococcus*, *Enterococcus*, *Citrobacter*, *Klebsiella*, *Enterobacter*), most species demonstrated moderate to poor capacity to utilize GOS as a metabolic substrate.

Matsumoto *et al.* (2004) investigated the ability of 62 different enterobacterial strains from 22 bacterial genera to grow in the presence of oligosaccharides purified from a GOS mixture. The purified oligosaccharides consisted of di-, tri-, and tetrasaccharides with β (1-4), β (1-6), β (1-2), and β (1-3) linkages. All bifidobacterial and lactobacilli species tested, *Bacteroides vulgatus*, *Clostridium perfringens*, *Enterococcus faecium*, *Streptococcus salivarius*, and *Escherichia coli* were able to grow using the disaccharides. The tri- and tetra-oligosaccharides showed a similar bacterial utilization pattern, and were utilized by all bifidobacterial species tested, 3

Lactobacillus species, *B. vulgatus*, *C. clostridiiforme*, *B. fragilis*, *Eubacterium rectale*, and *C. perfringens*. The tetra-oligosaccharide fraction of the GOS mixture was utilized by 5 of the bifidobacterial species tested, *B. vulgatus*, *L. reuteri*, *E. rectale*, and *C. perfringens*.

Tzortzis *et al.* (2005) utilized a 3-stage continuous fermenter to evaluate the ability of a GOS mixture (produced from lactose via a galactosidase derived from *Bifidobacterium bifidum* NCIMB 41171) to modulate the colonic microbiota. The model, validated by Macfarlane *et al.* (1998) against gut contents from human sudden death victims, consists of 3 vessels aligned in series. The first has nutrient-rich, fast transit, and acidic conditions; the third has much less substrate, slow, and neutral conditions. The first vessel is set to resemble the proximal colon, the second the transverse colon, and the third the distal colon. The numbers of *Bifidobacterium* species were significantly increased in the first and second vessel, representing the proximal and transverse colon, respectively.

(ii) Animals

The effect of feeding 5% (w/w) GOS in the diet (~5,000 mg GOS/kg body weight/day, calculated using U.S. FDA, 1993) for a period of 4 weeks on cecal pH and bacterial numbers was investigated in rats colonized with human fecal microflora (Rowland and Tanaka, 1993). The GOS (Yakult) used in the experiment contained lactose (1%), and tri- (50%), tetra- (35%), and penta- and hexa- (14%) galactooligosaccharides connected with β -(1-3), β -(1-4), and β -(1-6) linkages. A significant decrease in cecal pH and numbers of enterobacteria and a significant increase in total anaerobic bacteria, lactobacilli, and bifidobacteria were reported. Similarly, Kikuchi *et al.* (1996) investigated the effect of feeding GOS to male Fischer 344 rats inoculated with a human fecal suspension at levels of 0, 5, or 10% in the diet (equivalent to approximately 0, 2,153 or 4,424 mg/kg body weight/day) for a period of 4 weeks, on hydrogen and methane production, cecal pH, and cecal SCFA. The GOS mixture (Yakult) consisted of tri- (48%), tetra- (37%), and penta- and hexasaccharides (13%). No significant differences in methane production were reported. Hydrogen excretion was increased in both dose-groups but was not significantly different between the 2 dose-groups and the combination of methane and hydrogen excretion was significantly increased only in the 2,153 mg GOS/kg body weight/day dose group. Cecal pH was significantly decreased in both dose groups and rats receiving 4,424 mg GOS/kg body weight/day had significantly lower cecal pH than those receiving 2,153 mg GOS/kg body weight/day. Total SCFA were significantly increased in both dose groups relative to the control, but not significantly different from each other.

Smiricky-Tjardes *et al.* (2003b) also investigated the effect of adding GOS to the diets of T-cannulated pigs and examined ileal, and fecal bacterial populations, as well as ileal SCFA production. Crossbred pigs (PIC326 sire line X C22 dams) were administered a GOS-free casein control diet or a diet casein-cornstarch plus 3.5% GOS (Friesland Foods, The Netherlands). Pigs were fed their respective diet for a total of 7 days. Fecal samples were collected on Day 6 for microbiological analysis and ileal digesta were collected on Day 7 for

SCFA analysis. The GOS diets had no effect on ileal *lactobacilli* or *bifidobacilli* populations. However, relative to pigs fed the control diets, fecal *bifidobacteria* and *lactobacilli* were significantly increased ($P < 0.05$). The concentration of propionate and butyrate were significantly increased in the ileum of pigs fed the GOS diet relative to controls. The results of this study indicate that in pigs, significant quantities of GOS are metabolized by the ileal microflora early in the gastrointestinal tract following consumption.

Tzortzis *et al.* (2005) also investigated the bacterial populations in the proximal and distal colons of pigs after supplementation of their diet with 1.6% (~360 mg/kg body weight/day, calculated using Blackburn, 1988) or 4% (~900 mg/kg body weight/day, calculated using Blackburn, 1988) GOS mixture for 34 days. In the proximal colon, both the 1.6 and 4% GOS diets resulted in significant increases in *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* species relative to pigs administered the control diet. No effects of Clostridium species growth were observed. In the distal colon, only *Bifidobacterium*, and *Lactobacillus* numbers were significantly increased relative to the pigs consuming the unsupplemented diet.

(iii) Humans

The microbial fermentation of dietary GOS was investigated in healthy subjects by Tanaka *et al.* (1983). Quantities of 0.5 g GOS/kg body weight (~30 g for a 65 kg human) were administered to 5 volunteers and breath hydrogen excretion was analyzed. Breath hydrogen concentrations were above basal levels in all 5 subjects over a 4-hour time period, indicating that significant quantities of GOS are fermented by human gastrointestinal bacteria. As reported by Ishikawa *et al.* (1995). Similar increases in breath hydrogen were reported in 17 of 20 men administered a $\beta(1-4)$ -linked GOS (dose not reported) as measured over an 8-hour time period.

Chonan *et al.* (2004) measured breath hydrogen excretion in 16 healthy subjects administered a GOS mixture prepared from lactose via the transgalactosylation reaction of *Sporobolomyces singularis* cells and β -galactosidase from *Kluyveromyces lactis*. Excretion of breath hydrogen was significantly greater from 1.5 hours to 8 hours in the subjects who consumed a single ingestion of GOS (unclear as to whether the ingested amount of GOS was 34.7 g or 15 g, full article in Japanese) compared to subjects who consumed a placebo (placebo not identified in English abstract). The authors concluded that the GOS mixture was composed of non-digestible saccharides (Chonan *et al.*, 2004).

Matsumoto *et al.* (2004) also investigated the effect on fecal flora of feeding 2.5 or 5.0 grams of a GOS mixture/day for a period of 2 weeks in 22 healthy volunteers. The total number of anaerobes present was increased at 1 week in the 5.0 g/day dietary group and in both dietary groups following 2 weeks of feeding. In addition, the number of *Bifidobacterium* was increased in both dietary groups at 1 and 2 weeks of feeding, demonstrating the ability of this bacterial species to ferment GOS.

Numerous studies have investigated the effect of dietary GOS on gastrointestinal microflora in infants. The consumption of GOS supplemented infant formulas is consistently reported to increase the *Bifidobacteria* populations when included in infant formula at concentrations of 4, 8, or 10 g/L. Although a number of studies report that the consumption of GOS in infants are associated with significant increases in *Lactobacillus* populations and significant decreases in the numbers of *E. coli*, and *Clostridium* species in the feces of infants consuming GOS supplemented infant formulas, these observations have not been reported in all studies. That GOS preferentially support the growth of lactic acid bacteria in the gastrointestinal tract is also supported by biochemical changes in the feces. In general, GOS supplemented infant formulas reduce fecal pH over time, an effect that is attributed to fermentation by lactic acid bacteria and the production of significant increases in fecal concentrations of SCFA. The significant increases in SCFA production are dominated by increases in acetate and propionate production. *Bifidobacteria* do not synthesize butyrate, and fecal concentrations of this short-chain fatty acid tend to decrease in most studies. Changes in stool consistency are another consistent finding reported in infants consuming GOS supplemented infant formulas. Most studies report that GOS consumption results in softer/looser stools (Boehm *et al.*, 2002; Moro *et al.*, 2002; Fanaro *et al.*, 2005; Mihatsch *et al.*, 2006; Moro *et al.*, 2006; Ziegler *et al.*, 2007; Costalos *et al.*, 2008). In addition, slight increases in stool frequency are reported in some studies (Moro *et al.*, 2002; Ben *et al.*, 2004). Studies using breast milk fed comparator group show similar changes in microbial flora populations, short-chain fatty acid production, and fecal characteristics (Boehm *et al.*, 2002; Moro *et al.*, 2003; Ben *et al.*, 2004; Haarman and Knol, 2005; Knol *et al.*, 2005; Scholtens *et al.*, 2008), therefore these changes are considered beneficial effects.

The effect of GOS on fecal characteristics has been evaluated in a number of studies. Although conflicting observations have been reported, in general, GOS consumption is associated with a softening effect on feces in healthy adults, and may relieve constipation in the elderly. For example, the consumption of 2.5, 5, or 10 g of GOS on a daily basis for 1 week resulted in softer stools in healthy males, and constipation was reduced in young adults consuming 143 mg GOS/kg body weight over a 10-day period (Ito *et al.*, 1990; Liu *et al.*, 1994). Increased stool frequency in healthy subjects and elderly constipated subjects in association with GOS consumption has been reported as well (Teuri and Korpela, 1998; Teuri *et al.*, 1998). In contrast, the consumption of GOS in elderly females was reported to have no effect on constipation, stool frequency, or fecal hydration (Tamai *et al.*, 1992; Ito *et al.*, 1993; Bouhnik *et al.*, 1997; Teuri and Korpela, 1998).

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F. Toxicity Studies

(i) Acute Toxicity Studies

The LD₅₀ of GOS in rats when administered orally was reported to be greater than 15 g GOS/kg body weight; additional details pertaining to study design were not reported (Matsumoto *et al.*, 1993). No additional acute toxicity studies were identified.

(ii) Subchronic Toxicity Studies

The effects of GOS consumption on growth, and gastrointestinal function in male Sprague-Dawley (SD) rats was reported by Ohtsuka *et al.* (1990). Rats weighing 50 g were fed diets containing 0% (control), 5%, or 10%, GOS. These dietary concentrations would correspond to intakes of approximately 0, 5,000, or 10,000 mg/kg body weight (U.S. FDA, 1993). Two additional control groups also received experimental diets containing 10% lactose or 10% lactulose. Animals consumed each respective diet for 6 weeks, and the control animals consumed the basal diet, which was a corn-starch based chow supplemented with cellulose, casein, DL-methionine, corn oil, minerals and vitamins. The GOS used in the study was reported to contain a mixture of β -(1,4) linked galactooligosaccharides synthesized from lactose by *Cryptococcus laurentii*. Body weight and food intake were recorded every 2 days and feces were collected 4 days prior to sacrifice. Blood was collected at the time of sacrifice for determination of cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride, and phospholipid concentration. Immediately after sacrifice, the small intestine, cecum, colon, stomach, liver, heart, kidney, and spleen were removed and weighed. Body weight increased in both groups receiving GOS-supplemented diets; however, the increase was significant only in the high-dose group. Food intake was significantly increased in both groups receiving GOS in the diet, but there were no significant differences in the food conversion ratio (food/gain). Diarrhea was reported to occur in the rats receiving 10,000 mg GOS/kg body weight/day; however, the effect was transient and only lasted 2 weeks. A dose-related significant increase in relative cecum weight was reported for the rats receiving GOS when compared to those receiving the control diet, were cecum weights were increased 1.9- and 3.7-fold ($P < 0.01$) above controls in animals consuming the 5 and 10% GOS diets. Significant increases in colon weights ($\sim +20\%$; $P < 0.05$) also was reported for both groups receiving GOS compared to the control. Increases in cecal weights are typical following exposure to sugar alcohols (sorbitol, mannitol, and xylitol), lactose, and caramel, as well as some chemically-modified food starches and synthetic polydextrose (Newberne *et al.*, 1988). There were no significant differences in the weights of the small intestine, liver, heart, kidney, or spleen and no significant differences in serum or liver lipids when the values from the rats receiving GOS were compared to those receiving the control diet. Consistent with the fact that galactooligosaccharides are not metabolized by pancreatic or brush border enzymes in the small intestine, and are not absorbed intact, fecal matter was significantly increased in both GOS groups, and the lactose and lactulose controls relative to that reported in the rodents consuming the basal diet. Since

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cecal enlargement in association with polyol consumption in rodents is considered not relevant to humans (WHO, 1987), the no-observed-adverse-effects level (NOAEL) for male SD rats was 10,000 mg GOS/kg body weight/day, the highest dose tested.

Kobayashi *et al.* (2003) conducted a 90-day repeated-dose oral toxicity study of GOS in male and female Crj:CD (SD) IGS rats (Kobayashi *et al.*, 2003). The GOS test article was administered to each animal at doses of 0, (control), 500, 1,000, or 2,000 mg/kg body weight/day, dissolved in distilled water and administered in volumes corresponding to 5 mL/kg body weight *via* gavage for 90 days. Each treatment group contained 10 rats of each sex, except for the male control group and female high-dose groups which included only 9 rats of each sex. Although the composition of the GOS could not be obtained from the study report, the authors were affiliated with the Yakult Central Institute for Microbiological Research. Ito *et al.* (1990) of the Yakult Central Institute for Microbiological Research have published the compositional identity of the ingredient. The authors report that the GOS (Oligomate 55) is synthesized from lactose by transgalactosylation using β -galactosidases derived from *Aspergillus oryzae* and *Streptococcus thermophilus*. The sugar composition is 52% GOS with a structural formula of (Gal)_n-Glc (n=0-4), and the main GOS is a trisaccharide. In addition, the product contains 38% monosaccharides and 10% lactose. Body weight, food intake, and water consumption were recorded throughout the study period. Urinalysis⁶ was performed on samples taken over several days (number not specified). After the rats had been sacrificed, hematology⁷, and blood chemistry⁸ analyses were conducted and macroscopic and histopathological examinations of the tissues and organs (*i.e.*, bronchus, epididymis, heart, harderian gland, kidneys, lungs, liver, lymph nodes, prostate, pancreas, small intestine, stomach, seminal vesicle, thymus, and urinary bladder) were performed. Absolute and relative organ weights (brain, pituitary, thyroid, salivary gland, thymus, lungs, heart, liver, spleen, kidneys, adrenal glands, cecum, and where applicable testis, seminal vesicle, prostate, ovary, and uterus) also were measured. No significant differences in body weight, food intake, water intake, urinalysis, hematology values, blood chemistry values, or absolute organ weights were reported. When the rats were examined macroscopically there were a few minor sporadic abnormal findings; however, these effects were slight to moderate in nature, not dose-dependent, and were observed in each group (1 in each of the control, 500, and 2,000 mg GOS/kg body weight/day dose groups and 3 in the 1,000 mg GOS/kg body weight/day dose group). Sporadic abnormalities also were observed in histopathological examinations; however, once again, these were not dose-dependent and occurred at similar rates in the controls and the

⁶ volume, specific gravity, sodium (Na⁺), potassium (K⁺), chloride (Cl⁻), creatinine, pH, protein, glucose, ketones, bilirubin, red blood cells (RBC), and white blood cells (WBC)

⁷ RBC, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), WBC, prothrombin time (PT), activated partial thromboplastin time (APTT), and differential leukocyte count

⁸ [serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose, triglycerides, total cholesterol, total bilirubin, blood urea nitrogen (BUN), creatinine, calcium (Ca⁺), Na⁺, K⁺, Cl⁻, total protein (TP), albumin/globulin ratio (A/G), and protein fraction]

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groups treated with GOS. The mean weight of the cecum in male rats treated with 2,000 mg GOS/kg body weight/day was significantly greater (+20%; $P \leq 0.05$) than that of the control male rats; similar changes in cecum weights were not reported in the female. In addition, there were no histopathological abnormalities observed in the cecums of this dose-group, and thus the change was considered a physiological reaction to the intake of GOS and not of toxicological significance. Based on the above results, the NOAEL was 2,000 mg GOS/kg body weight/day, the highest dose tested.

A 90-day repeated dose toxicity study was conducted in male and female Sprague-Dawley Crl:CD (SD) IGS BR rats (6 weeks of age; 229.3 ± 11.15 g for males and 160.7 ± 10.44 g for females) administered a GOS product (Vivinal GOS; Friesland Foods, The Netherlands) (Anthony *et al.*, 2006). Animals were housed individually under standard experimental conditions. The test article (GOS) was provided as a syrup and was reported to contain 45% GOS (the majority of which are tri-saccharides and higher), 15% lactose and 10% glucose⁹. Rats were divided into 4 groups, each consisting of 15 males and 15 females, and 1 of 4 treatments: reverse osmosis deionized water (control); FOS reference control; 2,500 or 5,000 mg of GOS/kg body weight/day *via* gavage for a period of 89 to 91 days. Daily doses were adjusted based on the most recent body weight determinations. The authors reported that the low and high doses groups received 2,000 and 5,000 mg GOS/kg body weight/day. The FOS reference control was formulated to match the oligosaccharide and digestible sugars content of the high-dose GOS test material. Throughout the study, animals were routinely observed for signs of toxicity 2 hours following each dose, twice daily for general health status and signs of moribundity, and once weekly and just prior to sacrifice, detailed examinations were performed. Animal body weights were recorded on Day 1, weekly, and just prior to sacrifice, and food consumption was recorded weekly. Ophthalmologic examinations were conducted on Day 2 and Day 83 by a veterinary ophthalmologist. Blood was collected from the orbital plexus after an overnight fast and just prior to euthanasia for hematological¹⁰ and clinical chemistry¹¹ evaluations. Urinalysis (bilirubin, blood, glucose, ketones, leucocytes, nitrites, protein, specific gravity, urobilinogen) was performed on urine samples collected overnight prior to blood collection. After sacrifice, gross necropsy examinations were performed on all animals and included examination of external surfaces, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents. The weights of the adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, and uterus were recorded (paired organs were

⁹ Based on information obtained from the literature the GOS test article used in the study was produced from lactose using galactosidase derived from *B. circulans*, and was considered representative of GTC Nutrition's GOS (Goulas *et al.*, 2002; Montilla *et al.*, 2006; Anthony *et al.*, 2006; U.S.-FDA GRAS Notification 000238).

