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ORIGINAL SUBMISSION

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Soni & Associates Inc.

749 46th Square
Vero Beach, FL 32968, USA
Telephone: 772-299-0746
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GRN 000623

January 7, 2016

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

Subject: GRAS Notification for Fructooligosaccharides

Dear Sir/Madam:

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

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

Sincerely,

(b) (6)

Madhu G. Soni, Ph.D.

B. Name and Address of Notifier:

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

Tel: +86 766-8750999 / 8750777
Fax: +86 766-8750800
Email: hank.tsai@king-prebiotics.com

C. Common or usual name of the GRAS substance:

The common name of the substance of this GRAS assessment is fructooligosaccharides (FOS) or oligofructose. FOS for food uses will be marketed as standardized syrup and powder.

D. Conditions of use:

Fructooligosaccharides (FOS) are intended for use in food categories such as Acidophilus Milk; Analogs and Substitutes for Meat, Poultry or Fish; Bars; Breakfast Cereals; Beverages and Juices; Cakes; Cheese; Cream; Confectionery; Cookies; Crackers; Dessert Toppings and Fillings; Hard candy; Ice cream; Infant Foods; Jams and Jellies; Milk, flavored and unflavored; Milk, evaporated and condensed; Muffins and Quick Bread; Sauces, Gravies, and Condiments; Snacks; Sorbet and Sherbet; Soup; Toddler Foods (12-24 months); and Yogurt (all food categories mentioned in GRN 44 and in additional correspondence of June 1, 2007 between GTC Nutrition and FDA) at use levels of 0.4-6.7%. The estimated daily intake for the general population, excluding infants less than one year old, is estimated to be as high as 6.2 g/day for the mean consumer, and as high as 12.8 g/day for the 90th percentile consumer. The addition of FOS is safe for use in foods in general at levels up to 20 g/day in the general population and at levels up to 4.2 g/day in infants less than one year of age from the intended uses in infant foods.

FOS is also intended as a prebiotic in non-exempt infant formula at the maximum intended addition levels of 400 mg FOS/100 ml in starter formula as consumed and 500 mg FOS/100 ml in follow-on formula as consumed. For infant formula powders with a hydration rate of 12.7 g/100 ml, these levels are equivalent to 31.5 and 39.4 mg FOS/g powder, respectively. The intended uses and levels of FOS in non-exempt infant formula are similar to those described in GRN 537 (Ingredion, 2014). Based on energy intakes and the energy content of infant formula, the 90th percentile formula intake for males and females combined is estimated as 207 ml/kg body weight (bw)/day. The 90th percentile intake of FOS is estimated as 828 mg/kg bw/day from starter formula within the first month of life and about 800 mg/kg bw/day from the follow-on formula thereafter. FOS is not intended for use in meat and poultry products that come under USDA jurisdiction.

E. Basis for GRAS Determination:

#623

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GRN 000623

GRAS NOTIFICATION

I. Claim of GRAS Status

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

New Francisco Biotechnology Corporation (NFBC), China, has determined that fructooligosaccharides derived from enzymatic conversion of sucrose is Generally Recognized As Safe under the conditions of its intended use in conventional foods, including non-exempt infant formula and is therefore exempt from the requirement of premarket approval, under the conditions of its intended use. This determination is based on scientific procedures as described in the following sections, under the conditions of fructooligosaccharides's intended use in food, among experts qualified by scientific training and expertise. NFBC is also of the opinion that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

Signed,

(b) (6)

Date: 1/16/16

Madhu G. Soni, PhD, FACN, FATS

Agent for:

New Francisco Biotechnology Corporation
Yunfu City, Guangdong Province,
CHINA

In accordance with 21 CFR 170.30, FOS has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. A comprehensive search of the scientific literature was utilized for this determination. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data to determine safety-in-use for FOS. In recent years, FOS has been the subject of three GRAS notifications (GRN 537; GRN 392; and GRN 44). Among these notices, the GRAS notice GRN 392 was for a fructan oligosaccharide that includes FOS and inulin. Of the three GRAS notices, two (GRN 537 and GRN 392) were submitted for uses of the ingredient in infant formula. In response to all of these notices, FDA did not question the conclusions that the use of FOS is GRAS under the conditions of use described in the notices. The safety determination of FOS for the present GRAS assessment is based on the totality of available scientific evidence that includes human observations and a variety of preclinical and clinical studies. An Expert Panel was assembled to evaluate the health aspects of FOS for the intended uses. The Expert Panel unanimously concluded that, based on the available safety-related information, the estimated daily intake, if ingested daily over a lifetime, under the intended uses discussed in this dossier, FOS is safe.

F. Availability of Information:

The data and information that forms the basis of this FOS GRAS determination will be available at the above mentioned New Francisco Biotechnology Corporation (NFBC) address in China as well as at the following address in the USA:

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

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

FOS is derived from food grade sucrose via a transfructosylation catalyzed by β-fructofuranosidase enzyme derived from a non-pathogenic and non-toxigenic strain of *Aspergillus oryzae.*

A. Synonyms and Trade Name:

FOS; Oligofructose; short-chain fructooligosaccharide (scFOS or FOS); Neosugar. The systematic name of all fructans, including scFOS, is [α-D-glucopyranoside-(1-2)-]-β-D-fructofuranosyl-[(1-2)-β-D-fructofuranosyl]$_n$.

The subject of this GRAS assessment will be marketed under the trade name King-Prebiotics® FOS (FOS-300-P, FOS-500-S; FOS-550-S; FOS-700-S; FOS-750-S;; FOS-900-S; FOS-950-S; FOS-700-P; FOS-750-P; FOS-900-P; FOS-950-P).

B. Physical Characteristics

Colorless to light yellow syrup and off white color powder

C. Chemical Abstract Registry (CAS) Number

The CAS Registry Number for fructooligosaccharide is 308066-66-2.

D. Chemical Formula and Molecular Weight

Fructooligosaccharides (FOS) are a mixture of oligosaccharides consisting of a sucrose molecule (glucose - fructose disaccharide, GF1) linked to one (GF2), or two (GF3) or three (GF4) additional fructose units added by β2-1 glycosidic linkages to the fructose unit of the sucrose. Fructans can have degrees of polymerization (the number of fructose or glucose residues) ranging from 2 to over 60. Short chain FOS consists entirely of molecules with degrees of polymerization between 3 and 5, consisting of 2 to 4 fructose residues and a single terminal glucose residue. FOS, the subject of this present GRAS dossier, primarily consists of 3 different molecules, each containing a terminal glucose residue and 2, 3, or 4 fructose residues, designated as GF_2, GF_3, and GF_4, also called as 1-kestose, nystose, and fructofuranosylnystose, respectively.

E. Structure

The molecular formula for all fructans is $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$. The formulas of its three components are: 1-kestose - $C_{18}H_{32}O_{16}$, nystose - $C_{24}H_{42}O_{21}$, and fructofuranosylnystose - $C_{30}H_{52}O_{26}$. The molecular weight of scFOS is 700, representing the average of the molecular weights of its 3 components (505, 666, and 828, respectively), respectively. The structural formulas of 1-kestose, nystose, and fructofuranosylnystose are shown in Figure 1.

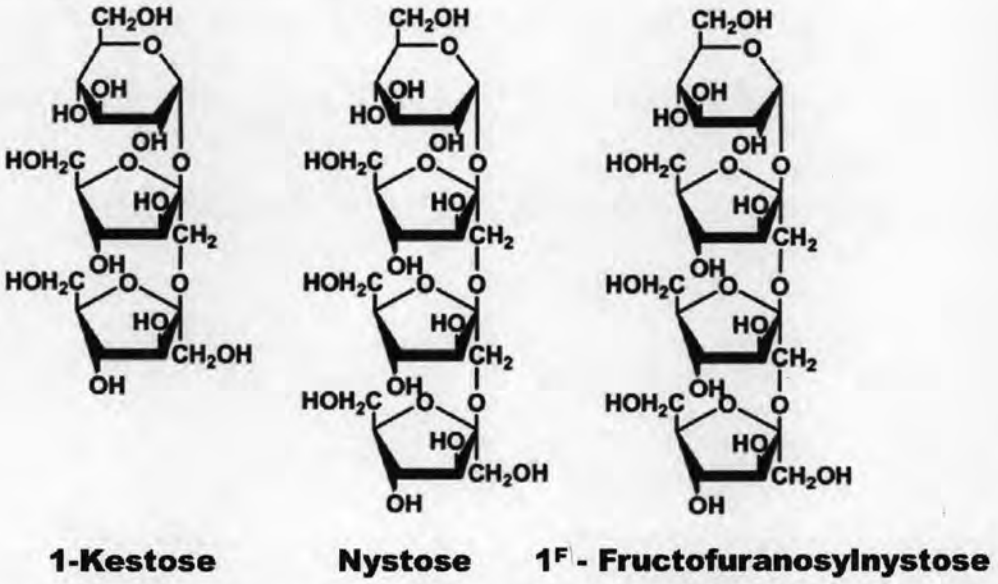

Figure II.E.1. Structural Formulas of FOS components 1-Kestose, Nystose, and Fructofuranosylnystose

F. Food Grade Specifications

Food grade specifications of FOS have been established by New Francisco Biotechnology Corporation (NFBC). FOS will be marketed in the U.S. in the form of powder and syrup. The specifications for FOS-300-P, FOS-550-S and FOS-950-P are presented in Tables II.F.1, II.F.2 and II.F.3, respectively. A copy of the specification sheet provided by NFBC for all the products FOS-300-P, FOS-500-S; FOS-550-S; FOS-700-S; FOS-750-S;; FOS-900-S; FOS-950-S; FOS-700-P; FOS-750-P; FOS-900-P; FOS-950-P is included as Appendix V (included at the end of this document). To demonstrate conformance with the food-grade

specifications, NFBC analyzed several batches of FOS. Analytical results from five lots (Tables II.F.1, II.F.2, II.F.3) suggest that FOS is consistently manufactured to meet the standard specifications. The specification parameters comprise physical appearance, purity, total FOS levels, moisture, sulphated ash, as well as limits for potential chemical and microbiological impurities, and contaminants. The average distribution ratio of FOS components [1-kestose (GF2), Nystose (GF3) and Fructofuranosylnystose (GF4)] for FOS-550-S and FOS-950-P is presented in Table II.F.2 and II.F.3. The subject of this GRAS determination, FOS, is substantially equivalent to the FOS that was the subject of the GRAS notified substances reviewed by the FDA without any questions [including GRN 537 (Ingredion, 2014) and GRN 44 (GTC Nutrition, 2000)].

Table II.F.1. Food Grade Physical and Chemical Specifications of FOS (FOS-300-P)

Parameters	**Standard Specifications**	**Batch #1401**	**Batch#1402**	**Batch #1404**	**Batch #1405**	**Batch #1406**
Appearance	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder
Taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste
G2+G3+G4(d.m.)	≥30%	31.2	31.5	31.7	30.8	30.6
Sucrose+Monosaccharides(d.m.)	≤26%	23.4	24.5	23.6	24.1	24.7
Maltodextrin(d.m.)	≤55%	47.5	48.4	46.7	48.9	48.5
pH (30%)	4.5-7.0	5.6	6.1	5.5	6.0	5.7
Moisture	≤3.5%	2.1	2.3	2.3	2.5	2.2
Sulphated ash	≤0.1%	0.02	0.02	0.01	0.02	0.03
Heavy metals						
Lead	≤0.02 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Total Arsenic	≤0.05 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	≤0.1 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Total mercury	≤0.01 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Melamine	≤0.01 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological limits						
Total plate count	≤1000 CFU/g	<10	<10	<10	<10	<10
Yeasts	≤20 CFU/g	<10	<10	<10	<10	<10
Moulds	≤20 CFU/g	<10	<10	<10	<10	<10
Coliforms	<30MPN/100g	<30	<30	<30	<30	<30
E. coli	<3.0MPN/100g	<3.0	<3.0	<3.0	<3.0	<3.0
Salmonella	Negative/25g	Negative	Negative	Negative	Negative	Negative
Shigela	Negative/25g	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative/25g	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	<0.3MPN/g	<0.3	<0.3	<0.3	<0.3	<0.3
Listeria	Negative/25g	Negative	Negative	Negative	Negative	Negative
Bacillus cereus	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0
Anaerobic sulfite-reducing clostridia	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0