¹⁰ RBC, Hct, Hb, MCH, MCHC, MCV, platelet count, reticulocyte count, total different leukocyte counts, APTT, PT, fibrinogen

¹¹ ALT, albumin, A/G ratio, ALP, AST, blood creatinine, BUN, Ca+, cholesterol, Na+, K+, Cl-, gamma glutamyl transpeptidase (GGT), globulin, glucose, phosphorus, total bilirubin, total serum protein

weighed together) for all animals. Histological examinations¹² were performed on all animals in the control group and the high-dose group, while histological examinations were only performed on the lungs, liver, and kidneys in the FOS control group and the low-dose group.

No deaths attributable to the test substance were reported; however, 2 females in the high-dose group died from gavage error, which was confirmed by the presence of perforation of the esophagus, fluid in the thoracic cavity, and dark red lobes in the lungs upon necropsy. Sporadic, non-dose dependent clinical abnormalities were observed, including hair loss, ocular discharge, dark material around the eyes and nose, malalignment, trimmed incisor(s) and broken incisor(s); however, these effects were not considered to be toxicologically significant. There were no significant differences in body weights or ophthalmologic examinations between the animals treated with GOS and either of the control groups. Mean food consumption was decreased at several time points in all groups treated with GOS except for the males receiving 2,500 mg/kg body weight/day when compared with the water controls; however, when compared to the FOS controls, there were no significant differences in mean food consumption in either sex in either dose-group. Hematological parameters were not significantly different in the GOS-treated rats when compared to the control groups, except for hemoglobin and hematocrit counts in the high-dose females, which were significantly higher than in the FOS controls. Clinical chemistry values also were not significantly different from the control groups, except for a significant decrease in ALT in the high-dose males when compared to the FOS controls. These differences were not dose-related and were within intralaboratory historical control data. Sporadic differences in urinalysis results (*i.e.*, mean specific gravity values) also occurred, but once again, were inconsistent and not dose-related. Similarly, sporadic, inconsistent, non-dose related changes in absolute and relative organ weights (*e.g.*, spleen, ovaries, liver) were reported, but were considered to be of no toxicological significance. No gross abnormalities were observed in the necropsy, and no test-article-related changes were reported from the histopathological examinations. In summary, there were no adverse toxicological effects attributable to GOS when administered to rats by gavage at doses of 2,500 or 5,000 mg/kg body weight/day for a period of 90 days. The authors determined a NOAEL of 5,000 mg/kg body weight/day, the highest dose administered.

The effects of oral GOS administration was examined in constipated dogs (n=6, species not specified) and cats (n=4, species not specified) for 30 days (Ohtsuka *et al.*, 1995). The dogs and cats were administered 100 mg GOS/kg body weight/day and were observed for side effects, changes in clinical chemistry (*e.g.*, levels of glucose, blood urea nitrogen, creatinine, liver enzymes, cholesterol, minerals, vitamins, and enzymes), palatability, and effect on fecal characteristics. No side effects were reported. The only effect attributable to GOS consumption

¹² Genital organs, adrenals, aorta, brain, cecum, colon, duodenum, esophagus, exorbital lachrymal glands, eyes, femur and bone marrow, ileum, jejunum, kidneys, liver, lungs with bronchi, mammary glands, lymph nodes, nasal cavity, pancreas, peripheral sciatic nerve, pituitary, prostate, rectum, skeletal muscle, skin, spinal cord, spleen, stomach, submaxillary salivary gland, testes/ovaries, tongue, trachea, and bladder.

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was the presence of softer stools in dogs and cats administered 100 mg/kg body weight/day of GOS.

A summary of the available animal toxicity studies is presented in Table IV.F.ii-1.

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Species Strain, No./Sex/Group	Study Duration	Administration Route	Test Item	Dose of GOS (mg/kg body weight/day)	NOAEL (mg/kg bw)	Observations	Reference
Rat [Sprague-Dawley Crj:CD (SD) IGS BR, 15/sex/group] 6 weeks of age	89 to 91 days	Gavage	GOS syrup (Friesland Foods, The Netherlands) Synthesized from lactose via β -galactosidase from <i>Bacillus circulans</i>	2,500 or 5,000	5,000	No significant adverse toxicological effects attributable to GOS NOAEL was 5,000 mg/kg body weight/day	Anthony <i>et al.</i> , 2006
Rat [Crj:CD (SD) IGS, 10/sex/group Male control and female high-dose groups n=9 ~200 g	90 days	Gavage	GOS (Yakult, Japan) Synthesized from lactose via β -galactosidases from <i>Aspergillus oryzae</i> and <i>Streptococcus thermophilus</i>	500, 1,000, or 2,000	2,000	↑ cecum weight in high-dose males	Kobayashi <i>et al.</i> , 2003
Dog (n = 6) Cat (n =4)	30 days	Oral (not specified as to in the diet or via gavage)	GOS Synthesized from lactose via transglycosylation activity of <i>Cryptococcus laurentii</i>	100	100 ²	No side effects were reported	Ohtsuka <i>et al.</i> , 1995
Rat Sprague-Dawley 6 Males/group ~50 g	6 weeks	Diet	GOS Synthesized from lactose via transglycosylation activity of <i>Cryptococcus laurentii</i>	~5,000 or 10,000 ¹	10,000 ²	Transient (2 weeks) diarrhea in high-dose group Dose-related significant increase in relative cecum weight Non-dose related significant increase in colon weight	Ohtsuka <i>et al.</i> , 1990

¹ Calculated using U.S. FDA, 1993

² Studies not conducted in-line with OECD or U.S-FDA Redbook guidelines for toxicity testing in animals.

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G. Mutagenicity and Genotoxicity Studies

Yasutake *et al.* (2003) investigated the genotoxicity of a GOS product under a standard genotoxicity battery: bacterial reverse mutation assay, a mouse micronucleus test, and a mammalian chromosomal aberration test. The GOS test material was produced from lactose via a 2-step process using *Sporobolomyces singularis* and β -galactoside from *Kluyveromyces lactis*.

The bacterial reverse mutation assay was conducted using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain WP2 *uvrA*. GOS was added at concentrations of 312.5, 625, 1,250, 2,500, or 5,000 $\mu\text{g}/\text{plate}$ both with and without metabolic activation by S9 mix. No mutagenic response was reported at any of the concentrations tested, up to 5,000 μg GOS/plate, either with or without metabolic activation (Yasutake *et al.*, 2003).

Crj:CD-1(1CR) mice were divided into 4 groups of 6 and GOS was administered as single oral doses 500, 1,000, or 2,000 mg/kg body weight in 10 mL volumes. Peripheral blood cells were collected from the tail vein and examined for the appearance of micronuclei at 48 and 72 hours. There were no significant differences in the number of micronucleated reticulocytes or the ratio of reticulocytes to total erythrocytes at any of the doses at either time point in the mice treated with GOS compared to the controls (Yasutake *et al.*, 2003).

Cultured mammalian cells (CHL/IU) were treated with 1,250, 2,500, or 5,000 μg GOS/mL either in the presence or absence of metabolic activation (S9 mix) for 6 hours and recovered after 18 hours, or were treated for 24 or 48 hours in the absence of metabolic activation and were recovered at the end of their respective treatment periods. There were no significant increases in the frequency of cells with structural or numerical aberrations or significant decreases in the cell proliferation ratios at any dose, whether with or without metabolic activation (Yasutake *et al.*, 2003). A summary of the identified genetic toxicity studies of GOS is presented in Table IV.G-1.

Table IV.G-1 Summary of Identified Genetic Toxicity Studies on GOS				
Test System	Type	Result	Concentration	Reference
<i>In vitro System</i>				
<i>Salmonella typhimurium</i> TA98, TA100, TA1535, and TA1537	mut ^a (+/-S9) ^b	Negative	312.5, 625, 1,250, 2,500, or 5,000 µg/plate	Yasutake <i>et al.</i> , 2003
<i>Escherichia coli</i> WP2 <i>uvrA</i>	mut(+/-S9)	Negative	312.5, 625, 1,250, 2,500, or 5,000 µg/plate	Yasutake <i>et al.</i> , 2003
Chinese hamster fibroblast cells	CA ^c (+/-S9)	Negative	1,250, 2,500, or 5,000 µg/mL	Yasutake <i>et al.</i> , 2003
<i>In vivo System</i>				
Mouse micronucleus assay	MR ^d , ret:RBC ^e	Negative	500, 1,000, or 2,000 mg/kg bw	Yasutake <i>et al.</i> , 2003

^a mutation

^b with or without metabolic activation

^c chromosomal aberration

^d micronucleated reticulocytes

^e ratio of reticulocytes to total erythrocytes

H. Additional Relevant Animal Studies

Two studies were identified reporting the repeat dose administration of GOS at high dietary concentrations for long durations. Although these studies were designed to investigate the effects of GOS on tumor growth, several safety related endpoints were obtained during the experiments; therefore these studies are reviewed below as additional supporting information.

Appel *et al.* (1997) examined the effect of dietary GOS on azaserine-induced pancreatic carcinogenesis and azaserine-induced fat-promoted pancreatic tumor development in male Wistar rats. The rats were injected with azaserine prior to weaning and at weaning were divided into 4 groups of 39 and fed a low fat-low GOS, low fat-high GOS, high fat-low GOS, or high fat-high GOS diet. GOS (Friesland Foods, Netherlands) was added to the diets as a syrup, and contained on dry matter basis, 58.8% GOS, 21.3% lactose, 19.3% glucose, and 1.1% galactose. The resulting GOS content of the diets ranged from 3 to 10% GOS. Consumption of these diets was estimated to result in GOS exposures of approximately 1500 to 3000 mg of GOS/kg body weight/day throughout the experiment among animals in the lowest GOS group (U.S. FDA, 1993). Similarly, intakes of GOS for animals randomized to the high dose group were estimated to be approximately 5,000 to 10,000 mg/kg body weight/day. Nine (9) rats per group were sacrificed at 6 months, and the remaining 30 rats per group were sacrificed at 12 months. At sacrifice, the pancreas and cecum were weighed, and the pancreas was examined for neoplastic changes. Dietary GOS did not affect food intake or body weight; however, high-dose GOS did result in a significant increase in the absolute and relative weight of the cecum. The authors concluded that dietary GOS did not modulate pancreatic carcinogenesis in

azaserine-treated rats. The authors also concluded that dietary GOS did not have an effect on the pancreatic tumor-promoting capability of a high-fat diet.

Wijnands *et al.* (1999) examined induced colorectal cancer development in rats fed a diet containing high or low levels of cellulose, or high or low levels of GOS, combined with low, medium, or high levels of dietary fat. Male specific pathogen-free Wistar WU rats [Cri:(WI)WU BR] were divided into groups of 39 and fed 1 of the 12 different diet combinations for 9 months (see Table IV.H-1). The low GOS diets contained GOS at a concentration of approximately 3% resulting in approximate GOS exposures of between 1500 to 3000 mg/kg body weight throughout the experiment (U.S-FDA, 1993). Rodents in the high-dose group consumed GOS in the diet at 10% resulting in approximate intakes of between 5,000 to 10,000 mg/kg body weight/day of GOS over the study period. The GOS syrup (Friesland Foods, Netherlands) contained 75% weight dry substance consisting of 58.8% GOS, 21.3% lactose, 19.3% glucose, and 1.1% galactose. Rats also were administered 1,2-dimethylhydrazine to induce tumor formation. Animals were monitored for clinical signs and body weights and food intake were recorded on a regular basis. After sacrifice, a thorough autopsy was performed and the colon was examined for neoplastic changes. Energy intake, and as a result terminal body weight, varied slightly between the treatment groups. Although rats receiving the high GOS diets had slightly softer feces, no incidences of diarrhea were reported. A trend for a decrease in tumor incidence was reported in the rats receiving the high GOS diet compared to those receiving the low-GOS diet, but this was not significant. The authors “concluded that GOS conferred a greater protection against colorectal cancer than did the diets containing non-fermentable cellulose.”

Diet Group	GOS¹ Content (wt%)	Cellulose Content (wt%)
LF/LC	-	4.5
LF/HC	-	23.5
MF/LC	-	4.8
MF/HC	-	22.6
HF/LC	-	5.2
HF/LF	-	24.5
LF/LGOS	3.1	-
LF/HGOS	10.3	-
MF/LGOS	3.3	-
MF/HGOS	10.0	-
HF/LGOS	3.6	-
HF/HGOS	10.8	-

LF, MF, and HF are low-, medium, and high-fat, respectively; LC and HC are low- and high-cellulose, respectively; and LGOS and HGOS are low- and high-GOS, respectively.

¹ GOS syrup contained approximately 75% weight dry substance consisting of 58.8% GOS, 21.3% lactose, 19.3% glucose, and 1.1% galactose.

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Wijnands *et al.* (2001) further investigated the development of aberrant crypt foci (ACF) and colorectal tumors in azoxymethane-treated rats fed a low- or high-GOS diet. Male specific-pathogen free Fischer 344 rats were divided into 2 groups of 102 animals each and fed a diet containing 1.91 or 7.64% w/w GOS syrup. The composition of the GOS syrup was as described in Wijnands *et al.* (1999), resulting in similar GOS intakes as described above. At Week 7, 18 rats from each group were sacrificed for ACF scoring and half of the remaining rats in each treatment group were switched to the alternate treatment, resulting in 4 treatment groups. At Week 13, 9 rats from each of the 4 treatment groups were sacrificed. The remaining rats continued on their diets until they were sacrificed 10 months after the start of the experiment. Food intake, body weight, and clinical signs were recorded regularly. Animals that were sacrificed at 10 months underwent a thorough autopsy and their colons were examined for the presence of neoplastic changes. Food consumption, calculated energy intake, and body weight were slightly increased in the rats receiving the low-GOS diet. The authors concluded that GOS is protective “against the development of colorectal tumours in rats”.

I. Human Studies

Infant formula's supplemented with GOS and FOS have been widely used in Europe for a number of years. As a result of patents owned by the Royal Numico company (Numico) pertaining to the use of GOS and long-chain FOS at a ratio of 9:1 in food, Numico has a significant world-wide marketshare for the use of its GOS:FOS ingredient. Based on the widespread use of Numico's GOS:FOS ingredient, and due to the extensive publication activities of Numico Research, the vast majority of studies conducted in humans are conducted with Numico's proprietary GOS:FOS mixture. Numico does not manufacture its own GOS, and as reported in the literature, Numico's GOS is sourced from Friesland foods (Vivinal GOS) and its FOS obtained from Orafti Active Food Ingredients (Raftiline HP FOS) (Scholtens *et al.*, 2006; Bakker-Zierikee, 2005a,b). In addition, given Numico's patent protection on the use of GOS:FOS in a ratio of 9:1, studies reporting the use of GOS:FOS in this ratio should be assumed to be obtained from Numico. Therefore, as discussed in Section IV, studies conducted with this material were considered to be representative of GTC Nutrition's GOS and can be considered applicable to the safety of their ingredient.

(i) Adults

Nineteen (19) studies – 17 studies in adults and 2 studies conducted in children – were obtained from the public domain containing data relevant to the safety evaluation of GOS under the intended uses described herein; although none of these studies contained standard safety measurements as primary endpoints, parameters related to tolerance (flatulence, bloating, abdominal cramping, *etc.*) and adverse event monitoring were reported in these investigations¹³. The majority of these studies were conducted in healthy adults, and typical intakes of GOS are between 5 to 15 g per person per day for periods of between 1 to 3 weeks. Studies reporting acute GOS intakes of 20 to 30 g/person also are reported in the literature (Tanaka *et al.*, 1983; Van den Heuvel *et al.*, 2000). In three studies intakes of GOS between 5.5 to 10 g are reported to be well tolerated without adverse events for durations of between one to 2.5 months (Ito *et al.*, 1990; Shadid *et al.*, 2007; Vulevic *et al.*, 2008; Silk *et al.*, 2008). A summary of relevant studies obtained from the literature are presented in the following paragraphs and in Table IV.H-1 below.