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Table II.F.2. Food Grade Physical and Chemical Specifications of FOS (FOS-550-S)

Parameters	Standard Specifications	Batch #1402	Batch#1403	Batch #1404	Batch #1405	Batch #1406
Appearance	Off white light yellow syrup	Off white light yellow syrup	Off white light yellow syrup	Off white light yellow syrup	Off white light yellow syrup	Off white light yellow syrup
Taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste
Total scFOS	≥55%	56.4	55.8	56.7	56.7	57.2
1-kestose (GF2)	NLT 24.0	25.5	25.3	25.8	26.4	25.2
Nystose (GF3)	NLT 24.0	26.1	25.8	26.6	25.8	27.2
Fructofuranosylnystose (GF4)	NLT 3.0	4.8	4.7	4.3	4.5	4.8
Sugars	≤45%	43.6	44.2	43.3	43.3	42.8
Dry matter(d.m.)	≥75%	75.5	76.0	75.5	76.5	76.0
pH (30%)	4.5-7.0	5.8	6.0	5.7	6.2	6.3
Ash	≤0.1%	0.01	0.01	0.02	0.03	0.02
Heavy metals						
Lead	≤0.02 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Total Arsenic	≤0.05 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	≤0.1 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Total mercury	≤0.01 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Melamine	≤0.01 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological limits						
Total plate count	≤1000 CFU/g	<10	<10	<10	<10	<10
Yeasts	≤20 CFU/g	<10	<10	<10	<10	<10
Moulds	≤20 CFU/g	<10	<10	<10	<10	<10
Coliforms	<30MPN/100g	<30	<30	<30	<30	<30
E. coli	<3.0MPN/100g	<3.0	<3.0	<3.0	<3.0	<3.0
Salmonella	Negative/25g	Negative	Negative	Negative	Negative	Negative
Shigela	Negative/25g	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative/25g	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	<0.30MPN/g	<0.30	<0.30	<0.30	<0.30	<0.30
Listeria	Negative/25g	Negative	Negative	Negative	Negative	Negative
Bacillus cereus	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0
Anaerobic sulfite-reducing clostridia	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0

Table II.F.3. Food Grade Physical and Chemical Specifications of FOS (FOS-950-P)

Parameters	Standard Specifications	Batch #1401	Batch#1402	Batch #1403	Batch #1404	Batch #1405
Appearance	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder	Off white powder
Taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste	Slightly sweet taste
Total scFOS	≥95%	96.7	96.5	96.2	95.9	95.8
1-kestose (GF2)	NLT 30.0	39.3	36.4	35.8	37.2	35.9
Nystose (GF3)	NLT 40.0	46.1	49.1	48.4	45.9	48.2
Fructofuranosylnystose (GF4)	NLT 5.0	11.3	11.0	12.0	12.8	11.7
Sugars	≤5%	3.3	3.5	3.8	4.1	4.2
pH (30%)	4.5-7.0	5.8	6.1	5.6	6.2	6.0
Moisture	≤3.5%	2.2	2.1	2.3	2.5	2.3
Ash	≤0.1%	0.02	0.03	0.02	0.01	0.02
Heavy metals						
Lead	≤0.02 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Total Arsenic	≤0.05 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium	≤0.1 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Total mercury	≤0.01 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Melamine	≤0.01 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01
Microbiological limits						
Total plate count	≤1000 CFU/g	<10	<10	<10	<10	<10
Yeasts	≤20 CFU/g	<10	<10	<10	<10	<10
Moulds	≤20 CFU/g	<10	<10	<10	<10	<10
Coliforms	<30MPN/100g	<30	<30	<30	<30	<30
E. coli	<3.0MPN/100g	<3.0	<3.0	<3.0	<3.0	<3.0
Salmonella	Negative/25g	Negative	Negative	Negative	Negative	Negative
Shigela	Negative/25g	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative/25g	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	<0.3MPN/g	<0.3	<0.3	<0.3	<0.3	<0.3
Listeria	Negative/25g	Negative	Negative	Negative	Negative	Negative
Bacillus cereus	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0
Anaerobic sulfite-reducing clostridia	<3.0MPN/g	<3.0	<3.0	<3.0	<3.0	<3.0

G. Production Process

FOS is manufactured according to current good manufacturing practices (cGMP) and ISO standards, as outlined in Figure II.G.1, at New Francisco (Yunfu) Biotechnology Corporation (NFBC) facilities located at Swan-kan-chiau Ind. Dist., Kaofong Village, Yunfu City, Guangdong, Zip: 527343, China. The manufacturing details of different products form of powder and syrup are shown in Figure 2. Fructooligosaccharides (FOS) such as 1-kestose, nystose, and fructosyl-nystose are produced by the treatment of sucrose with a food-grade preparation of β-

fructofuranosidase. In general, β-fructofuranosidase hydrolyzes sucrose to glucose and fructose. At high concentrations of sucrose, some β-fructofuranosidases can transfer the fructosyl residue to the sucrose molecule, in which fructosyl residues are transferred to sucrose by β-2,1 glycosidic bonds.

The enzyme, β-fructofuranosidase, used in the manufacturing of FOS is derived from *Aspergillus oryzae*. It is a well-known commercial enzyme commonly used for the production of FOS. The β-D-fructofuranosidases enzyme preparation meets the general and additional requirements for enzyme preparations as outlined in the monograph on Enzyme Preparations in the Food Chemicals Codex. The β-D-fructofuranosidases preparation is produced in accordance with current good manufacturing practices, using ingredients that are acceptable for general use in foods, and under conditions that ensure a controlled fermentation. These methods are based on generally available and accepted methods used for production of microbial enzymes (Aunstrup, 1979; Aunstrup et al., 1979, Enzyme Applications, 1994).

The β-D-fructofuranosidases enzyme preparation is derived from a pure culture of a nonpathogenic, nontoxigenic strain of *Aspergillus oryzae*, which is registered with the American Type Culture Collection (ATCC). The specific *A. oryzae* strain used in the production of FOS is considered as proprietary. *A. oryzae* is the source organism for many enzyme preparations that are considered to be GRAS. These include glucose oxidase (GRASN # 106), lipase (GRASN #43, #75, and #103), aspartic proteinase (GRASN #34), exopeptidase (GRASN #10), pectin esterase (GRASN # 8), and protease and carbohydrase (GRASN #90). Enzymes from *A. oryzae* are accepted as a constituent of foods (JECFA, 1987). A. *oryzoe* has been used to produce soy sauce in the United States since before 1958 (Barbesgaard, 1992; IFBC, 1990) and carbohydrase and protease (GRN #90). Therefore, ingredients from *A. oryzae* meet the criterion of "common use in foods in the US before 1958" and can be considered "generally recognized as safe" (IFBC, 1990).

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

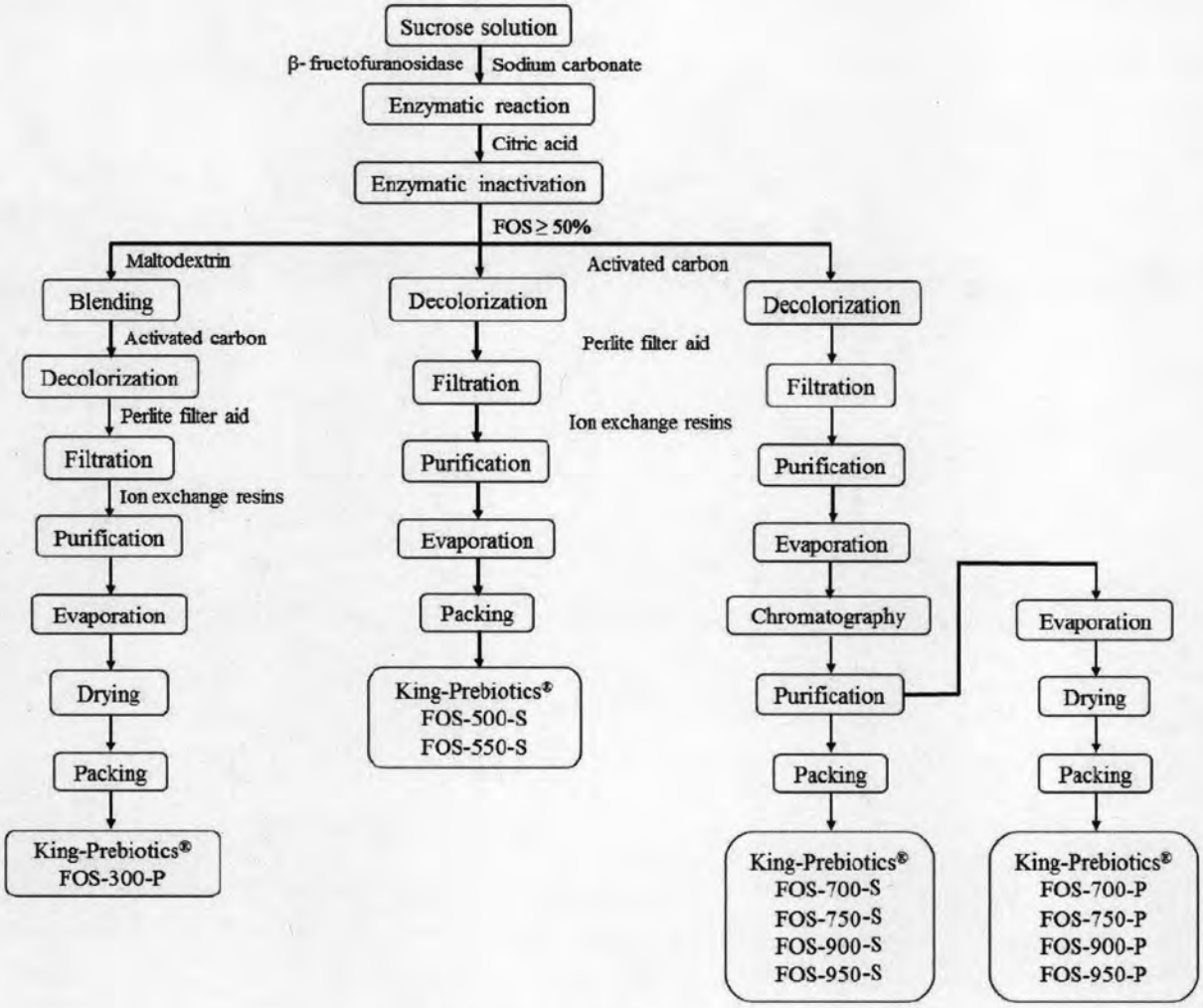

Figure II.G.1. Manufacturing Process of FOS

III. Summary of the Basis for NFBC's Determination that FOS is GRAS

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by New Francisco Biotechnology Corporation (NFBC) to determine the Generally Recognized As Safe (GRAS) status of FOS. The Expert Panel consisted of the following individuals: Professor Douglas L. Archer, Ph.D. (University of Florida and Retired FDA Deputy Director, CFSAN); Robert L. Martin, Ph.D. (Retired FDA Deputy Director of DBGNR/OFAS); and Madhusudan G. Soni, PhD, FACN, FATS (Food Ingredient Safety Consultant). Given Dr. Archer's background in microbiology, he was also assigned to specifically review the safety aspects related to the use of microorganism, *Aspergillus oryzae* and the enzyme, β-fructofuranosidase, derived from it.