Matsumoto *et al.* (2004) reported the effect of GOS supplementation on various microbial metabolic indices in 22 healthy volunteers consuming 2.5 or 5.0 g of GOS per day over a 2-week interval. No increases in fecal ammonia, indole, phenol, p-cresol, β -glucuronidase were

¹³ Ito *et al.*, 1990; Ishikawa *et al.*, 1995; Bouhnik *et al.*, 1997; Deguchi *et al.*, 1997; Teuri and Korpela, 1998; Teuri *et al.*, 1998; Alles *et al.*, 1999; van Dokkum *et al.*, 1999; van den Heuvel *et al.*, 2000; Alander *et al.*, 2001; Satokari *et al.*, 2001; Scholtens *et al.*, 2006; Bouhnik *et al.*, 2004; Matsumoto *et al.*, 2004; Sazawal *et al.*, 2004; Shadid *et al.*, 2007; Depeint *et al.*, 2008; Silk *et al.*, 2008; Vulevic *et al.*, 2008.

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reported at the end of treatment relative to baseline. Also, no change in 1° or 2° bile acids were noted.

The long-term consumption of a GOS supplemented milk in children (1 to 3 years of age) was investigated by Sazawal *et al.* (2004). Six hundred and thirty four (634) children selected from a peri-urban population in south Delhi were randomized to receive either unsupplemented milk or milk supplemented with GOS (2.4 g/100 g) and *B. lactis* HN019 (10^7 to 10^8 CFU/100 g) on a daily basis for up to one year. General health, growth, iron status, and hematological parameters were evaluated during the study. Children consuming the GOS supplemented milk had better growth at 6 and 12 months, and improved iron status. Significant reductions in the incidence of blood diarrhea and non-significant reductions in diarrhea were reported.

Overall, no reports of adverse or severe adverse events attributable to the consumption of GOS were reported in the literature, which included several studies administering GOS at intakes (10 to 20 g/person) that are comparable to the estimated GOS exposure under the proposed uses of GTC Nutrition's GOS. Among the studies that included tolerance endpoints, side-effects are limited to reports of flatulence when GOS is consumed on a repeat basis at quantities of between 10 to 15 grams (Ito *et al.*, 1990; Deguchi *et al.*, 1997; Teuri *et al.*, 1998; Alles *et al.*, 1999; Ito *et al.*, 1990); however, this effect is not consistently reported in all studies at these intakes (Bouhnik *et al.*, 1997; Teuri and Korpela, 1998; van Dokkum *et al.*, 1999; Bouhnik *et al.*, 2004; Shadid *et al.*, 2007). Similar observations of increased flatulence have been reported following the consumption fructooligosaccharides (15 g) over a 7-day period (Alles *et al.*, 1996), and is an effect that is expected in association with the consumption of indigestible fiber in large quantities. The results of these studies indicate that the consumption of GOS under the proposed uses of GTC Nutrition's GOS will be well tolerated.

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
Randomized, blind (single or double not reported), crossover trial	59 healthy volunteers Age: 28 to 41	Treatment: <i>Phase I:</i> 7 g/day [Vegetable fat-filled milk powder] <i>Phase II:</i> 3.6 or 7 g/day [Vegetable fat-filled milk powder] Control: [Vegetable fat-filled milk powder]	7 days 7 day washout	-Fecal evaluation	<i>Phase I:</i> - ↑ fecal vifidobacteria -No adverse symptoms reported <i>Phase II:</i> -↑ bifidobacteria -No adverse effects in low-dose group -2 volunteers in the high-dose group reported abdominal discomfort and diarrhea	Depeint <i>et al.</i> , 2008
Randomized, single-blind, placebo controlled, cross-over trial	42 patients with Irritable Bowel Syndrome (IBS) Age: 20 to 79	Treatment: 3.5 or 7.0 g/day [water] Control: Maltodextrin [water]	4 weeks 2 week washout	-Bowel movement frequency -stool assessment -Adverse events	-↑ bifidobacterium -↓ <i>C. perfringes</i> subgroup <i>histolyticum</i> -significant improvement in abdominal discomfort, stool consistency, and quality of life -1 individual reported increase in diarrhea and 2 individuals reported nausea	Silk <i>et al.</i> , 2008
Randomized, double-blind, placebo-	44 volunteers Age: 64 to 79 yrs	Treatment: 5.5 g/day	10 weeks 4 week	- analysis of fecal samples	-↑ <i>Bifidobaterium</i> ,, <i>Lactobacills-Enterococcus</i> and <i>C. coccoides-E. rectale</i>	Vulevic <i>et al.</i> , 2008

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
controlled, crossover trial		[water] Control: Maltodextrin [water]	washout period	-blood samples, blood pressure, weight, use of medication -adverse events	-↓ <i>Bacteroides</i> , <i>C. histolyticum</i> , <i>E. coli</i> , and <i>Desulfovibrio</i>	
Randomized, double-blind, placebo-controlled trial	48 pregnant women Age: 22 to 48 yrs	Treatment: 9 g/day (GOS:FOS; 9:1) [added to milk or water] Control: Maltodextrin [added to milk or water]	Wk 25 gestation until delivery (~12 wks)	-Stool characteristics - Infant immunity - Digestive tolerance adverse effect monitoring	-Bifidobacteria increased in maternal gut - No effect on lactobacillus - No effect on fetal bacteria counts or immunity - No major side-effects and overall tolerance was good	Shadid <i>et al.</i> , 2007
Randomized, double-blind, placebo-controlled trial	20 children Age: 4 to 6 months	Treatment: 4.05 g/day (Friesland Foods, Netherlands) [weaning products (solid food)] Control: Maltodextrin	6 weeks	-Stool frequency, stool consistency, -gastrointestinal symptoms, -microbial analysis, pH, and SCFA content	- Significant ↑ in the percentage of <i>Bifidobacteria</i> in GOS supplemented infants compared to baseline - ↓ in % butyrate in GOS supplemented infants, but no significant difference compared to control group - No significant differences in SCFAs or pH between the GOS and control groups - No change in stool	Scholtens <i>et al.</i> , 2006

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
					consistency or frequency - No adverse events were reported	
Randomized, double-blind, placebo-controlled trial	<p><i>Phase I:</i> 64 volunteers (8/group)</p> <p><i>Phase II:</i> 136 volunteers (32/group except placebo with 8 volunteers)</p> <p>Age: 18 to 54 yrs</p>	<p>Treatment:</p> <p><i>Phase I:</i> 10 g/d</p> <p>(GOS: Nissin Sugar),</p> <p><i>Phase II:</i> 0, 2.5, 5.0, 7.5, or 10 g/d</p> <p>Control:</p> <p>Sucrose:Maltodextrin (1:1)</p>	<p><i>Phase I:</i> 7-day</p> <p><i>Phase II:</i> 7-day</p>	<p>- Digestive tolerance</p> <p>- Fecal bacteria counts</p>	<p><i>Phase I:</i></p> <p>- GOS, resulted in significantly higher fecal <i>Bifidobacteria</i> counts on Day 15 relative to placebo</p> <p>- Reports of Excess flatus, bloating, borborygmi, and abdominal pain were increased over time in all groups, however effect not significant relative to placebo group.</p> <p>- Diarrhea not reported in any groups</p> <p><i>Phase II:</i></p> <p>- Significantly higher fecal bifidobacteria counts relative to baseline</p> <p>- Significant increase in excess flatus, bloating, and abdominal pain relative to baseline.</p> <p>Diarrhea not reported in any groups</p>	Bouhnik <i>et al.</i> , 2004

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
Randomized, placebo-controlled trial (blinding not reported)	634 children Age: 1 to 3 yrs	Treatment: 2.4 g/day (GOS source not reported) [consumed as milk drink, also containing <i>B. lactis</i> HN019] Control: [Milk]	12 months	General health growth	- GOS consumption associated with ↓ incidence of dysentery, severe illness days, days with fever, ear infections - GOS consumption resulted in better growth at 6 months and 1 year - Higher weight gain in GOS group - ↓ number of iron deficient children in GOS group	Sazawal <i>et al.</i> , 2004
Not reported	22 healthy volunteers Age: 29 to 49	Treatment: 2.5 or 5 g/day [GOS source not reported] Control: Not reported	14 days	-Microflora analysis, organic acid concentration, pH, water content, ammonia, phenols, β-glucuronidase activity, bile acid concentrations	- No changes in fecal pH, water content, ammonia, phenols, and β-glucuronidase activity	Matsumoto <i>et al.</i> , 2004
Randomized, single-blind, cross-over trial (sample handlers blinded, volunteers not blinded)	30 healthy volunteers Age: 22 to 47 yrs	Treatment: 4.05 g/day (GOS source not reported) [yogurt] Control: [Plain yogurt]	14 days	-Fecal microflora	- ↓ <i>Clostridium perfringens</i> - One volunteer in GOS group reported pain from intestinal bloating, and one started antibiotic therapy; otherwise, GOS was well-tolerated	Alander <i>et al.</i> , 2001

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
Randomized, single blind, crossover trial	30 healthy adults Age: 22 to 47 yrs (3 M, 27 F)	Treatment: 8.1 g/day (Friesland Foods, Netherlands) [in yogurt] Control: [Plain yogurt]	14 days	- <i>Bifidobacterium</i> population	- No effect on the qualitative composition of indigenous <i>Bifidobacterium</i> populations. - One subject reported abdominal discomfort during the feeding trial; however, Vivinal GOS was well tolerated in all other subjects.	Satokari <i>et al.</i> , 2001
Randomized, double blind, crossover trial	12 postmenopausal women Age: 55 to 65 yrs	Treatment: Days 1,2: 10 g Days 3,4: 15 g Days 5 - 9: 20 g (Friesland Foods, Netherlands) [200 mL yogurt drink] Control: [Plain yogurt]	9 days 19 day washout	-Calcium absorption - Gastrointestinal effects	- Calcium absorption increased by 16% in treated women - No adverse events were reported (gastrointestinal effects or changes in stools)	van den Heuvel <i>et al.</i> , 2000
Blinded (single or double and randomization not reported), placebo-controlled, parallel trial	40 healthy adults Age: 18 to 58 (22 M, 18 F)	Treatment: 0, 8.5, or 14.4 g/day (Friesland Foods, Netherlands) [Added to fruit juice]	21 days	-Defecation frequency Signs of illness Gastrointestinal complaints Fecal parameters (weight, pH, SCFA, bile acids, nitrogen, TOS, ammonia, indoles,	- At 14.4 g/day: increase fecal nitrogen density, and breath hydrogen - Some volunteers in the low-GOS and high-GOS groups reported flatulence	Alles <i>et al.</i> , 1999

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
		Control: [fruit juice]		skatole) Microbiological analysis of feces	- No effect on mean fecal concentrations of ammonia, indoles, and skatoles.	
Randomized, Latin square, double blind, diet-controlled, crossover trial	12 healthy males Age: 20 to 26 yrs	Treatment; 15 g/day (GOS source not reported) [Added to orange juice] Control: [Orange juice]	21 days	-Subjective evaluation of gastrointestinal complaints, intestinal transit time, amount of hydrogen in expired air, fecal pH, concentration and composition of SCFAs, concentration and composition of fecal bile acids, fecal enzyme activity, blood lipids, and blood glucose levels	- ↓ percentage of fecal dry weight - ↑ concentration of acetic acid - No change in terms of intestinal transit time, fecal pH, amount of hydrogen in expired air, fecal bile acids, fecal neutral steroids, fecal enzyme activity, blood lipid concentrations, and blood glucose levels - GOS was reported to be well-tolerated and no adverse events were reported	van Dokkum <i>et al.</i> , 1999
Intervention trial, not placebo-controlled, not randomized (1-week run-in period)	Phase I: 12 healthy volunteers Age: 25 to 55 yrs (3 M, 9 F)	Treatment: 15 g/day [added to yoghurt] Control: [yoghurt]	2 weeks	-Frequency of defecation -Subjective assessments of appetite, flatulence, loose stools, diarrhea, hard stools, abdominal distension, any other abdominal symptoms	- ↑ frequency of defecation - ↑ flatulence - ↑ sum of GI symptoms	Teuri <i>et al.</i> , 1998

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
	<i>Phase II:</i> 6 of the 12 from the first phase			-Fecal samples (1 day): Total lactic acid bacteria, bifidobacteria, pH	- ↑ total number of anaerobes (medium was not selective enough for total lactic acid bacteria)	
Randomized, double blind, , placebo- controlled cross-over trial	14 elderly females Age: 69 to 87 yrs	Treatment: 9 g/day (Friesland, Netherlands) [Added to yogurt] Control: [Yogurt]	14 days No washout period	-Defecation frequency -Consistency of stools -Ease of defecation -GI symptoms (flatulence, abdominal pain, abdominal distension) -Fecal pH	- ↑ frequency of defecation - No adverse events were reported	Teuri and Korpela, 1998
Not placebo- controlled, further details not reported	8 healthy volunteers Age: 20 to 32 yrs (4 M, 4 F)	Treatment: 10 g/day (GOS source unknown) [powder] Control: NA	21 days	-Stool weight, fecal water, fecal pH, hydrogen excretion, methane production, fecal concentrations of <i>Bifidobacteria</i> and <i>Enterobacteria</i> -Tolerance (gas, bloating, borborygmi, abdominal pains)	- ↑ hydrogen production - ↑ <i>Bifidobacteria</i> numbers - No changes with respect to other variables studied. - No adverse events were reported.	Bouhnik <i>et</i> <i>al.</i> , 1997
Survey	78 healthy females Age: not reported in abstract	Treatment: 2.5 or 5.0 g/day [beverage]	7 days	-Constipation symptoms	At 5 g/day: - ↑ defecation frequency - Softer feces -No adverse events	Deguchi <i>et</i> <i>al.</i> , 1997

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
		Control: NA			reported	
	50 healthy adults, Age: not reported in abstract (9 M, 41 F)	Treatment: 10 g/day [beverage] Control: NA	7 days	-Constipation symptoms	- ↑ defecation frequency - Softer feces - ↑ flatus and bowel movement frequency - No adverse events reported	
Open-label feeding trial	20 males Age: not reported	Treatment: 2.5 or 10 g/day (GOS source not reported) Control: NA	3 weeks	-Fecal microflora content, fecal bile acids, SCFAs, fecal pH, stool weight, water content, ammonia, phenols, β-glucuronidase activity	In 2.5 g/d group: - ↑ <i>Bifidobacterium</i> was the only reported changes in all parameters tested In 10 g/d group: - ↑ <i>Bifidobacterium</i> and <i>Staphylococci</i> -↓ total SCFA - No changes were reported in any of the other parameters tested - No adverse events reported.	Ishikawa <i>et al.</i> , 1995
Randomized, Latin square, Single blind, crossover trial	12 healthy males, Age: 26 to 48 yrs	Treatment: 0, 2.5, 5.0, or 10.0 g/day	7 days	-Colonic function and fecal microflora -Fullness	- Dose-dependent ↑ of <i>Bifidobacteria</i> and <i>Lactobacilli</i>	Ito <i>et al.</i> , 1990

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Table IV.H-1 Summary of Galactooligosaccharides Supplementation Studies in Adults and Children: Studies Containing Relevant Safety Endpoints						
Study Design	Subjects	Dose in g/day (source of GOS) [vehicle of administration]	Duration of Treatment	Variables Studied	Results and Adverse Events	Reference
		(Oligomate-50) [Added to apple juice] Control: [Apple juice]		-Flatulence Abdominal pain	- No changes in stool weight or frequency - No adverse event reported at any of the doses - Subjects administered the highest dose reported a greater sensation of fullness, flatulence, and abdominal pain in comparison with the other dose groups.	

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(ii) Infants

As discussed in Section IV.B, human milk is reported to contain significant quantities of oligosaccharides of similar chemical structure to GOS. The physiological role of oligosaccharides in human milk is unclear, however, these compounds are believed to impart nutritional effects in the infant related to the development of immunity and maintenance of stool consistency; therefore, to date, the consumption of GOS by infants has been extensively evaluated. Twenty-two (22) studies that included various safety endpoints from tolerance related parameters (e.g., crying, regurgitation, and vomiting) to complete anthropometric monitoring were obtained during a search of the public domain¹⁴. This information as reviewed in this exemption claim is not included to support the use of GOS in infant formula; however, it was considered relevant to the safety evaluation of GOS under the intended uses described herein.

Collectively, GOS supplemented infant formula has been administered to 1801 infants for a total of 9,271 subject years at concentrations of up to 9 g/L (~5.6 g GOS/kg body weight)¹⁵ without reports of adverse events, and overall has been reported to be well tolerated in these subjects. The longest duration of GOS consumption was reported by Moro *et al.* (2006), where 102 infants consumed an infant formula containing GOS at a concentration of 7.2 g/L/day (~2.2 g GOS/kg body weight)¹⁶ for a period of 6 months. No adverse events, tolerance issues, or adverse effects on weight gain were observed. Furthermore, these subjects were followed up for an additional 18 months without evidence of adverse effects attributable to GOS consumption (Arslanoglu *et al.*, 2008). To date, no reports of adverse effects on anthropometric indices of growth or effects on water balance leading to diarrhea have been reported.

J. Information Pertaining to the Safety of the Bacterial Enzymes

GTC Nutrition's GOS is produced from lactose in a process catalyzed by β -galactosidase derived from *B. circulans* LOB 377. *B. circulans* is a member of the *Bacillus* genus that contains a large number of bacterial strains used for industrial production of a number of enzymes applicable to food production (Schallmeyer *et al.*, 2004). A summary of the current taxonomic assignment (based on the 8 kingdom classification scheme) of *B. circulans* is presented in Table IV.J-1.