A comprehensive search of the scientific databases for safety and toxicity information on FOS was conducted through November 2015. Additionally, safety and regulatory evaluations by national and international agencies were also searched and considered for the present assessment.

The Expert Panel also reviewed all accessible information in the GRAS Notices on FOS that are publicly available in FDA's GRAS Notice inventory. Based on a critical evaluation of the pertinent data and information summarized herein, and employing scientific procedures, the Expert Panel members have individually and collectively determined that the addition of FOS to the foods [such as Acidophilus Milk; Analogs and Substitutes for Meat, Poultry or Fish; Bars; Breakfast Cereals; Beverages and Juices; Cakes; Cheese; Cream; Confectionery; Cookies; Crackers; Dessert Toppings and Fillings; Hard candy; Ice cream; Infant Foods; Jams and Jellies; Milk, flavored and unflavored; Milk, evaporated and condensed; Muffins and Quick Bread; Sauces, Gravies, and Condiments; Snacks; Sorbet and Sherbet; Soup; Toddler Foods (12-24 months); and Yogurt (all food categories mentioned in GRN 44 and in additional correspondence of June 1, 2007 between GTC Nutrition and FDA) at use levels of 0.4-6.7%], and to non-exempt infant formula at the maximum intended addition levels of 400 mg FOS/100 ml in starter formula as consumed and 500 mg FOS/100 ml in follow-on formula as consumed, when not otherwise precluded by a Standard of Identity, meeting the specification cited above and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use, as specified herein.

In arriving at this decision that FOS is GRAS, the Expert Panelists relied upon the conclusions that neither FOS nor any of its constituents pose any toxicological hazards or safety concerns at the intended use levels in conventional foods and in non-exempt infant formula, as well as on published toxicology studies and other articles relating to the safety of the product. It is also the opinion of the Expert Panelists that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

The GRAS Panel did not prepare a separate report or statement, but reviewed the entire GRAS dossier. FOS was the subject of three GRAS notifications (GRN 537; GRN 392; and GRN 44) to the FDA for use as a food ingredient, including infant formula. The safety information and other relevant information are hereby incorporated by reference into this document and was considered in evaluating the GRAS status of NFBC's proposed use of FOS in conventional foods and in non-exempt infant formula. A synopsis of the pertinent information in these documents is presented below.

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

TABLE OF CONTENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF FRUCTOOLIGOSACCHARIDES AS FOOD INGREDIENT

1. EXECUTIVE SUMMARY

At the request of New Francisco Biotechnology Corporation (NFBC), China, a comprehensive search of the scientific literature for safety and toxicity information on FOS was conducted by Soni & Associates Inc. to determine the Generally Recognized As Safe (GRAS) status of FOS as a food ingredient. NFBC intends to use FOS as a food ingredient in Acidophilus Milk; Analogs and Substitutes for Meat, Poultry or Fish; Bars; Breakfast Cereals; Beverages and Juices; Cakes; Cheese; Cream; Confectionery; Cookies; Crackers; Dessert Toppings and Fillings; Hard candy; Ice cream; Infant Foods; Jams and Jellies; Milk, flavored and unflavored; Milk, evaporated and condensed; Muffins and Quick Bread; Sauces, Gravies, and Condiments; Snacks; Sorbet and Sherbet; Soup; Toddler Foods (12-24 months); and Yogurt (all food categories mentioned in GRN 44 and in additional correspondence of June 1, 2007 between GTC Nutrition and FDA) at use levels of 0.4-6.7%. In addition to its use in the above described food categories, FOS is also intended for use in non-exempt infant formula at the maximum intended addition levels of 400 mg FOS/100 ml in starter formula as consumed and 500 mg FOS/100 ml in follow-on formula as consumed. The exposure from added FOS in the proposed food and non-exempt infant formula is considered as safe. As described below, the weight of evidence clearly supports the safety and GRAS status of FOS, when produced in accordance with cGMP to food-grade specifications, for its intended use. No studies were identified showing any adverse effects when this amount of FOS is added to the diet.

1.1. Background

In recent years, fructooligosaccharides (FOS) have received considerable attention for their potential health benefits. FOS occurs naturally in many plants (Mitsuoka et al. 1987, Spiegel et al. 1994, Tashiro et al. 1992) and has been shown to exhibit beneficial health effects by stimulating the growth of bifidobacteria in the human colon (Gibson and Roberfroid 1995, Hidaka et al. 1986, Tomomatsu, 1994). The health benefits derived from the colonic fermentation of FOS in humans are well documented (Gibson and Roberfroid 1995). FOS have a number of interesting properties, including a low sweetness intensity. They are also low calorie, non-cariogenic and are considered as soluble dietary fiber. Additionally, FOS has been claimed for physiological effects such as improved mineral absorption and decreased levels of serum cholesterol, triacylglycerols and phospholipids (Sabater-Molina et al., 2009). Because of their prebiotic effects, currently FOS are increasingly included in food products and infant formulas. Their consumption increases fecal bolus and the frequency of depositions, and may help reduce constipation. Furthermore, FOS can improve food taste and texture. Given the potential health benefits of FOS, NFBC intends to use it as a food ingredient in selected food categories.

1.2. Description, Manufacturing Process and Specifications

As described earlier, the subject of this GRAS determination, FOS is off white to light yellow color syrupy liquid or powder with a slight sweet taste. It is produced by enzymatic biotransformation of sucrose by the action of catalyzed by β-fructofuranosidase enzyme derived from *Aspergillus oryzae*. The microorganism used in the production of enzyme is non-toxigenic and non-pathogenic. FOS is a mixture composed of fructose chains with a terminal glucose unit.