¹⁴ Boehm *et al.*, 2002; Moro *et al.*, 2002, 2003, 2006; Boehm *et al.*, 2003; Savino *et al.*, 2003; Schmelzle *et al.*, 2003; Ben *et al.*, 2004, 2008; Bakker-Zierikzee *et al.*, 2005, 2006; Fanaro *et al.*, 2005; Haarman and Knol, 2005; Knol *et al.*, 2005; Rinne *et al.*, 2005; Haarman and Knol, 2006; Mihatsch *et al.*, 2006; Savino *et al.*, 2006; Scholtens *et al.*, 2006; Alliet *et al.*, 2007; Arslanoglu *et al.*, 2007; Puccio *et al.*, 2007; Ziegler *et al.*, 2007; Arslanoglu *et al.*, 2008; Chouraqui *et al.*, 2008; Costalos *et al.*, 2008; Magne *et al.*, 2008; Nakamura *et al.*, 2008; Scholtens *et al.*, 2008; Van Hoffen *et al.*, 2008

¹⁵ Based on estimate of 1.6 kg infant consuming 1L of infant formula per day

¹⁶ Based on estimate of 3.3 kg infant consuming 1L of infant formula per day.

Table IV.J-1 Taxonomic Assignment: <i>Bacillus circulans</i> LOB 377 *	
Taxonomy	Taxonomic Assignment
Kingdom	Bacteria
Subkingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	<i>Bacillus</i>
Species	<i>Bacillus circulans</i>
Strain	LOB 377*

*ATTC No. 31382

A search of the literature revealed limited data on the potential pathogenic or toxigenic effects of *B. circulans*, and although foodborne illness from *Bacillus spp.*, in general is rare (with the exception of *Bacillus cereus*), isolates of *B. circulans* have been obtained from blood samples of patients with sepsis (Rowan *et al.*, 2001). In addition, several articles were identified indicating that some species of *B. circulans* contain functional genes encoding enterotoxigenic compounds (Beattie and Williams, 1999; Rowan *et al.*, 2001; Phelps and McKillip, 2002). For example, a strain of *B. circulans* isolated from whole milk was shown to express a number of genes encoding hemolytic and non-hemolytic enterotoxigenic proteins (*hblC*, *hblD*, *hblA*; *nheA*, *nheB*). These genes were also shown to display functional activity as beta-hemolysis was apparent following incubation of the strain on sheep blood agar plates (Phelps and McKillip, 2002). It is important to note that evidence for the presence of virulence factors in some strains of *B. circulans* does not preclude the use of enzymes isolated from the species in the production of food ingredients, but does illustrate the need to properly characterize the safety of the specific *B. circulans* strain.

In the EU, *B. circulans* is an approved source for the enzyme cycloglycosyltransferase used in the production of *beta*-cyclodextrin (Commission Directive 2003/95/EC) (Commission of the European Communities, 2003). The use of GOS in various foods and infant formula produced from lactose using a β -galactosidase, derived from *B. circulans* LOB 377 has recently been notified to FDA without objection from the agency (GRN000236). In addition, enzymes derived from several *Bacillus* species are considered GRAS: α -amylase preparations from *Bacillus licheniformis*; pullulanase enzyme preparations from *Bacillus subtilis* and *Bacillus licheniformis*; and pectate lyase from *Bacillus subtilis* (GRN, 000020, 000022, 000024, 000072, 000079, 0000114, 0000205) (U.S. FDA, 1999a,b, 2000, 2001a,b, 2003, 2006a). In addition, carbohydrase and protease enzyme preparations derived from *Bacillus subtilis* are affirmed as GRAS for use as direct food ingredients (21 CFR §184) (U.S. FDA, 2008), and α -acetolactate decarboxylase from recombinant *Bacillus subtilis* is currently regulated by the FDA under 21

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CFR § 173.115 as a secondary direct food additive permitted for use in food for human consumption (U.S. FDA, 2008).

Importantly, the manufacture of GOS involves a number of extensive purification steps where potential metabolic impurities and/or toxin(s) produced during fermentation are expected to be removed during the activated carbon filtration, ion-exchange and chromatography separation stages. Additional unpublished safety studies supporting that the β -galactosidase is obtained from a non-pathogenic, non-toxicogenic microorganism were provided by the manufacturer of the enzyme, and are presented below as additional corroborating safety data.

In a report by Konuma (2005), the acute toxicity of *B. circulans* LOB 377 was investigated. Thirteen (13) SPF mice (S1c:ICR) of each sex (4 weeks of age; 5/group/sex) were randomly assigned to receive a single gavage inoculation of 0.5 mL of cell culture medium containing 7.0×10^8 CFU of *B. circulans* LOB 377 or 0.5 mL of culture medium alone (control group). The animals were monitored for general health and signs of morbidity for 14 days following the administration of the control or test article. Multiple observations were made on the day of inoculations (*i.e.*, on Day 0 observations were made once prior to inoculation, over 3 continuous hours post-inoculation, twice between 3 and 5 hours post-inoculation) and once a day until the completion of the study at Day 14. In addition to observation of signs of general health and morbidity the mice were weighed periodically throughout the study (*i.e.*, on Day 0, 1, 3, 7, 10, and 14). At necropsy (Day 14) the mice were examined for macroscopic and microscopic abnormalities, while individual organs (*e.g.*, brains, livers, spleens, and kidneys) were sectioned and tested for the number of remaining viable bacteria, as well as examined for histopathological endpoints. Konuma (2005) reported that *B. circulans* had no effect on general health, body weight, macroscopic, microscopic, or histopathological endpoints. No deaths were reported. Konuma (2005) concluded that *B. circulans* was “non-pathogenic *via* oral inoculation” in ICR mice.

Yamaguchi (2005) evaluated the subchronic toxicity of the β -galactosidase enzyme preparation derived from *B. circulans* LOB 377, as used in the manufacture of GTC Nutrition’s GOS ingredient. Groups containing 12 Sprague-Dawley rats of each sex were administered gavage doses of 0 (control), 3,288, 6,575, or 13,150 mg/kg/day of β -galactosidase concentrate for 91 days starting at 6 weeks of age. No deaths or test article effects on body weight, food and water intake, clinical signs, urinalysis, ophthalmological examination, hematological examination, blood chemistry examination, organ weight, necropsy or histological endpoints were reported in any of the groups. Males administered 13,150 mg/kg/day of β -galactosidase (*i.e.*, high-dose group) were reported to have higher salivary gland weights compared with the control group; however, the salivary gland weights were only slightly higher in the high-dose group compared to the control group and no abnormalities were present upon histopathological examination. The slightly higher salivary gland weights in the high-dose group were concluded to be of little toxicological significance. The authors concluded that the “no observed adverse

effect level of the test article under the conditions of this study was estimated to be higher than 13,150 mg/kg/day (potency of the test article: 5200 SU/g) for both males and females”.

The mutagenicity/genotoxicity of the β -galactosidase enzyme preparations derived from *B. circulans* LOB 377 also was evaluated using reverse mutation and chromosome aberration assays (Mochizuki, 2004). Mochizuki (2004) examined the mutagenicity of β -galactosidase against four tester strains of *S. typhimurium* (e.g., TA100, TA98, TA1535, and TA1537) and one tester strain of *E. coli* (i.e., WP2 *uvrA*) in the presence or absence of metabolic activation (i.e., SD rat S9 mix). A dose-range finding test was conducted with 8 concentrations of β -galactosidase (e.g., 0.00305, 0.0122, 0.0488, 0.195, 0.781, 3.13, 12.5, and 50.0 mg/mL) prepared and diluted with water, while 6 concentrations were prepared for the main assay (1.56, 3.13, 6.25, 12.5, 25.0, and 50.0 mg/mL). Water (i.e., the vehicle) was used as the negative control, while 2-aminoanthracene and benzo[a]pyrene were used as positive controls for plates with metabolic activation and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, sodium azide, and 2-methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine dihydrochloride were used as positive controls for plates without metabolic activation. Three plates per test article treatment group were utilized in the dose-range finding and main assay. No growth inhibition of tester strains or precipitation, crystallization or coloration of the medium for any tester strain, in the presence and absence of metabolic activation at any dose level in the dose-range finding test. Furthermore, no increases were reported in the number of revertant colonies for plates treated with 0.305 to 5,000 μ g/plate β -galactosidase (i.e., 0.00305 to 50.0 mg/mL) with or without metabolic activation compared to the control in the dose-range finding test. Similar results were reported for the main assay for plates treated with 156 to 5000 μ g/plate (i.e., 1.56 to 50.0 mg/mL) of β -galactosidase. The study director concluded that the β -galactosidase enzyme preparation “has no reverse mutation inducing activity (negative) on bacteria under the conditions of this study.”

The clastogenicity of β -galactosidase was evaluated in an *in vitro* assay in Chinese hamster lung fibroblast cells incubated with 0 (control), 0.010, 0.020, 0.039, 0.078, 0.156, 0.313, 0.625, 1.250, 2.500, or 5.000 mg/mL of the enzyme concentrate in the absence or presence of metabolic activation (SD rat S9 mix) (Yokota, 2004). Specifically, in the growth inhibition test the cells were incubated with 0 (control), 0.010, 0.020, 0.039, 0.078, 0.156, 0.313, 0.625, 1.250, 2.500, or 5.000 mg/mL (+/-S9), while the doses for the chromosome aberration test were selected based on the results of the growth inhibition test. The negative control group was incubated with water and the positive control group was incubated with mitomycin C (MMC). Clastogenicity was determined based on the incidence (i.e., the percent) of cells with chromosome aberrations according to the criteria of Sofuni *et al.* (1998) where the test is negative, equivocal, or positive when the incidence of chromosome aberrations (e.g., the incidence of structural chromosome aberration and polyploidy cells) are judged to be $\leq 5\%$, $\geq 5\%$, and $< 10\%$, or $\geq 10\%$, respectively. In the cell growth inhibition test (i.e., short-term treatment method with a 6-hour incubation period) the concentration reported to result in 50% cell growth

inhibition was not less than 5.000 mg/mL in the absence (-S9) and presence (+S9) of metabolic activation. In the 24- and 48-hour cell growth inhibition test the 50% cell growth inhibition concentration was reported to be 0.055 and 0.009 mg/mL, respectively. Based on these results, the doses selected for the short-term chromosome aberration test were 1.25, 2.50, and 5.00 mg/mL (+/-S9); 0.020, 0.040, 0.060, and 0.080 mg/mL for the 24-hour continuous treatment method; and 0.0050, 0.0075, 0.0100, 0.0125 mg/mL for the 48-hour continuous treatment method. The incidence of cells with structural chromosome aberrations and the incidence of polyploidy cells following incubation with β -galactosidase were reported to be less than 5% (+/-S9) with the use of the short-term method, as well as with the use of the 24- and 48-hour continuous treatment method. The incidence of cells with structural chromosome aberrations in the short-term assay were 13.5% and 42.5% for cells treated with MMC in the absence and presence of metabolic activation, respectively. The incidence of cells with structural chromosome aberrations or polyploidy in the negative control group was reported to be less than 5% indicating that there were neither spontaneous nor vehicle-related increases in the number of abnormal cells. Similarly, with the use of the 24- and 48-hour methods the incidence of chromosome aberrations and polyploidy were reported to be less than 5% in the negative control group. In the positive control group the incidence of cells with structural chromosome aberrations were 41% and 53% with the use of the 24- and 48-hour method, respectively. Yokota (2004) concluded that the test article, β -galactosidase "was judged to have no clastogenic potential against Chinese hamster lung fibroblasts (CHL/IU) cells under the conditions of the study".

The β -galactosidase preparation also was analyzed for the presence of several mycotoxins including, aflatoxin (B_1 , B_2 , G_1 , and G_2), ochratoxin A, sterigmatocystin, zearalenone, and T-2 toxin), and for antibacterial activity against common bacterial strains [*Staphylococcus aureus* (ATCC 6538), *E. coli* (ATCC 11229), *B. cereus* (ATCC 2), *B. circulans* (ATCC 4516), *Streptococcus pyogenes* (ATCC 12344), and *Serratia marcescens* (ATCC 14041)]. No mycotoxins were identified at the limit of detection for each compound and no evidence of antibacterial activity was observed.

K. Allergy

A single case report of a female individual experiencing an anaphylactic reaction to a beverage containing GOS syrup was identified in the literature (Lee *et al.*, 2004). The 32-year-old female experienced anaphylaxis immediately following the consumption of the beverage containing the GOS syrup. Skin prick testing demonstrated that the individual was sensitized to the entire beverage formulation itself, as well as to the GOS syrup. Lee *et al.* (2004) reported that the GOS syrup contained trace amounts of protein and further testing demonstrated serum specific IgE binding to GOS syrup in an enzyme-linked immunosorbent assay (ELISA). Although the individual was demonstrated to be allergic to the GOS formulation, Lee *et al.* (2004) suggested that the allergic reaction may have resulted from an impurity (*e.g.*, the trace amounts of protein)

rather than the GOS. No further case reports of allergic reactions resulting from the consumption of GOS-containing food products were identified in the literature.

GTC Nutrition's GOS ingredient is manufactured from lactose obtained from a milk source (Whey), thus potential exposure to milk protein in the final product should be considered. Allergy to cow's milk is recognized as one of the most common food allergies (Bush and Hefle, 1996). A number of milk proteins are known to be allergenic or immunogenic to humans, and most humans react to more than one milk protein. Major milk protein allergens include caseins, a family of related proteins with each protein having genetic variants that may be post-translationally modified, as well as β -lactoglobulin a major whey protein that also has genetic variants. Other minor allergens have been identified in milk (e.g., α -lactalbumin and bovine serum albumin), but these proteins are of less concern. The minimum quantity of milk proteins required to produce sensitization or elicit an allergic reaction is not currently known (Bush and Hefle, 1996); however, as reported in the FDA guidance documentation "Approaches to Establish Thresholds for Major Food Allergens and for Gluten in Food" available evidence from clinical trials currently indicate that the lowest-observable-adverse-effect level (LOAEL) for allergic reactions to milk proteins is between 0.36 to 3.6 g of protein (U.S. FDA, 2006b). Thresholds specific to infants were not identified in the literature or public domain. Analytical data for GTC Nutrition's GOS ingredient using the Kjeldahl assay indicate that protein is absent from the product within a detection limit of 10 ppm. GOS is intended for use in various foods at quantities of up to 1.28 g/serving. Based on the absence of protein in the ingredient (detection limit 10 ppm), exposure to trace amounts of milk protein from exposure to GOS under the intended uses in various traditional foods is unlikely to result in allergic responses in milk sensitive individuals. Nevertheless, GTC Nutrition recognizes that all foods containing its GOS ingredient will be required to comply with the Food Allergen Labeling and Consumer Protection act of 2004, where exemption from allergy labeling would require notification to, and approval by, the FDA.

L. Summary and Basis for GRAS

GTC Nutrition intends to market a GOS, produced from lactose, as a food ingredient. The ingredient is manufactured under cGMP using suitable food grade ingredients and processing aids. Analytical data support that GOS is consistently manufactured to suitable food grade specifications, and that GOS are stable compounds with bulk stability studies supporting a shelf-life of at least 6 months. GTC's GOS also was shown to be stable in solution under a variety of temperatures and over a broad pH range supporting the use of GOS as an ingredient under the intended uses in food described herein.

GTC Nutrition's GOS ingredient is produced *via* the transgalactosylation of lactose using the enzyme β -galactosidase isolated from *B. circulans* LOB 377. Based on the widespread use of enzymes derived from strains of *B. circulans* in the U.S. and throughout the world, in conjunction with the extensive purification steps (activated carbon, and cation/anion-exchange

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chromatography) employed during the manufacturing process, the β -galactosidase, derived from *B. circulans* LOB 377 was concluded to be generally recognized as safe for its use in the production of GTC Nutrition's GOS. Similarly, the use of GOS in various foods and infant formula produced from lactose using a β -galactosidase, derived from *B. circulans* LOB 377 has recently been notified to FDA without objection from the agency (GRN000236). Unpublished toxicity studies conducted with enzyme and organism, as well as additional analytical data supporting the non-pathogenicity and non-toxic status of the organism and enzymes derived thereof were provided by the manufacturer of the enzyme, which further corroborate the safe use of the enzyme during the manufacture of GTC Nutrition's GOS.

GTC Nutrition intends to market GOS as a food ingredient in the United States in a variety of food products including baby, infant and toddler foods (excluding infant formula), beverages and beverage bases, dairy product analogs, milk products, bakery products, beverages, cereal and other grain products, desserts, fruit and fruit juices, snacks, soups, and soft and hard candy, at use levels of 0.48 to 12.21% per serving. GTC Nutrition's GOS is not intended for use in meat or poultry containing products. Approximately 100% of the total U.S. population was identified as consumers of GOS from the proposed food-uses (8,168 actual users identified). Consumption of these types of foods by the total U.S. population resulted in estimated mean and 90th percentile all-user intakes of GOS of 9.2 g/person/day (171 mg/kg body weight/day) and 15.4 g/person/day (351 mg/kg body weight/day), respectively (Tables IV.B-1 and IV.B-2). On an individual population basis, the greatest mean and 90th percentile all-user exposures were estimated to occur in male teenagers (aged 12 to 19 years), at 12.1 g/person/day (192 mg/kg body weight/day) and 20.2 g/person/day (335 mg/kg body weight/day), respectively. On a body weight basis, mean and 90th percentile all-user intakes of GOS were highest in infants, ages 0 to 2 years, with intakes of 499 and 815 mg/kg body weight/day, respectively. Based on the limitations of the methodology used to estimate GOS consumption, which include the use of short-term surveys, inclusion of numerous infrequently consumed foods, and additional assumptions related to market share and ubiquitous use of GOS within all foods of a given food category, it is reasonable to conclude that these estimates represent gross-overestimates of the actual GOS exposure in the U.S population that are expected under the proposed uses in food described herein.