The number of fructose units varies from two to four. The first fructose unit that is attached to glucose is joined by an α-1-1' glycosidic linkage. The remaining fructose units are joined to the first fructose unit in a chain by β-2-1 glycosidic linkages. The identity and specifications of FOS have been fully developed (see Section II). Food grade specifications of FOS are presented in Tables II.F.1 – II.F.3. The manufacturing process is summarized in Figure II.G.1.

1.3. Natural Occurrence

Fructooligosaccharides (FOS) are oligosaccharides that occur naturally in plants such as onion, chicory, garlic, asparagus, banana, artichoke, Jerusalem artichokes, lettuce, rye, among many others (GTC Nutrition, 2000; Bornet et al., 2002; Sabater-Molina et al., 2009). Given their natural presence in commonly consumed vegetables, FOS is regularly consumed by humans in foods. Some grains and cereals, such as wheat and barley, also contain FOS (Campbell et al., 1997). The Jerusalem artichoke and its relative yacon[1] together with the Blue Agave plant have been reported to contain the highest concentrations of FOS of cultured plants. Campbell et al. (1997a) extensively analyzed and characterized the naturally occurring FOS levels in a variety of plants. Of the 25 samples analyzed for FOS content, 20 showed detectable levels of FOS. In these samples, the FOS content ranged from 0.1-0.2 mg/g for most (12/20) of the fruits. The highest FOS content was found in ripe bananas, which contained 2.0 mg/g FOS. Of the 40 vegetable samples analyzed, 16 did not contain FOS. An additional 6 vegetables contained 0.1 or 0.2 mg/g FOS, while the remaining 16 vegetables contained from 0.3 to 58.4 mg/g FOS.

The available information suggests that humans consume FOS on a daily basis following ingestion of plants that naturally contain FOS. An estimate of FOS intake from commonly consumed plants was provided in GRN 44 (GTC Nutrition, 2000). For this analysis, data provided by Campbell et al. (1997) for the content of FOS was used along with food intake data available for the U.S. population from the 1994-96 United States Department of Agriculture's (USDA) Continuing Survey of Food Intakes by Individuals (CSFII). Based on the foods included in the analysis reported by Campbell et al. (1997), the mean FOS intake for adults in the U.S. was estimated as 114 mg/day. For adults, an upper bound estimate of daily FOS intake, based on the 90th percentile food intake was determined as 248 mg/day. The food types that contributed the most to FOS consumption were onions, bananas, lettuce, and wheat (in rough and bran forms).

1.4. Current Uses

FOS and other prebiotic ingredients are increasingly being recognized as useful dietary tools for the modulation of the colonic microflora toward a healthy balance. FOS represents only a fraction of the inulin class of carbohydrates known as fructans. This class includes different chain length polymers such as inulin, oligofructose and FOS. Thus, inulin is a composite oligosaccharide that contains several FOS molecules. These polymers are chemically similar entities and share the same basic structure of β (2-1) linked fructosyl units, sometimes ending with a glucosyl unit. As all these fractions are mixtures of molecules that differ only in chain length, they can be described by their range and average degree of polymerization. Various terms describing fructans have been used interchangeably in the published literature. Currently, there are several commercial sources of FOS, inulin, and oligofructose. These products are sold and

[1] The yacón is a species of perennial daisy traditionally grown in the northern and central Andes from Colombia to northern Argentina for its crisp, sweet-tasting, tuberous roots.

consumed as fat replacements and sugar substitutes for use in a variety of foods such dairy products, candies and chocolates, spreads, baked goods and breakfast cereals, meat products, ice cream and frozen yogurt (GTC Nutrition, 2000). In the U.S., FOS is sold as a nutritional supplement at recommended doses of up to 4 to 8 g/day to promote the growth of bifidobacteria, and as an ingredient in nutritional supplement liquids as a source of dietary fiber.

Based on information from FDA's GRAS Notice Inventory[2] website as of April 28, 2015, the agency has received three notices on FOS and provided "no questions" letters to all of the notifiers. In May 01, 2000, GTC Nutrition Company submitted GRAS notification (GRN 44) to FDA for use of FOS in different food categories (GTC Nutrition 2000). On November 22, 2000, FDA issued "no questions" letter for this GRAS notice (FDA, 2000). Subsequently, two GRAS notifications were submitted to FDA for use of FOS in infant formulas by: Pfizer Nutrition (2011; GRN 392) and by Ingredion Incorporated (2014; GRN 537). Both these firms received a "no questions" letter from FDA (FDA, 2011, 2015, respectively). A closely related oligosaccharide, galacto-oligosaccharide, has also been determined to be GRAS for use in a variety of foods in nine GRAS notifications to the FDA. All these GRAS notices are available at FDA's GRAS Notice Inventory.

1.5. Intended Use Levels and Food Categories

FOS is intended for use in the same foods and at identical use levels mentioned in the GRN 44 and in the subsequent additional correspondence letter of June 1, 2007 for GRAS notice 44. There are no new food uses proposed by NFBC for FOS. The substance mentioned in GRN 44 (GTC Nutrition, 2000) has been reported to contain $\geq$ 95% FOS, which is similar to the subject of this GRAS determination. Thus, NFBC intends to use FOS as a food ingredient in Acidophilus Milk; Analogs and Substitutes for Meat, Poultry or Fish; Bars; Breakfast Cereals; Beverages and Juices; Cakes; Cheese; Cream; Confectionery; Cookies; Crackers; Dessert Toppings and Fillings; Hard candy; Ice cream; Infant Foods; Jams and Jellies; Milk, flavored and unflavored; Milk, evaporated and condensed; Muffins and Quick Bread; Sauces, Gravies, and Condiments; Snacks; Sorbet and Sherbet; Soup; Toddler Foods (12-24 months); and Yogurt (all food categories mentioned in GRN 44 and in additional correspondence of June 1, 2007 between GTC Nutrition and FDA) at use levels of 0.4-6.7%. The food categories and the intended use levels of FOS described in GRN 44 and additional correspondence are summarized in Table 2. In the January 26, 2007 letter to FDA, GTC Nutrition mentioned that the maximum daily intake of FOS, when used in foods, beverages and supplements, for the general population, one year of age or greater, is 20 g and 4.2 g for infants (from uses in infant foods, not from infant formula) less than one year of age in accordance to good manufacturing practices.