The safety of GTC Nutrition's GOS under the proposed uses was based on scientific procedures using generally available data. The test articles used in the studies obtained from the public domain were concluded to be representative of GTC Nutrition's GOS, and therefore product specific studies were not considered necessary to support the safe use of GTC Nutrition's GOS under the intended uses in food. A critical evaluation of this data by GTC Nutrition did not reveal any potential for adverse effects in individuals consuming GOS under the proposed conditions of use.

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Galactooligosaccharides and similar molecules (fructooligosaccharides) are naturally present in the diet, and therefore have an established history of apparent safe consumption.

Galactooligosaccharides, like most beta-linked sugars are not digested by human pancreatic or brush border enzymes, and the compounds are not expected to be absorbed intact.

Galactooligosaccharides therefore travel intact through the gastrointestinal tract, where they are metabolized by the resident microflora of the colon to innocuous metabolites of normal microbial fermentation.

Published studies indicate that GOS are of low toxicity in animals. The acute toxicity of GOS has been reported to be >15 g/kg body weight in rats. Subchronic toxicity studies administering GOS *via* gavage were unremarkable. NOAEL determinations of 2 and 5 g/kg body weight per day, the highest doses administered have been reported. The consumption of large dietary concentrations of GOS also was reported to be well tolerated in rats at repeat intakes in excess of ~10 g GOS/kg body weight over a period 6 weeks to 1 year. The only effect reported in animal studies in association with repeat exposure to GOS is increased cecal and colon weights, a phenomenon that is not considered to have toxicological relevance to humans. The mutagenicity/genotoxicity of GOS was evaluated in the bacterial reverse mutation test and an *in vitro* mammalian chromosome aberration test and an *in vivo* mouse micronucleus assay; GOS was concluded not to be genotoxic or mutagenic.

The consumption of GOS preparations manufactured from lactose using various β -galactosidases has been evaluated in a large number of studies in healthy adults and children. Among these studies GOS has been administered at quantities of up to 30 g per person, and typical intakes of between 5 to 15 g per person have been reported to be well tolerated for periods of between 6 days to 3 months. No adverse effects have been reported in any study to date. Overall, the results of these studies indicate the consumption of GOS under the proposed uses in food will be well tolerated.

While not the subject of this notification (*i.e.*, infant formula use), the administration of GOS to infants has been extensively investigated, collectively GOS supplemented infant formula has been safely administered to 1,801 infants for a total of 9,271 subject years. GOS exposures in these studies are several-fold in excess, on a mg/kg basis, to those estimated under the proposed uses of GTC Nutrition's GOS in food described in Appendix A. For example, GOS has been shown to be safe and well tolerated by infants consuming infant formulas supplemented with GOS at concentrations of up to 9 g/L (~5.6 g GOS/kg body weight)¹⁷, and infant formula concentrations of 7.2 g/L resulting in approximate dietary exposures of ~2.2 g GOS/kg body weight¹⁸ have been shown to be safe and well tolerated over a 6-month repeat consumption exposure period. Overall, no reports of adverse effects on anthropometric indices

¹⁷ Based on estimate of 1.6 kg infant consuming 1 L of infant formula per day

¹⁸ Based on estimate of 3.3 kg infant consuming 1 L of infant formula per day.

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of growth or effects on water balance leading to diarrhea have been reported in infants exposed to GOS. All of these studies were conducted in healthy infants, and safety data generated from these studies would be considered relevant to adults.

General recognition of safety for the use of GTC Nutrition's GOS for use in a food as described in table A-1 (Appendix A) at use levels of between 0.48 to 12.21%, is based the opinion of an Expert Panel of scientists qualified by scientific training and experience to evaluate the safety of GOS for use in food, and is further supported by the opinions of international regulatory bodies (The EU's Scientific Committee on Food and Food Standards Australia New Zealand), where the safe use of GOS:FOS mixtures produced from food grade lactose using enzymes derived from non-toxigenic non-pathogenic organisms were determined to be safe for use in traditional foods and in infant formula up to total GOS concentration of 7.2 to 8 g/L (~5 g/kg body weight). Finally, similar uses of GOS produced from lactose using a β -galactosidase derived from *B. circulans* have recently been notified to the U.S FDA without objection from the agency (GRN000236).

M. Conclusion

Based on the data and information summarized above, it can be concluded that GTC Nutrition's GOS ingredient produced from lactose *via* a β -galactosidase isolated from *B. circulans*, meeting appropriate food-grade specifications and manufactured in accordance with cGMP, is GRAS for the intended uses in traditional food products as described herein based on scientific procedures.

Therefore, the use of GTC's GOS in food as described herein is exempt from the requirement of premarket approval (Section 409 of the *Federal Food, Drug and Cosmetic Act*).

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101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	170.3	Definitions
173—Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins
	173.115	<i>Alpha</i> -acetolactate decarboxylase (α -ALDC) enzyme preparation derived from a recombinant <i>Bacillus subtilis</i>
177—Indirect food additives: Polymers	177.2260	Filters, resin-bonded
182—Substances generally recognized as safe	182.1057	Hydrochloric acid
184—Direct food substances affirmed as generally recognized as safe	184.1763	Sodium hydroxide

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APPENDIX A

PROPOSED FOOD USES OF GTC NUTRITION'S GOS IN THE U.S.

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GALACTOOLIGOSACCHARIDE GRAS NOTICE

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Purimune and Corresponding Use-Levels for GOS in the U.S.						
Food Category	Proposed Food Uses	Serving Size ¹ (g)	Purimune ² Use-Levels		GOS Use-Levels	
			Use-Level (g/serving)	Use-Level (%)	Use-Level (g/serving)	Use-Level (%)
Baby, Infant, and Toddler Foods ³	Cereals, Baby Food	15 (dry, instant) 110 (RTS)	1.0	6.67 0.91	0.86	5.70 0.78
	Cereals, Toddler (RTE)	20	1.5	7.5	1.28	6.41
	Cookies, Crackers, and Puffs, Baby Food	7	1.0	14.29	0.86	12.21
	RTS Fruit Based Baby/Toddler Food	60 (strained)	1.0	1.67	0.86	1.43
		110 (junior)	1.0	0.91	0.86	0.78
		125 (toddler)	1.5	1.20	1.28	1.03
	Fruit Juices, Baby Food	125	1.0	0.80	0.86	0.68
	RTS Dinners, Baby/Toddler Food	60 (strained)	1.0	1.67	0.86	1.43
110 (junior)		1.0	0.91	0.86	0.78	
170 (toddler)		1.5	0.88	1.28	0.75	
RTS Desserts, Baby Food	60 (strained) 110 (junior)	1.0	1.67 0.91	0.86	1.43 0.78	
RTF Vegetable Based Baby/Toddler Food	60 (strained) 110 (junior) 70 (toddler)	1.0 1.0 1.5	1.67 0.91 2.14	0.86 0.86 1.28	1.43 0.78 1.83	
Beverages and Beverage Bases	RTD Energy, Sport, and Isotonic Beverages	244	1.5	0.61	1.28	0.53
	RTD Non-Milk Based Meal Replacements and Protein Beverages	266	1.5	0.56	1.28	0.48
Dairy Product Analogs	RTD Soy Beverages	243	1.5	0.62	1.28	0.53

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GALACTOOLIGOSACCHARIDE GRAS NOTICE

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Purimune and Corresponding Use-Levels for GOS in the U.S.						
Food Category	Proposed Food Uses	Serving Size ¹ (g)	Purimune ² Use-Levels		GOS Use-Levels	
			Use-Level (g/serving)	Use-Level (%)	Use-Level (g/serving)	Use-Level (%)
Milk Products	RTD Flavored Milk and Milk Drinks	250	1.5	0.60	1.28	0.51
	RTD Milk-Based Meal Replacements and Protein Beverages	266	1.5	0.56	1.28	0.48
	Yogurt	225	1.5	0.67	1.28	0.57
Bakery Products	Breads, rolls	50g	1.5	3.00	1.28	2.56
	Brownies	40g	1.5	3.75	1.28	3.20
	Cakes, heavy weight	125g	1.5	1.20	1.28	1.02
	Cakes, medium weight	80g	1.5	1.88	1.28	1.60
	Cakes, light weight	55g	1.5	2.73	1.28	2.33
	Coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads, muffins, toaster pastries	55g	1.5	2.73	1.28	2.33
	Cookies ⁴	30g	1.5	5.00	1.28	4.27
	Crackers that are usually used as snacks ⁴	30g	1.5	5.00	1.28	4.27
	Variety mixes (dry mix)	40g	1.5	3.75	1.28	3.20
	Grain-based bars with or without filling or coating	40g	1.5	3.75	1.28	3.20
	Pies, cobblers, fruit crisps, turnovers, other pastries	125g	1.5	1.20	1.28	1.02
Waffles	85g	1.5	1.76	1.28	1.51	
Beverages	Carbonated and non-carbonated beverages, water ⁴	244g	1.5	0.61	1.28	0.53

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GALACTOOLIGOSACCHARIDE GRAS NOTICE

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Purimune and Corresponding Use-Levels for GOS in the U.S.						
Food Category	Proposed Food Uses	Serving Size ¹ (g)	Purimune ² Use-Levels		GOS Use-Levels	
			Use-Level (g/serving)	Use-Level (%)	Use-Level (g/serving)	Use-Level (%)
Cereal and other Grain Products	Breakfast cereals RTE	30g	1.5	5.00	1.28	4.27
	Pastas, plain (dry)	55g	1.5	2.73	1.28	2.33
Desserts	Ice cream, ice milk, frozen yogurt	66g	1.5	2.27	1.28	1.94
	Custards or pudding RTE	108g	1.5	1.39	1.28	1.19
Dessert Toppings and Fillings	Other dessert toppings, fruits, syrups	39g	1.5	3.85	1.28	3.28
Fruit and Fruit Juices	All other fruits, canned	140g	1.5	1.07	1.28	0.91
	Juices, nectars, fruit drinks	244g	1.5	0.61	1.28	0.53
Snacks	All varieties	30g	1.5	5.00	1.28	4.27
Soups ³	All varieties	245g	1.5	0.61	1.28	0.52
Soft and Hard Candy	All other candies	40g	1.5	3.75	1.28	3.2

RTD = ready-to-drink; RTE = ready-to-eat; RTF = ready-to-feed; RTS = ready-to-serve

¹ Serving sizes based on Reference Amounts Customarily Consumed per Eating Occasion (RACC; 21 CFR §101.12 – U.S. FDA, 2008).

² Purimune: refers the GTC's GOS preparation (~90% GOS on a dry basis. Product is ~5% moisture, 85.5% GOS and 9.5% non-GOS carbohydrates on an as is basis)

³ GTC Nutrition's GOS is not intended for use in meat or poultry containing products

⁴ Select food codes

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Appendix B

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APPENDIX B

**EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY
RECOGNIZED AS SAFE (GRAS) STATUS OF GOS FOR USE AS AN INGREDIENT
IN TRADITIONAL FOOD PRODUCTS, AND INFANT FORMULA**

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EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF GTC NUTRITION'S GALACTOOLIGOSACCHARIDES (PURIMUNE) FOR USE IN TRADITIONAL FOOD PRODUCTS, AND INFANT FORMULA

INTRODUCTION

On 17 October 2008, at the request of GTC Nutrition (GTC), an Expert Panel (the "Panel") of independent scientists qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients was convened. The Panel conducted an independent critical and comprehensive evaluation of the available pertinent data and information on the use of a galactooligosaccharide (GOS) preparation (Trade Named Purimune) as a food ingredient in various traditional food products, including baby, infant and toddler foods as described in Table 2, would be safe and suitable, and Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Dr. Ronald E. Kleinman MD. (Pediatric Gastroenterology and Nutrition Unit, Massachusetts General Hospital), Dr. David J. Brusick, Ph.D. (Consultant), and Dr. Ian C. Munro (Cantox Health Sciences International).¹

At the request of GTC, the above Expert Panel independently and collectively, critically examined a comprehensive package of data pertaining to the intended use of GTC's GOS ingredient, which included data pertaining to the method of manufacture and product specifications of the ingredient, supporting analytical data, the intended use levels in specified food products, consumption estimates for all intended uses, background dietary consumption of galactooligosaccharides and similar ingredients (fructooligosaccharides, inulin, etc.), and a comprehensive assessment of the available scientific literature pertaining to the safety of galactooligosaccharides and other oligosaccharide ingredients.

Following independent critical evaluation of such data and information, the Panel unanimously concluded that GTC Nutrition's GOS ingredient synthesized from lactose *via* a β -galactosidase obtained from *Bacillus circulans* LOB 377, meeting appropriate food-grade specifications and manufactured according to current Good Manufacturing Practices (GMP), are safe under the intended uses as described in Table 2.0. The Panel further concluded that these uses would be GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

¹ *Curricula vitae* evidencing the qualifications of each Panel member is provided in Attachment 1.

DESCRIPTION OF GTC-NUTRITION'S GOS INGREDIENT

Common Name or Usual Name

Galactooligosaccharides; GOS

Trade Names

Purimune

Chemical Name

GOS are of the general formula $\alpha\text{-D-Glu-(1}\rightarrow\text{4)-}[\beta\text{-D-Gal-(1}\rightarrow\text{6)-}]_n$, where $n=2$ to 5 (Playne and Crittenden, 1996). The common or usual name for the mixture is galactooligosaccharides or GOS.

Chemical and Physical Characteristics

The GOS ingredient is a highly hygroscopic white powder with a maximum moisture content of not more than 10%. Chemical and physical characteristics are presented in Table 1, and the general structure of a galactooligosaccharide is illustrated in Figure 1 below.

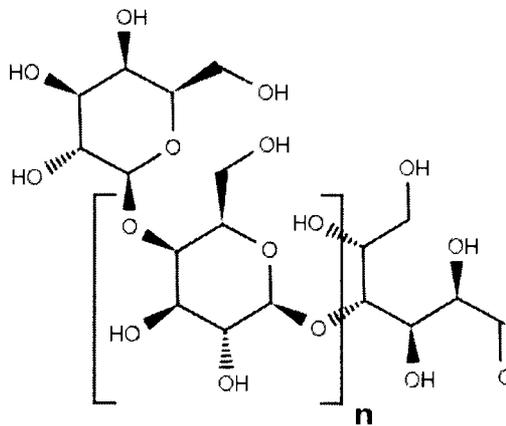


Figure 1. Basic Structure of a GOS Polymer: $\alpha\text{-D-Glu-(1}\rightarrow\text{4)-}[\beta\text{-D-Gal-(1}\rightarrow\text{6)-}]_n$

Manufacturing Process

GOS is manufactured from food grade lactose *via* a transgalactosylation reaction catalyzed by a β -galactosidase obtained from the non-toxicogenic non-pathogenic microorganism, *B. circulans* LOB 377. All raw materials and processing aids used in the manufacture of GOS are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations. GTC

Nutrition's GOS is produced through a multi-step process that begins with the dissolution of lactose powder in heated water in the saccharification tank. Following pH adjustment using sodium hydroxide (NaOH) and hydrochloric acid (HCl) solutions as required, the galactosidase is then added to the solution where it reacts with lactose to produce GOS². The hydrolysate formed during saccharification is then pumped through a heat exchanger where the solution is heated to approximately 80°C, resulting in inactivation of the β -galactosidase, and optimization of the decolorization step. The inactivated enzyme is removed *via* a Celite filter (plankton diatomite), and decolorization occurs using a fixed-bed continuous decolorization system. The organic impurities are adsorbed by the active carbon granules, which are discharged, replaced by a fresh carbon layer, and regenerated in the furnace for later use. The decolorized syrup is then cooled *via* a heat exchanger and then proceeds through a 3-column ion exchange process (*i.e.*, cation column with strongly acidic cation exchange resin; anion column with intermediate basic anion exchange resin; and a mixed bed column that has a combination of both strongly acidic and strongly basic resins) in order to remove ionic impurities (*e.g.*, calcium, chlorides, sulfates, phosphates, and other ionic components including amino acids, peptides and proteins).

Following filtration through active carbon and removal of cations and anions in the ion exchange columns, the purified GOS syrup is concentrated using an evaporator to produce a syrup. The concentrated GOS syrup then proceeds through a chromatographic separation process where glucose, galactose, and lactose are separated from the GOS mixture. The separated products are recovered from the adsorbent bed through displacement with pure water. Following chromatographic purification, the oligosaccharide fraction is composed of greater than 90% GOS, while the secondary fraction is composed of approximately 3 to 7% lactose, 10 to 15% oligosaccharide, 20 to 25% galactose, and 60 to 65% dextrose. The oligosaccharide fraction is then further refined through a second round of ion exchange, activated carbon and evaporative concentration treatments. The final purified and concentrated syrup is then retained in a storage tank where the composition of the GOS syrup is approximately 90 to 92% oligosaccharide, 7 to 10% lactose, 0 to 1.0% dextrose, and 0 to 0.5% galactose (dry weight basis). The syrup is then pumped through a heat exchanger to increase the temperature of the syrup prior to hot air spray drying, where the final product, a purified white GOS powder (97% dry solids) is produced. The final product is packaged in poly-lined craft paper bags and stored at room temperature.

² β -galactosidases are generally known as enzymes that catalyze the hydrolysis of β -D-galactopyranosides such as lactose, however, the enzyme also catalyzes transgalactosylation of these sugars, and when lactose is present at high concentrations, the transgalactosylation reaction predominates (Playne and Crittenden, 1996).

Product Specifications

The product specifications for GTC's GOS ingredient are presented below in Table 1, and includes physical and chemical specifications, as well as limits corresponding to microbiological contamination. Analytical data of 3 non-consecutive lots of the ingredient were reviewed by the Panel, verifying that the manufacturing processes produced a consistent product in compliance with the product specifications. The Panel also evaluated stability information provided by GTC, demonstrating that GOS are stable for at least 6 months under suitable storage conditions. GTC also supplied information indicating that its GOS ingredient is stable in solution at ambient (30°C) and elevated (50, 75, 100°C) temperature over a wide pH range (3 to 7). The results of the stability data indicate that GTC's GOS ingredient is expected to be stable under the proposed food uses.

Table 1 Product Specifications for GOS		
Specification Parameter	Specification	Method (Reference)
Physical/Chemical		
GOS Contents (%DB)	90.0 to 92.0	CPSMA S30 (internal validated) Based on AOAC 979.23
Moisture (%)	Maximum 10	CPSMA M50 (internal validated) (AOAC)
Carbohydrate Profile		CPSMA S30 (internal validated) Based on AOAC 979.23
Dextrose (%DB)	0 to 1.0	
Galactose (%DB)	0 to 0.5	
Lactose (%DB)	7.0 to 10.0	
Galactooligosaccharides (%DB)	90.0 to 92.0	
Disaccharides	16.0 to 21.0	
Trisaccharides $\beta(1,3)$ (1,4)	14.0 to 19.0	
Trisaccharides $\beta(1,4)$ (1,4)	16.0 to 20.0	
Trisaccharides $\beta(1,6)$ (1,3)	8.0 to 13.0	
Tetrasaccharides and greater	25.0 to 29.0	
Granular size (% through 40 mesh)	100	-
Ash (% w/w)	0.05	CPSMA A90 (Internal validated) (Based on AOAC method)
Protein (%)	Negative (LOD = 10 ppm)	CPSMA P60 (internal validated) Based on Kjeldhal method (USP "chemical tests:<461> Nitrogen Determination". "Maltodextrin NF Monograph")
pH (in 10% solution)	4.0 to 7.0	CPSMA P40 (internal validated) (Based on USP method)
Lead (ppm)	<0.01	CPSMA L40 (internal validated*)
Arsenic (as AS ₂ O ₃)	≤1	CPSMA A80 (internal validated) (Based on FCC method)
Foreign substance	Negative	-

Table 1 Product Specifications for GOS		
Specification Parameter	Specification	Method (Reference)
Appearance	White powder	Visual inspection
Foreign taste and odor	Negative	Sensory test
Microbiological (counts/g)		
Mesophilic bacteria	300	CPSMA Microbiological Methods (internal validated)
Mold and yeast	20	
Coliforms	Negative	Based on Microbiological Methods of the Member Companies of the Corn Refiners Association
Anaerobic thermophilic spores	<10	
Aerobic thermophilic spores	<10	
Anaerobic mesophilic spores	<10	
Aerobic mesophilic spores	<10	
<i>Salmonella</i>	Negative	
<i>Staphylococcus aureus</i>	Negative	
<i>Escherichia coli</i>	Negative	
Listeria (tested in 50 g)	Negative	Food Codex of the Korean Food and Drug Administration

CPSMA = Corn products of America Standard Methods of Analyses; CRA = Corn Refiners Association; Association of Official Analytical Chemists (AOAC), United States Pharmacopeia (USP), Food Chemicals Codex (FCC); LOD = limit of detection.

*Methodology for lead analyses based on the following references: (1) Corn Industries Research Foundation Division of Corn Refiners Association, Inc.: Standard Analytical Methods. Sixth Edition. Washington; (2) CPC International: Moffet Center - Analytical Methods L20. Determination of Lead by Dithizone: Spectrophotometric Procedure. 1961; (3) Food Chemicals Codex. Current Edition. Appendix III. Lead Limit Test; (4) The United States Pharmacopeia. Current Edition. "Chemical Tests: <251> Lead".

INTENDED LEVELS OF USE AND EXPOSURE ESTIMATES

GTC Nutrition intends to market GOS as a food ingredient in the United States in a variety of food products including baby, infant and toddler foods (including infant formula and follow-on formula), beverages and beverage bases, dairy product analogs, milk products, bakery products, beverages, cereal and other grain products, desserts, fruit and fruit juices, snacks, soups, and soft and hard candy, at use levels of 0.86 to 1.28 g per serving. On a percent basis the intended food uses of GOS result in use levels of between 0.48 to 12.21%. The individual proposed food uses and use levels for GOS are located in Table 2. Food codes representative of each proposed food use were chosen from the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Survey (NHANES) (CDC, 2006; USDA, 2008) and were grouped in food use categories according to Title 21, Section §170.3 of the *Code of Federal Regulations* (U.S. FDA, 2008).

Table 2 Summary of the Individual Proposed Food-Uses and Use-Levels for Purimune and Corresponding Use-Levels for GOS in the U.S.

Food Category	Proposed Food Uses	Serving Size ¹ (g)	Purimune ² Use-Levels		GOS Use-Levels	
			Use-Level (g/serving)	Use-Level (%)	Use-Level (g/serving)	Use-Level (%)
Baby, Infant, and Toddler Foods ³	Cereals, Baby Food	15 (dry, instant) 110 (RTS)	1.0	6.67 0.91	0.86	5.70 0.78
	Cereals, Toddler (RTE)	20	1.5	7.5	1.28	6.41
	Cookies, Crackers, and Puffs, Baby Food	7	1.0	14.29	0.86	12.21
	RTS Fruit Based Baby/Toddler Food	60 (strained)	1.0	1.67	0.86	1.43
		110 (junior)	1.0	0.91	0.86	0.78
		125 (toddler)	1.5	1.20	1.28	1.03
	Fruit Juices, Baby Food	125	1.0	0.80	0.86	0.68
	RTS Dinners, Baby/Toddler Food	60 (strained)	1.0	1.67	0.86	1.43
		110 (junior)	1.0	0.91	0.86	0.78
170 (toddler)		1.5	0.88	1.28	0.75	
RTS Desserts, Baby Food	60 (strained) 110 (junior)	1.0	1.67 0.91	0.86	1.43 0.78	
Infant Formula and Follow-on Formula	157 ⁵	1.3	0.84	1.13	0.72	
RTF Vegetable Based Baby/Toddler Food	60 (strained)	1.0	1.67	0.86	1.43	
	110 (junior)	1.0	0.91	0.86	0.78	
	70 (toddler)	1.5	2.14	1.28	1.83	
Beverages and Beverage Bases	RTD Energy, Sport, and Isotonic Beverages	244	1.5	0.61	1.28	0.53
	RTD Non-Milk Based Meal Replacements and Protein Beverages	266	1.5	0.56	1.28	0.48
Dairy Product Analogs	RTD Soy Beverages	243	1.5	0.62	1.28	0.53
Milk Products	RTD Flavored Milk and Milk Drinks	250	1.5	0.60	1.28	0.51
	RTD Milk-Based Meal Replacements and Protein	266	1.5	0.56	1.28	0.48

280000

Table 2 Summary of the Individual Proposed Food-Uses and Use-Levels for Purimune and Corresponding Use-Levels for GOS in the U.S.						
Food Category	Proposed Food Uses	Serving Size ¹ (g)	Purimune ² Use-Levels		GOS Use-Levels	
			Use-Level (g/serving)	Use-Level (%)	Use-Level (g/serving)	Use-Level (%)
	Beverages					
	Yogurt	225	1.5	0.67	1.28	0.57
Additional Categories						
Bakery Products	Breads, rolls	50	1.5	3.00	1.28	2.56
	Brownies	40	1.5	3.75	1.28	3.20
	Cakes, heavy weight	125	1.5	1.20	1.28	1.02
	Cakes, medium weight	80	1.5	1.88	1.28	1.60
	Cakes, light weight	55	1.5	2.73	1.28	2.33
	Coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads, muffins, toaster pastries	55	1.5	2.73	1.28	2.33
	Cookies ⁴	30	1.5	5.00	1.28	4.27
	Crackers that are usually used as snacks ⁴	30	1.5	5.00	1.28	4.27
	Variety mixes (dry mix)	40	1.5	3.75	1.28	3.20
	Grain-based bars with or without filling or coating	40	1.5	3.75	1.28	3.20
	Pies, cobblers, fruit crisps, turnovers, other pastries	125	1.5	1.20	1.28	1.02
	Waffles	85	1.5	1.76	1.28	1.51
	Beverages	Carbonated and non-carbonated beverages, water ⁴	244	1.5	0.61	1.28
Cereal and other Grain Products	Breakfast cereals RTE	30	1.5	5.00	1.28	4.27
	Pastas, plain (dry)	55	1.5	2.73	1.28	2.33
Desserts	Ice cream, ice milk, frozen yogurt	66	1.5	2.27	1.28	1.94
	Custards or pudding RTE	108	1.5	1.39	1.28	1.19

Table 2 Summary of the Individual Proposed Food-Uses and Use-Levels for Purimune and Corresponding Use-Levels for GOS in the U.S.

Food Category	Proposed Food Uses	Serving Size ¹ (g)	Purimune ² Use-Levels		GOS Use-Levels	
			Use-Level (g/serving)	Use-Level (%)	Use-Level (g/serving)	Use-Level (%)
Dessert Toppings and Fillings	Other dessert toppings, fruits, syrups	39	1.5	3.85	1.28	3.28
Fruit and Fruit Juices	All other fruits, canned	140	1.5	1.07	1.28	0.91
	Juices, nectars, fruit drinks	244	1.5	0.61	1.28	0.53
Snacks	All varieties	30	1.5	5.00	1.28	4.27
Soups ³	All varieties	245	1.5	0.61	1.28	0.52
Soft and Hard Candy	All other candies	40	1.5	3.75	1.28	3.2

RTD = ready-to-drink; RTE = ready-to-eat; RTF = ready-to-feed; RTS = ready-to-serve

¹ Serving sizes based on Reference Amounts Customarily Consumed per Eating Occasion (RACC; 21 CFR §101.12 – U.S. FDA, 2008).

² Purimune: refers the GTC's GOS preparation (~90% GOS on a dry basis. Product is ~5% moisture, 85.5% GOS and 9.5% non-GOS carbohydrates on an as is basis)

³ GTC Nutrition's GOS is not intended for use in meat or poultry containing products

⁴ Select food codes

⁵ 5 fluid ounce serving = 157 g at density of 1.06 g/mL.

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Approximately 100% of the total U.S. population was identified as potential consumers of GOS from the proposed food-uses (8,168 actual users identified). Consumption of these types of foods by the total U.S. population resulted in estimated mean and 90th percentile all-user intakes of GOS of 9.3 g/person/day (171.5 mg/kg body weight/day) and 15.4 g/person/day (350.6 mg/kg body weight/day), respectively (Tables 3 and 4). On an individual population basis, the greatest mean and 90th percentile all-user exposures were estimated to occur in male teenagers (aged 12 to 19 years), at 12.1 g/person/day (192.0 mg/kg body weight/day) and 20.2 g/person/day (335.2 mg/kg body weight/day), respectively. On a body weight basis, mean and 90th percentile all-user intakes of GOS were highest in infants, ages 0 to 2 years, with intakes of 498.5 and 814.5 mg/kg body weight/day, respectively.

It was noted that the methodology used to estimate consumption of GOS described above is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimate. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently. Thus, the estimated intakes reported in tables 3 and 4 below were considered to be a gross over-estimates of the actual expected intake of GOS in the U.S population.

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (g)	90 th Percentile (g)	Mean (g)	90 th Percentile (g)
Infant	0-2	90.1	838	5.4	9.7	5.7	9.8
Child	3-11	100	1,287	9.5	14.2	9.5	14.2
Female Teenager	12-19	99.9	991	9.4	14.6	9.4	14.6
Male Teenager	12-19	100	999	12.1	20.2	12.1	20.2
Female Adult	20 and Up	99.8	2,125	8.1	14.0	8.1	14.0
Male Adult	20 and Up	99.9	1,928	10.4	17.8	10.4	17.8
Total Population	All Ages	98.8	8,168	9.2	15.4	9.3	15.4

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (mg/kg)	90 th Percentile (mg/kg)	Mean (mg/kg)	90 th Percentile (mg/kg)
Infant	0-2	90.1	838	466.8	804.8	498.5	814.5
Child	3-11	100	1,287	363.2	617.0	363.2	617.0
Female Teenager	12-19	99.9	991	164.2	274.9	164.4	274.9
Male Teenager	12-19	100	999	192.0	335.2	192.0	335.2
Female Adult	20 and Up	99.8	2,125	113.5	196.4	113.9	197.2
Male Adult	20 and Up	99.9	1,928	122.9	207.2	122.9	207.2
Total Population	All Ages	98.8	8,168	170.8	350.6	171.5	350.6

GTC Nutrition intends also intends to market GOS derived from lactose as a food ingredient in the United States for use in infant formula and follow-on formula at concentrations not to exceed 0.72% (7.2 g of GOS per liter of infant formula) of the final reconstituted or ready-to-serve product (1.13 g per 157 g serving³). Assuming 100% marketshare and the inclusion of GOS in all infant formula sold in the U.S., approximately 80% of the infant population aged 0 to 6 months and 7 to 12 months respectively were identified as consumers of GOS under the proposed food uses (202 actual users aged 0 to 6 months and 138 users 7 to 12 months identified). Toddlers aged 1 to 2 years represented a small proportion of users at 3.7% (n=19). The consumption of GOS containing infant formula by infants aged 0 to 6 months is expected to result in estimated mean and 90th percentile all-user intakes of GOS of 5.9 g/person/day (0.9 g/kg body weight/day) and 8.5 g/person/day (1.5 g/kg body weight/day), respectively (Tables 5 and 6). Infants aged 7 to 12 months were estimated to consume 5.2 and 7.9 g GOS/person/day for mean and 90th percentile all-users, corresponding to exposures of 0.6 and 0.9 g/kg body weight respectively on a body weight basis. The use of GOS containing infant formula by toddlers resulted in mean and 90th percentile all-user estimated intakes of 2.8 (0.3 g/kg body weight) and 6.6 g (0.6 g/kg body weight) per person respectively.

Additional exposure to GOS from the consumption of GOS containing baby and toddler foods (baby foods, cereals, crackers *etc.*) currently present or intended for use in the U.S. market was considered, particularly with respect to exposures by pre-weanling infants. However, exposure to these foods will be limited in this age group (0 to 4 months). Furthermore, exposures to GOS by weanling infants *via* the consumption of traditional food products supplemented with GOS products is expected to partially replace GOS exposures from infant formula, and therefore not

³ 5 fluid ounce serving = 157 g at density of 1.06 g/mL.

appreciably alter the estimated exposures in mean and 90th percentile users as presented in tables 5 and 6 below.

Table 5 Summary of the Estimated Daily Intake of GOS from Infant Formulas and Follow-On Formulas in the U.S. by Population Group (2003-2004 NHANES Data)							
Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (g)	90 th Percentile (g)	Mean (g)	90 th Percentile (g)
Infants	0 to 6 months	80.8	202	4.8	8.5	5.9	8.5
Infants	7 to 12 months	81.2	138	4.5	7.6	5.2	7.9
Toddlers	1 to 2	3.7	19	*0.1	*NA	*2.8	*6.6

*Due to the small sample size data that may be statistically unreliable. NA = Not available due to small number of users in this age group.

Table 6 Summary of the Estimated Daily Per Kilogram Body Weight Intake of GOS from Infant Formulas and Follow-On Formulas in the U.S. by Population Group (2003-2004 NHANES Data)							
Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (g/kg)	90 th Percentile (g/kg)	Mean (g/kg)	90 th Percentile (g/kg)
Infants	0 to 6 months	80.8	202	0.8	1.4	0.9	1.5
Infants	7 to 12 months	81.2	138	0.5	0.9	0.6	0.9
Toddlers	1 to 2	3.7	19	* < 0.1	*NA	*0.3	*0.6

*Due to the small sample size data that may be statistically unreliable. NA = Not available due to small number of users in this age group.

BASIS FOR GRAS STATUS OF GTC'S GOS INGREDIENT

In the absence of product specific safety studies supporting the GRAS use of GTC Nutrition's galactooligosaccharides, published studies using GOS products determined to be comparable to GTC Nutrition's ingredient were used to support the safety of the ingredient for its intended uses. Currently, all GOS products are manufactured in a similar manner using lactose as a starting material followed by GOS synthesis *via* a beta-galactosidase(s) obtained from various non-toxicogenic strains of bacteria. Based on the widespread use of a standard manufacturing process for GOS ingredients currently on the market, the panel concluded that differences between various GOS products produced using standard manufacturing processes, using cGMP, would be limited to slight variations in the compositional distribution of the GOS oligomers, and to differences in the residual levels of lactose and water in the final product. Thus, the panel concluded that provided GOS is manufactured using a beta-galactosidase

obtained from a non-toxigenic organism using standard manufacturing techniques, and in accordance with cGMP, the safety information obtained from peer reviewed scientific literature on other GOS products would support the safety GTC Nutrition's ingredient. As such, product specific safety studies were not considered necessary to determine that the uses of GTC Nutrition's ingredient are GRAS. This conclusion is consistent with the European Food Safety Authority (EFSA) and Food Standards Australia New Zealand's (FSANZ) regulatory opinions of the use of GOS in traditional food products and infant formula. These agencies have determined that it was not practical to develop specifications for the use of these products in traditional food products or infant formula, and a generic⁴ approval of the use of these products has been granted (SCF, 2001a,b; FSANZ, 2008). Pursuant to the regulations in these countries, Novel Food applications for the use of GOS in traditional food products and infant formula would not be required provided the GOS ingredient is not manufactured using novel processes.