In addition to the above described general food categories, NFBC also intends the use of FOS in non-exempt infant formula. The maximum intended addition levels of FOS are 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed. For infant formula powders with a hydration rate of 12.7 g/100 ml, these levels are equivalent to 31.5 and 39.4 mg FOS/g powder, respectively. The intended uses and use levels for infant formula are same as those described in GRN 537 (Ingredion, 2014).

[2]Accessible at: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true.

Table 2. Typical Use Levels of Fructooligosaccharides in General Food Categories*

Food Category	Standard Serving Size	Level of Use per Serving (percent)
Acidophilus Milk	240 milliliters (ml)	0.4
Analogs and Substitutes for Meat, Poultry or Fish	15-85 grams (g)	1.2-6.7
Bars	40-70 g	1.4-2.5
Breakfast Cereals	40-55 g	1.8-2.5
Beverages and Juices	240 ml	0.4
Cakes	55 g	1.8
Cheese	30-110 g	0.9-3.3
Cream	15-30 g	3.3-6.7
Confectionery	40 g	2.5
Cookies	30 g	3.3
Crackers	15-30 g	3.3-6.7
Dessert Toppings and Fillings	30 g	3.3
Hard candy	15 g	6.7
Ice cream	68 g	1.5
Infant Foods ** (0-12 months)	7-60 g	0.4-3.6
Jams and Jellies	20 g	5.0
Milk, flavored and unflavored	240 ml	0.4
Milk, evaporated and condensed	30 ml	2.6-3.1
Muffins and Quick Bread	50-55 g	1.8-2.0
Sauces, Gravies, and Condiments	30-125 g	0.8-3.3
Snacks	30 g	3.3
Sorbet and Sherbet	85 g	1.2
Soup	245 g	0.4
Toddler Foods (12-24 months)	15-125 g	0.8-6.7
Yogurt	225 ml	0.4

Adapted from GRN 44 Additional Correspondence (FDA, 2007)

*Use levels may be different from the intended use in the original notice.

**This category excludes infant formula; it should be noted that infant formula uses are separately described.

Table 3. Typical Use Levels of Fructooligosaccharides in Infant Formula

Formula type	Use levels mg/100 ml	Infant Formula Powder* mg/g powder
Starter Formula	400	31.5
Follow-on-Formula	500	39.4

*Hydration rate of 12.7 g/100 ml

1.5.1. Estimated Daily Intake from the Intended Uses

As indicated above, FOS is intended for use in the same foods and at identical levels of addition as notified by GTC Nutrition in GRN 44 and in the subsequent correspondence. The intended food use categories and use levels of FOS are presented in Table 2. The intended use of FOS in the same foods and at the same levels as those in GRN 44 is not expected to noticeably affect the intake of FOS in the overall diet of the public from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. Based on a statistical analysis of potential dietary intake, in the GRN 44 notice it was estimated that dietary exposure to FOS from its intended use as a bulking agent would range from approximately 3.9 to 12.8 g/person/day at the 90th percentile consumption level. For these estimations, the 94-96 CSFII survey data was used. The estimated intake of FOS for the general public, excluding infants less than one year old, associated with its proposed uses in 18 food categories ranged from 3.9 to 6.2 g/day (mean intake) and from 7.1 to 12.8 g/day (90th percentile intake) for consumers of these food products. The estimated intake of FOS for infants less than one year old for the proposed use of FOS in the same 18 food categories were reported as 1.6 and 3.1 g/day for the mean and 90th percentile consumers of these food products, respectively. Subsequently in 2007, GTC Nutrition informed FDA of their determination that the addition of FOS is GRAS for use in foods in general, excluding meat and poultry products from its proposed uses in infant foods, at levels up to 20 g/day in the general population and at levels up to 4.2 g/day in infants less than one year of age.

The intake of FOS from the intended uses in infant formula was determined based on the daily energy intake by infants (GRN 537). As reported by Fomon (1993) about daily energy intake by formula-fed infants, the subpopulation of infants with the highest intake per kg body weight are boys age 14-27 days. The FDA typically uses the 90th percentile of the intake distribution to represent extreme intakes. The 90th percentile energy intake by this group is 141.3 kcal/kg bw/day. Among girls, the highest energy intake is found in the same age group, 14-27 days, and is nearly as high as boys: 138.9 kcal/kg bw/day. These estimates from 1993 are corroborated by data from the 2008 Feeding Infants and Toddlers Study (Butte et al. 2010). Most standard infant formulas provide 0.676 kcal/ml. Therefore, in order to obtain 141.3 or 138.9 kcal energy/kg bw, an infant must consume 209.0 or 205.5 ml/kg bw of formula, respectively. The 90th percentile formula intake for both sexes combined is 207 ml/kg bw/day.

The addition of FOS to infant formula at a maximum concentration of 500 mg/ml is estimated to be 1035 mg/kg bw/day at the 90th percentile. This maximum is based on the addition level of FOS in follow-on formula. The 90th percentile daily intake of FOS from starter formula, with a maximum concentration of 400 mg/100 ml, is estimated as 828 mg/kg bw/day. There are no other sources of fructans in the diets of formula-fed infants and, therefore, the above estimated daily intake constitutes the total daily exposure of the infant to FOS.