Therefore, the data supporting the GRAS status GTC Nutrition's GOS ingredient for the intended uses as described in Table 2.0, was obtained from a series of published studies conducted in animals and in humans using sources of GOSs that were considered comparable to GTC Nutrition's ingredient. These studies, summarized below, consist of information characterizing background exposure to GOSs and similar beta-linked carbohydrates in the diet, studies investigating the metabolic fate, microbial fermentation and toxicity of GOSs. The Panel also reviewed several studies conducted in healthy adults, and safety studies conducted with infants administered GOS supplemented infant formulas. Information pertaining to mineral absorption, and allergenicity also was considered by the Panel. Finally, unpublished toxicity studies conducted with the beta-galactosidase and source organism (*B. circulans* Jordan LOB 377) used in the manufacture of GTC Nutrition's GOS also were reviewed by the panel as additional information supporting safety.

Background Exposure

Galactooligosaccharides are naturally present in the diet, and although comprehensive estimates of background GOS consumption are not available, Delzenne (2003) has estimated that the intake of fructooligosaccharides may range from 3 to 13 g/person/day. Higher background intakes of naturally occurring oligosaccharides have been reported by the FSANZ, who estimated that the combined baseline intakes of inulin derived substances and GOS in Australia and New Zealand are between 5 to 17 g per person per day for the mean and 90th percentile intakes in toddlers aged 9 months to 1 year (FSANZ, 2008). Importantly, GOSs are

⁴ In Australia and New Zealand, GOSs used in traditional food products or infant formula must meet the general requirements of standard 1.3.4 pertaining to identity and purity whereby GOS is defined as "a mixture of those substances produced from lactose by enzymatic action, comprised of between two and eight saccharide units, with one of these units being a terminal glucose and the remaining saccharide units being galactose, and disaccharides comprised of two units of galactose."

reported to be present in breast milk, although in limited quantities. However human milk has been shown to contain oligosaccharides with comparable β -linked oligomer structures in significant quantities; concentrations between 8 to 12 g/L have been reported in mature human milk and levels as high as 25 g/L have been observed in colostrum (Kunz *et al.*, 1999; Kunz *et al.*, 2000). Thus, the estimated exposures to GOS under the intended uses of Purimune in food are expected to be comparable to background exposures from the consumption of similar β -linked oligosaccharides in the diet.

Metabolic Fate

Dietary fibre has been defined as the sum of lignin and non-starch polysaccharides (NSP), which consist of cellulose, hemicellulose, β -glucans, pectin, gums, and mucilage. Resistant alpha-linked starches and non-digestible oligosaccharides also have been added to this list (Cho and Dreher, 2001). Dietary fibre can be further divided into soluble and insoluble forms, a distinction based on their solubility characteristics in hot aqueous buffer solutions (Cho and Dreher, 2001). The American Association of Cereal Chemists has defined dietary fibre as "the edible parts of plants or analogues of carbohydrates that are resistant to digestion and absorption in the small intestine, with complete or partial fermentation in the large intestine" (American Association of Cereal Chemists, 2001).

The human digestive tract can efficiently hydrolyze glucose polymers linked by alpha-glycosidic linkages, like those present in starch and glycogen (Wisker *et al.*, 1985); however, with the exception of brush border beta-galactosidases (lactases), which are responsible for the degradation of lactose, luminal digestion of dietary sugars linked *via* beta-glycosidic linkages does to occur to any significant degree (Wisker *et al.*, 1985). For example, Ohtsuka *et al.* (1990) and Chonan *et al.* (2004) reported that GOS are not hydrolyzed by human salivary amylase, hog pancreatic α -amylase, or artificial gastric juice, and as shown by Engfer *et al.*, (2000) the incubation of GOS with human pancreatic juice and brush border membrane isolates did not provide evidence of GOS hydrolysis (i.e., liberation of monosaccharides in solution as measured via HPLC/MS). All undigested and therefore unabsorbed dietary fibre passes into the colon where it is metabolized by the colonic microflora, resulting in the production of short-chain fatty acids - mainly acetate and propionate. Microbial fermentation of GOS also is expected to liberate small amounts carbon dioxide, hydrogen, and methane gas (Tanaka *et al.*, 1983; Ishikawa *et al.*, 1995; Chonan *et al.*, 2004). Rats have been reported to have a high fermentation capacity for GOS, and following dietary administration of large doses (5,000 mg/kg body weight), Ohtsuka *et al.* (1991) reported that no GOS was detectable in the feces. Fermentation may be incomplete in humans, at least in infants. Moro *et al.* (2005) reported that GOS was detectable [*via* high-performance liquid chromatography (HPLC)] in the feces in infants administered an infant formula supplemented with 8 g/L of GOS. All of the metabolic products of GOS fermentation in the colon are considered innocuous substances, and are metabolized by well characterized pathways.

Various enterobacterial strains are capable of utilizing GOS as an energy source (e.g., *Bifidobacterium*, *Bacteroides*, *Lactobacillus*, *Enterobacteriaceae*, *Clostridium*, and *Enterococcus faecium*, *E. coli*, and *S. salivarius*); however, β -linked GOS are preferentially metabolized by *Bifidobacteria* and *Lactobacilli* (Tanaka *et al.*, 1983; Ishikawa *et al.*, 1995; Matsumoto *et al.*, 2004; Tzortzis *et al.*, 2005). Although not consistently reported in all animal and human studies, the repeat consumption of GOS tends to have bifidogenic effects, promoting the growth of *Bifidobacteria* in the gastrointestinal tract. This effect is transient, and requires continuous daily exposures.

Toxicity Studies

Galactooligosaccharides are of low toxicity in animals. As reported by Matsumoto *et al.* (1993), the acute toxicity of a GOS was >15 g/kg body weight in rats. The results of repeat dose toxicity studies conducted in rats also support the low toxicity of GOS (Ohtsuka *et al.*, 1990; Kobayashi *et al.*, 2003; Anthony *et al.*, 2006). In a sub-chronic toxicity study conducted by Anthony *et al.* (2006), a GOS preparation synthesized from lactose via β -galactosidase from *B. circulans* was administered to male and female SD rats (6 weeks of age) for a period of 90-days; a no-observed-adverse-effect level (NOAEL) of 5,000 mg GOS/kg body weight, the highest dose tested was determined by the authors. Based on available evidence from the literature characterizing the composition and manufacturing of various commercial GOS ingredients (Goulas *et al.*, 2002; Montilla *et al.*, 2006; Anthony *et al.*, 2006; U.S.-FDA GRAS Notification 000238), it was concluded that the test article used in the subchronic toxicity study by Anthony *et al.* (2006) was representative of GTC Nutrition's GOS ingredient (Purimune), and therefore applicable for use as pivotal data supporting a GRAS determination of the ingredient (see Attachment 2, Table A.2-1). Similar observations were reported by Kobayashi *et al.* (2003) who administered a GOS preparation prepared from lactose via β -galactosidases from *A. oryzae* and *S. thermophilus* to groups of male and female SD rats (~200 g) for a period of 90 days. Following standard toxicological evaluations, a NOAEL of 2,000 mg GOS/kg body weight, the highest dose tested was determined. Higher repeat intakes of GOS have been reported to be well tolerated up to doses of ~10,000 mg GOS/kg body weight over a period of 6 weeks when administered in the diet to weanling SD rats (Ohtsuka *et al.*, 1990). Overall, the only effect reported in animal studies in association with repeat exposure to GOS is increased cecal and colon weights (Shimura *et al.*, 1991; Chonan and Watanuki, 1995; Chonan *et al.*, 1995, 1996; Appel *et al.*, 1997; Kobayashi *et al.*, 2003; Ohtsuka *et al.*, 1990). However, increased cecum weights following the consumption of other indigestible carbohydrates (sorbitol, mannitol, xylitol, caramel, and polydextrose) in rodents is a well established phenomenon, and is not considered to have toxicological relevance to humans (WHO, 1987).

Mutagenicity/Genotoxicity

Short-term genotoxicity assays demonstrated no mutagenic or genotoxic effect of GOS in the bacterial reverse mutation test and the mammalian chromosome aberration test *in vitro* or the mouse micronucleus test *in vivo* (Yasutake *et al.*, 2003).

Human Studies

Infant formula's supplemented with GOS and FOS have been widely used in Europe for a number of years. As a result of patents owned by the Royal Numico company (Numico) pertaining to the use of GOS and long-chain FOS at a ratio of 9:1 in food, Numico has a significant world-wide marketshare for the use of its GOS:FOS ingredient. As a result of the widespread use of Numicos GOS:FOS ingredient, and due to the extensive publication activities of Numico Research, the vast majority of studies conducted in humans are conducted with Numico's proprietary GOS:FOS mixture. Numico does not manufacture its own GOS, and as reported in the literature, Numico's GOS is sourced from Friesland foods (Vivinal GOS) and its FOS obtained from Orafit Active Food Ingredients (Raftiline HP FOS) (Scholtens *et al.*, 2006; Bakker-Zierikee, 2005a,b). In addition, given Numico's patent protection on the use of GOS:FOS in a ratio of 9:1, studies reporting the use of GOS:FOS in this ratio should be assumed to be obtained from Numico. Therefore, as discussed above, studies conducted with this material were considered to be representative of GTC Nutrition's GOS and therefore directly relevant to the safety of their ingredient.

Adults

Nineteen (19) studies – 17 studies in adults and two studies conducted in children – were obtained from the public domain containing data relevant to the safety evaluation of GOS under the intended uses described herein; although none of these studies contained standard safety measurements as primary endpoints, parameters related to tolerance (flatulence, bloating, abdominal cramping, *etc.*) and adverse event monitoring were reported in these investigations⁵. The majority of these studies were conducted in healthy adults, and typical intakes of GOS are between 5 to 15 g per person per day for periods of between 1 to 3 weeks. Studies reporting acute GOS intakes of 20 to 30 g/person also are reported in the literature (Tanaka *et al.*, 1983; Van den Heuvel *et al.*, 2000). In three studies intakes of GOS between 5.5 to 10 g are reported to be well tolerated without adverse events for durations of between one to 2.5 months (Ito *et al.*, 1990; Shadid *et al.*, 2007; Vulevic *et al.*, 2008; Silk *et al.*, 2008). A summary of relevant studies obtained from the literature are presented in the following paragraphs and in table IV.H-1 below.

⁵ Ito *et al.*, 1990; Ishikawa *et al.*, 1995; Bouhnik *et al.*, 1997; Deguchi *et al.*, 1997; Teuri and Korpela, 1998; Teuri *et al.*, 1998; Alles *et al.*, 1999; van Dokkum *et al.*, 1999; van den Heuvel *et al.*, 2000; Alander *et al.*, 2001; Satokari *et al.*, 2001; Scholtens *et al.*, 2006; Bouhnik *et al.*, 2004; Matsumoto *et al.*, 2004; Sazawal *et al.*, 2004; Shadid *et al.*, 2007; Depeint *et al.*, 2008; Silk *et al.*, 2008; Vulevic *et al.*, 2008.

Overall, no reports of adverse or severe adverse events attributable to the consumption of GOS were reported in the literature, which included several studies administering GOS at intakes (10 to 20 g/person) that are comparable to the estimated GOS exposure under the proposed uses of GTC Nutrition's GOS. Among the studies that included tolerance endpoints, side-effects are limited to reports of flatulence when GOS is consumed on a repeat basis at quantities of between 10 to 15 grams (Ito *et al.*, 1990; Deguchi *et al.*, 1997; Teuri *et al.*, 1998; Alles *et al.*, 1999; Ito *et al.*, 1990); however, this effect is not consistently reported in all studies at these intakes (Bouhnik *et al.*, 1997; Teuri and Korpela, 1998; van Dokkum *et al.*, 1999; Bouhnik *et al.*, 2004; Shadid *et al.*, 2007). Similar observations of increased flatulence have been reported following the consumption fructooligosaccharides (15 g) over a 7-day period (Alles *et al.*, 1996), and is an effect that is expected in association with the consumption of indigestible fiber in large quantities. The results of these studies indicate that the consumption of GOS under the proposed uses of GTC Nutrition's GOS will be well tolerated.

Infants

Of the studies reporting the effects of GOS administration *via* infant formula, 7 studies meeting the following safety criteria were identified: Galactooligosaccharide administration initiated within the first 2 weeks of life, for a duration of at least 3 months; and studies that included complete anthropometric safety monitoring (Schmelzle *et al.*, 2003; Ben *et al.*, 2004; Moro *et al.*, 2006; Puccio *et al.*, 2007; Ziegler *et al.*, 2007; Arslanoglu *et al.*, 2008; Costalos *et al.*, 2008). Of these studies 3 were sufficiently powered throughout the entire study period to detect clinically significant reductions in weight gain of ≥ 3 g/day (Moro *et al.*, 2006; Arslanoglu *et al.*, 2008; Costalos *et al.*, 2008). All of these studies reported that GOS was well tolerated up to concentrations of 7.2 g/L, the highest concentration used, and no significant differences in anthropometric indices of growth were reported in any of the studies. Ziegler *et al.* (2007) reported that a significant increase in diarrhea was observed in infants consuming a GOS:polydextrose (50:50) supplemented formula (4 g/L), relative to control infants receiving the unsupplemented formula (18 vs. 4% respectively, $P=0.008$). Increased incidence of eczema (GOS 4g/L vs. control; 18 vs. 4% respectively, $P=0.008$) and irritability (GOS:polydextrose:lactulose, 8 g/L vs. control; 16 vs. 4% $P=0.27$) also were reported in the study. In addition, Ziegler *et al.* (2007) reported that subjects leaving the study due to feeding intolerance tended to weigh higher in the high dose GOS group. The significance of these findings are unclear: the treatment groups were administered a heterogeneous mixture of GOS, polydextrose and or lactulose; findings were not dose-responsive – significant increases in diarrhea and eczema were apparently not reported in the high dose group; studies measuring the development of allergic manifestations in susceptible infants, as primary endpoints, do not report similar findings (Moro *et al.*, 2006; Arslanoglu *et al.*, 2008); and finally, these effects although undesirable did not affect weight gain of the infants.

Several additional studies investigating the consumption of GOS (GOS alone or GOS:FOS in ratio of 9:1) supplemented infant formula were reviewed⁶. In these studies the authors administered GOS supplemented infant formulas to various age groups at concentrations ranging from 2.4 to 9 g/L over periods of 14 days to 8 months. Two studies were conducted in pre-term infants administered GOS supplemented formulas at concentrations of 10 g/L for 14 and 28 days (Boehm *et al.*, 2002; Mihatsch *et al.*, 2006). In all of these studies the GOS supplemented infant formulas were well tolerated and no adverse effects on weight gain were reported. The only effects noted in these studies are changes in gastrointestinal microflora (Bifidogenic effects, increases in fecal concentrations of acetate and propionate short-chain fatty acids and lowered fecal pH), and softer looser stools relative to infants receiving unsupplemented infant formulas; based on observations from studies directly comparing GOS supplementation to those of breast fed control, these effects are considered comparable to those of in infants fed breast milk (Boehm *et al.*, 2002; Moro *et al.*, 2003; Bakker-Zierikzee *et al.*, 2005a,b; Haarman and Knol, 2005; Knol *et al.*, 2005; Scholtens *et al.*, 2008).

Overall, 23 studies including 1,836 infants consuming GOS supplemented infant formulas for a total of 8,757 subject years were reviewed as part of the safety evaluation of GTC Nutrition's GOS ingredient for use in infant formula. These studies included 1,059 infants consuming GOS supplemented infant formula's with GOS concentration's of 7.2 g/L or higher for a period of 1,483 subject years. No adverse events, adverse effects on anthropometric indices of growth or effects on water balance leading to diarrhea have been reported to date. These studies also included 7 studies conducted in infants where GOS infant formula was administered within the first 2 weeks of life, for a duration of at least 3 months, and with sufficient power to detect clinically meaningful changes in weight gain (Schmelzle *et al.*, 2003; Ben *et al.*, 2004; Moro *et al.*, 2006; Puccio *et al.*, 2007; Ziegler *et al.*, 2007; Chouraqui *et al.*, 2008; Costalos *et al.*, 2008); two of these studies included GOS supplementation at a concentration of 7.2 g/L (Schmelzle *et al.*, 2003; Moro *et al.*, 2006), one of which administered GOS supplemented infant formula for 6 months and included anthropometric follow-up visits at 12 and 24 months-post GOS consumption (Moro *et al.*, 2006; Arslanoglu *et al.*, 2008).

In conclusion, based on the safe use of GOS supplemented infant formula as reported by Schmelzle *et al.*, (2003) and Moro *et al.*, (2006), the totality of evidence from the large body available literature investigating the consumption of GOS by infants, and unremarkable observations reported in rodent toxicity studies, supports the safe use of GTC Nutrition's GOS as an ingredient in infant formula and follow-on formula at intended use levels resulting in GOS concentrations of up to 7.2 g/L. The safe use of GTC Nutrition's GOS in infant formula is further

⁶ Knol *et al.* (2001, 2002, 2005); Rigo *et al.* (2001); Boehm *et al.* (2002, 2003); Moro *et al.* (2002, 2005, 2006); Napoli *et al.* (2003); Savino *et al.* (2003); Schmelzle *et al.* (2003); Ben *et al.* (2004); Bakker-Zierikzee *et al.* (2005a,b); Fanaro *et al.* (2005); Haarman and Knol, (2005, 2006); Rinne *et al.* (2005); Mihatsch *et al.* (2006); Scholtens *et al.* (2006); Kukkonen *et al.* (2007); Puccio *et al.* (2007); Ziegler *et al.* (2007); Arslanoglu *et al.* (2008); Costalos *et al.* (2008); Magne *et al.* (2008).

supported by the fact that human milk is reported to contain significant concentrations of oligosaccharides of similar core molecular structure at concentrations of between 8 to 12 g/L in mature milk to as high as 25 g/L in colostrum.

Allergenicity

GTC Nutrition's GOS ingredient (Purimune) is manufactured from lactose obtained from a milk source (Whey), thus potential exposure to milk protein in the final product should be considered. Allergy to cow's milk is recognized as one of the most common food allergies (Bush and Hefle, 1996). A number of milk proteins are known to be allergenic or immunogenic to humans, and most humans react to more than one milk protein. Major milk protein allergens include caseins, a family of related proteins with each protein having genetic variants that may be post-translationally modified, as well as β -lactoglobulin a major whey protein that also has genetic variants. Other minor allergens have been identified in milk (e.g., α -lactalbumin and bovine serum albumin), but these proteins are of less concern. The minimum quantity of milk proteins required to produce sensitization or elicit an allergic reaction is not currently known (Bush and Hefle, 1996); however, as reported in the FDA guidance documentation "Approaches to Establish Thresholds for Major Food Allergens and for Gluten in Food" available evidence from clinical trials currently indicate that the lowest-observable-adverse-effect level (LOAEL) for allergic reactions to milk proteins is between 0.36 to 3.6 g of protein (U.S. FDA, 2006). Analytical data for Purimune using the Kjeldahl assay indicate that protein absent from the product within a detection limit of 10 ppm. Purimune is intended for use in various foods at quantities of 1 to 1.5 g/serving. Based on the absence of protein in the ingredient (detection limit 10 ppm), exposure to trace amounts of milk protein from exposure to Purimune under the intended use in food is unlikely to result in allergic responses in milk sensitive individuals. Nevertheless, GTC Nutrition recognized that foods containing GOS will be required to comply with the Food Allergen Labeling and Consumer Protection act of 2004, and exemption from allergy labeling would require notification to, and approval by, the FDA.

Although the content of non-GOS residual sugars in GTC Nutrition's ingredient are low, The Panel noted that exposure to the residual galactose under the intended uses of the ingredient could be a concern if the product is added to infant formula's used by infants with galactosemia. As such, it was recommended that the residual sugar content on the specification clearly indicate the presence of galactose so that the end-user of the product is aware that this by-product is present in foods containing GTC Nutrition's GOS.

Safety of Production Organism and Enzyme

GTC Nutrition's GOS ingredient is produced *via* the transgalactosylation of lactose using the enzyme β -galactosidase isolated from *B. circulans* LOB 377. Numerous enzymes derived from various species of *Bacillus* are widely used in the manufacture of foods and food ingredients. For example, carbohydrase and protease enzyme preparations derived from *Bacillus subtilis* are

affirmed as GRAS for use as direct food ingredients (21 CFR §184) (U.S. FDA, 2008), and α -acetolactate decarboxylase from recombinant *B. subtilis* is currently regulated by the FDA under 21 CFR § 173.115 as a secondary direct food additive permitted for use in food for human consumption (U.S. FDA, 2008). Moreover, the use of GOS in various foods and infant formula produced from lactose using a β -galactosidase, derived from *B. circulans* LOB 377 has recently been notified to FDA without objection from the agency (GRN000236). Finally, the use of *B. circulans* as a source of cycloglycosyltransferase for the production of *beta*-cyclodextrin is permitted in the European Union (EU) (Commission Directive 2003/95/EC) (Commission of the European Communities, 2003). Based on the widespread use of enzymes derived from strains of *B. circulans* in the U.S. and throughout the world, in conjunction with the extensive purification steps (activated carbon, and cation/anion-exchange chromatography) used during the manufacturing process, β -galactosidase, derived from *B. circulans* LOB 377 was concluded to be generally recognized as safe for its use in the production of GTC Nutrition's GOS. Unpublished toxicity studies conducted with enzyme and organism, as well as additional analytical data supporting the non-pathogenicity and non-toxic status of the organism and enzymes derived thereof were provided by the manufacturer of the enzyme, which further corroborate the safe use of the enzyme during the manufacture of GTC Nutrition's GOS.

Summary and Basis for GRAS Status of GTC Nutrition's GOS Ingredient Through Scientific Procedures

GTC Nutrition intends to market GOS, produced from lactose, as a food ingredient. The ingredient is manufactured under current Good Manufacturing Practices (cGMP) using suitable food grade ingredients and processing aids. Analytical data support that GOS is consistently manufactured to suitable food grade specifications, and that GOS are stable compounds with bulk stability studies supporting a shelf-life of at least 6 months. GTC's GOS also was shown to be stable in solution under a variety of temperatures and over a broad pH range supporting the use of GOS as an ingredient under the intended uses in food described herein.

GTC Nutrition's GOS ingredient is produced *via* the transgalactosylation of lactose using the enzyme β -galactosidase isolated from *B. circulans* LOB 377. Based on the widespread use of enzymes derived from strains of *B. circulans* in the U.S. and throughout the world, in conjunction with the extensive purification steps (activated carbon, and cation/anion-exchange chromatography) used during the manufacturing process, β -galactosidase, derived from *B. circulans* LOB 377 was concluded to be generally recognized as safe for its use in the production of GTC Nutrition's GOS. Similarly, the use of GOS in various foods and infant formula produced from lactose using a β -galactosidase, derived from *B. circulans* LOB 377 has recently been notified to FDA without objection from the agency (GRN000236). Unpublished toxicity studies conducted with enzyme and organism, as well as additional analytical data supporting the non-pathogenicity and non-toxic status of the organism and enzymes

derived thereof were provided by the manufacturer of the enzyme, which further corroborate the safe use of the enzyme during the manufacture of GTC Nutrition's GOS.

GTC Nutrition intends to market GOS as a food ingredient in the United States in a variety of food products including baby, infant and toddler foods, beverages and beverage bases, dairy product analogs, milk products, bakery products, beverages, cereal and other grain products, desserts, fruit and fruit juices, snacks, soups, and soft and hard candy, at use levels of 0.48 to 12.21% per serving. Consumption of these types of foods by the total U.S. population resulted in estimated mean and 90th percentile all-user intakes of GOS of 9.3 g/person/day (171.5 mg/kg body weight/day) and 15.4 g/person/day (350.6 mg/kg body weight/day), respectively. On an individual population basis, the greatest mean and 90th percentile all-user exposures were estimated to occur in male teenagers (aged 12 to 19 years), at 12.1 g/person/day (192.0 mg/kg body weight/day) and 20.2 g/person/day (335.2 mg/kg body weight/day), respectively. On a body weight basis, mean and 90th percentile all-user intakes of GOS were highest in infants, ages 0 to 2 years, with intakes of 498.5 and 814.5 mg/kg body weight/day, respectively.

Under infant formula uses GOS is intended for use as a food ingredient in infant formula and follow-on formula at concentrations not to exceed 0.72% (7.2 g of GOS per liter of infant formula) of the final reconstituted or ready-to-serve product. Under the conditions of intended use, estimated mean and 90th percentile intakes of GOS from the consumption of GTC's ingredient by infants aged 0 to 6 months of age were 5.9 and 8.5 g per person per day respectively (0.9 and 1.5 g/kg body weight respectively). Infants aged 7 to 12 months were estimated to consume respective quantities of 5.2 g (0.6 g/kg body weight) and 7.9 g (0.9 g/kg body weight) of GOS per person per day among mean and 90th percentile all-users. In toddlers aged 1 to 2, respective intakes of GOS for mean and 90th percentile all-users was 2.8 (0.3 mg/kg body weight) and 6.6 g (0.6 mg/kg body weight).

The safety of GTC Nutrition's GOS was based on the scientific procedures using generally available data. The test articles used in the studies obtained from the public domain were concluded to be representative to GTC Nutrition's GOS, and therefore product specific studies were not considered necessary to support the safe use of GTC Nutrition's GOS under the intended uses in food. A critical evaluation of this data by GTC Nutrition did not reveal any potential for adverse effects in infants and toddlers consuming GOS under the intended conditions of use.

Galactooligosaccharides and similar molecules (fructooligosaccharides) are naturally present in the diet, and therefore have an established history of apparent safe consumption.

Galactooligosaccharides, like most beta-linked sugars are not digested by human pancreatic or brush border enzymes, and the compounds are not absorbed intact in small intestine.

Galactooligosaccharides therefore travel intact through the gastrointestinal tract, where they are metabolized by the resident microflora of the colon to innocuous metabolites of normal microbial fermentation.

Published studies indicate that GOS are of low toxicity in animals. The acute toxicity of GOS has been reported to be >15 g/kg body weight in rats. Subchronic toxicity studies administering GOS *via* gavage or in the diet were unremarkable, with respective NOAEL determinations of 2,000 and 5,000 mg/kg body weight per day, the highest doses administered. The consumption of large dietary concentrations of GOS also was reported to be well tolerated in rats at repeat intakes in excess of ~10,000 mg GOS/kg body weight over a period 6 weeks. The only effect reported in animal studies in association with repeat exposure to GOS is increased cecal and colon weights a phenomenon that is not considered to have toxicological relevance to humans. The mutagenicity/genotoxicity of GOS was evaluated in the bacterial reverse mutation test and an mammalian chromosome aberration test and an *in vivo* mouse micronucleus assay; GOS was concluded not to be genotoxic or mutagenic.

The consumption of GOS preparations manufactured from lactose using various β -galactosidases has been evaluated in a large number of studies in humans administered GOS at quantities of 1 to 20 g (most studies administered 10 to 15 g) for periods of 6 days to 4 weeks. No adverse effects have been reported in any study to date.

In total, 23 studies including 1,836 infants consuming GOS supplemented infant formulas for a total of 8,757 subject years were reviewed as part of the safety evaluation of GTC Nutrition's GOS ingredient for use in infant formula. These studies included 1,059 infants consuming GOS supplemented infant formula's with GOS at concentration's of 7.2 g/L or higher for a period of 1,483 subject years. No adverse events, adverse effects on anthropometric indices of growth or effects on water balance leading to diarrhea have been reported to date. These studies also included 7 studies conducted in infants where GOS infant formula was administered within the first 2 weeks of life, for a duration of at least 3 months, and with sufficient power to detect clinically meaningful changes in weight gain. Two of these studies included GOS supplementation at a concentration of 7.2 g/L, one of which administered GOS supplemented infant formula for 6 months and included anthropometric follow-up visits at 12 and 24 months-post GOS consumption.

Finally, general recognition of safety is supported, in part, by the opinions of international regulatory bodies – The EU's Scientific Committee on Food and Food Standards Australia New Zealand – where the safe use of GOS:FOS mixtures produced from food grade lactose using enzymes derived from non-toxicogenic non-pathogenic organisms were determined to be safe in traditional food products and in infant formula at a maximum GOS concentration of 8 g/L.

CONCLUSION

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that GTC Nutrition's galactooligosaccharide (GOS) ingredient (trade-named Purimune) synthesized using a β -galactosidase obtained from *Bacillus circulans* LOB 377, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practice, is safe and suitable for use in the food products and infant formulas listed in Table 2.

We further conclude that the intended uses of GTC's GOS (trade named Purimune) ingredient synthesized from lactose using a β -galactosidase obtained from *B. circulans* LOB 377, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practice, are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

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List of U.S. FDA (2008) CFR Sections Referenced (Title 21—Food and Drugs)		
Part	Section §	Section Title
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	170.3	Definitions
173—Secondary direct food additives permitted in food for human consumption	173.115	Alpha-acetolactate decarboxylase (α -ALDC) enzyme preparation derived from a recombinant <i>Bacillus subtilis</i>
184—Direct food substances affirmed as generally recognized as safe		

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ATTACHMENT 1
EXPERT PANEL CURRICULA VITAE

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ATTACHMENT 2
GOS COMPARATIVE ANALYSES

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GOS COMPARATIVE ANALYSES

As shown in Table A.2-1 below, the composition of GTC Nutrition's GOS ingredient (Purimune) can be considered comparable to the GOS test article used in the subchronic toxicity study by Anthony *et al.* (2006). Both ingredients are synthesized from lactose using a beta-galactosidase obtained from *B. circulans* Jordan. The main difference between the 2 ingredients relates to the lower concentrations of residual lactose, and differences in moisture content where Vivinal GOS is marketed as a syrup and GTC Nutrition's ingredient is a spray-dried powder. However, as seen in Table A.2-1, the compositional distribution of the GOS oligomers is comparable between the 2 products, and with the exception of residual lactose and water, no additional differences were noted between the 2 products.

Table A.2-1 Comparison of GTC Nutrition's GOS to other Commercial Preparations		
Parameter*	Vivinal GOS Anthony <i>et al.</i> (2006)	Purimune Lot 200802001
Manufacturing		
Starting Material	Lactose	Lactose
Enzyme	β -Galactosidase	β -Galactosidase
Enzyme: Source Organism	<i>Bacillus circulans</i> Jordan LOB 377*	<i>Bacillus circulans</i> Jordan LOB 377
Ingredient Composition		
Monosaccharide (%DM)	19%	0 to 1%
Lactose (%DM)	31.5%	7 to 10%
GOS(%DM)	73 - 78%	90 to 92%
GO-2 (% GOS DM)	14%	16.8
GO-3 (% GOS DM)	44.1%	44.7
\geq GO-4 (% GOS DM)	36.5%	28.7
Nitrogen	Max 0.016%	Negative
Ash	Max 0.3%	Max 0.05%
Lead	N/A	Max 0.01 ppm
Arsenic	N/A	Max 1.0 ppm

N/A = Information not available; * The test article used in the study by Anthony *et al.* (2006) (Vivinal GOS) is a product of Friesland Foods. Information characterizing the composition of the ingredient (Vivinal GOS) was obtained from Goulas *et al.* (2002); Montilla *et al.* (2006); Anthony *et al.* (2006); GRN000236.

*ATCC 31382

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APPENDIX C
BATCH ANALYSES

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GALACTOOLIGOSACCHARIDE GRAS NOTICE

Table C-1 Chemical and Microbiological Specifications: Analytical Data				
Specification Parameter	Specification	Manufacturing Lot		
		20080103	200802001	20080305
Physical/Chemical				
Appearance	White Powder	White Powder	White Powder	White Powder
Moisture	Max 10.0	2.41	2.76	2.30
pH	4.5 – 7.0	5.82	5.73	5.51
Oligosaccharide (%DB)	90 – 92.0	91.5	90.2	91.8
Carbohydrate profile				
Residual Sugar (%DB)	8.0 – 10.0	8.5	9.8	8.2
Galactooligosaccharide (%DB)	90.0 – 92.0	91.5	90.2	91.8
GO-2 (%DB)	16.0 – 21.0	18.8	16.8	17.7
GO-3 (%DB)	14.0 – 19.0	18.7	18.8	18.0
GO-3 (β-1,4)(%DB)	16.0 – 20.0	19.1	17.7	17.5
GO-3 (β-1,6)(%DB)	8.0 – 13.0	9.0	8.2	10.4
GO-4 + (%DB)	25.0 – 29.0	25.9	28.7	28.2
Granule Size (% Through 40 mesh)	100	100	100	100
Ash (%w/w)	Max 0.05	0.01	0.01	0.02
Protein (ppm) (LOD = 10 ppm)	Negative	Negative	Negative	Negative
Lead (mg/kg)	Max 0.01	ND	ND	ND
Arsenic (as AS ₂ O ₃) (mg/kg)	Max 1.0	ND	ND	ND
Foreign Substance	Negative	Negative	Negative	Negative
Foreign taste and odor	Negative	Negative	Negative	Negative
Microbiological (counts/g)				
Mesophilic bacteria	Max 300	55	100	150
Mold and yeast	Max 20	13	10	8
Coliforms	Negative	Negative	Negative	Negative
Anaerobic thermophilic spores	Max 10	Negative	Negative	Negative
Aerobic thermophilic spores	Max 10	Negative	Negative	Negative
Anaerobic mesophilic spores	Max 10	Negative	Negative	Negative
Aerobic mesophilic spores	Max 10	Negative	Negative	Negative
<i>Salmonella</i>	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative	Negative	Negative	Negative
<i>Escherichia coli</i>	Negative	Negative	Negative	Negative
Listeria (tested in 50 g)	Negative	Negative	Negative	Negative

NA = not available; CFU = colony forming units; DB = dry basis; ND = Not detected; LOD = Limit of Detection

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SUBMISSION END

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