2. DATA PERTAINING TO SAFETY

2.1. Preamble

The safety in use of FOS has been extensively evaluated by national and international regulatory agencies such as FDA, EFSA (SCF) and FSANZ. These agency reviews demonstrate that FOS is safe for its intended use as an ingredient in food, including infant formula. The toxicity potentials of FOS have been summarized in multiple published experimental studies and

review articles. These studies include metabolic (*in vitro* and *in vivo*) experiments, short- and long-term toxicity studies in experimental animals, as well as human clinical studies. The currently marketed FOS products are manufactured using sucrose as a starting material that is converted to FOS using β-fructofuranosidase enzymes obtained from different non-toxigenic or non-pathogenic strains of microorganisms. Given the use of similar manufacturing processes, the differences between various FOS products would be limited to minor variations in the compositional distribution of the glucose-fructose disaccharides (FOS), and to differences in the residual levels of other sugars. This also suggests that the safety information on FOS products can be interchangeably used. This assumption is consistent with the SCF (2001a; 2001b) and FSANZ (2008) regulatory opinions for the use of FOS in traditional food products and infant formulas. Additionally, FDA also did not question such an assumption. However, it should be noted that in their evaluation SCF and FSANZ primarily considered the high molecular weight FOS.

In recent years, as the additional uses for FOS have been requested, the regulatory agencies, particularly FDA, have also updated their evaluations for the intended uses of FOS in non-exempt infant formula and additional conventional foods. The available FOS studies are extensively summarized in the 2014 FDA notification on small chain FOS (Ingredion, 2014). FDA did not question the acceptability and suitability of the available evidence to support the proposed uses described in the three GRAS notices, including its uses in infant formula, and replied to all these notifications that the agency received with recognition of the notifier's request and a statement that the agency had 'no questions' regarding the conclusions that the FOS is GRAS for the intended applications. Given the similarity between the FDA notices and the subject of this present GRAS assessment, it is instructive to review the information presented in these documents on FOS from a safety perspective. In the following section, an attempt has been made to present the relevant safety-related data of FOS to support its intake from the intended uses in conventional foods and non-exempt infant formula described in this dossier.

2.2. GRAS Notices on FOS

2.2.1. GRN 537

In this recent GRAS notice, Ingredion Incorporated informed the FDA that the use of short chain FOS as an ingredient in term infant formulas at levels up to 400 mg/100 ml in starter formula (as consumed) and 500 mg/100 ml in follow-on formula (as consumed) is GRAS through scientific procedures (Ingredion, 2014). The subject of GRAS notice (GRN 537) is derived from sucrose and is a fructan oligosaccharide. The FOS was described as being made up of linear chains of fructose units termed GF2, GF3, and GF4 linked by β (2-1) fructosyl-fructose linkages with a terminal glucose residue. The manufacturing process involved dissolving granular sucrose in water, then adding β-fructofuranosidase enzyme from *Aspergillus japonicus.* The specification and compositional analysis for the subject FOS included total FOS content of > 95%, with component limits for GF2 (> 30.0%), GF3 (> 45.0%), and GF4 (> 5.0%). The 90th percentile intake of FOS was estimated as 828 mg/kg bw/day from starter formula within the first month of life and about 800 mg/kg bw/day from the follow-on formula thereafter (Ingredion, 2014).

In this GRAS notice, the notifier extensively summarized and discussed the available published studies supporting the safety of FOS and other fructans. Ingredion (2014) noted that

studies conducted in Wistar rats and in humans, as well as an *in vitro* study, demonstrated that FOS, like other fructans, is not hydrolyzed by intestinal enzymes, is fermented by gut microorganisms, and is not absorbed. Additionally, several studies of FOS and other fructans, including acute, subacute, subchronic, chronic, and developmental toxicological studies conducted in multiple animal models (including neonatal piglets) did not reveal treatment-related adverse effects at the highest exposures. In subchronic and chronic feeding studies conducted in rats, the highest overall exposures of FOS at 4680 mg/kg bw/day, and at 2170 mg/kg bw/day, respectively, did not reveal any adverse effects. *In vitro* studies and studies conducted in animals showed that exposure to scFOS and other fructans did not reveal carcinogenicity, mutagenicity, or clastogenicity potentials. The notifier also discussed several published studies conducted with infants given formulas or infant foods that were supplemented with FOS or other fructans. Based on the totality of the evidence, the notifier (Ingredion, 2014) concluded that ingestion of scFOS from the proposed use results in intakes by infants that remain within safe limits established by published animal and human studies. The subject of GRAS, scFOS, has been sufficiently characterized to ensure that it is a food-grade product. No evidence exists in the available information on scFOS, other fructans, or other oligosaccharides that demonstrates, or suggests reasonable grounds to suspect, a hazard to infants when scFOS is added as a prebiotic ingredient to non-exempt infant formula at levels up to 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed. In a response letter to the notifier on February 5, 2015, FDA stated that the agency has 'no questions" regarding the conclusion that FOS is GRAS under the intended conditions of use (FDA, 2015).

2.2.2. GRN 392

In another GRAS notice, Pfizer Nutrition and BENEO-Orafti informed FDA that the use of oligofructose as an ingredient in milk-based term infant formula powder at a maximum level of 3 g/L of formula as consumed is GRAS (Pfizer, 2011). The subject of this GRAS notice was described as a member of a group of compounds designated as fructan oligosaccharides, which also includes FOS and inulin. Fructan oligosaccharides are described as predominately linear chains of fructose linked in a β-(2-1) configuration with a potential for branching and typically possess a terminal glucose moiety. Individual fructan products may be distinguished by their source, method of production, or degree of polymerization (DP-the number of fructose or glucose residues in the chain). Inulin is a naturally occurring fructan with a DP ranging from 2 to 60. Inulin can be enzymatically hydrolyzed to produce FOS or oligofructose. FOS generally refers to fructans with a DP < 10. Oligofructose refers to fructans with a DP of < 10 and more specifically with > 25% of the molecules having a DP ≥ 5 and < 75% with a DP ≤ 4. The oligofructose that is the subject of GRN 392 has a DP generally in the range of 2 to 8 and predominantly (90%) in the range of 3 to 6 (Pfizer, 2011).

In this GRAS notice, the notifier summarized published studies to support the safety-in-use of the oligofructose product. Some studies were conducted with FOS in general, while other studies were conducted with the notifier's specific oligofructose product. The studies were conducted in animal models as well as in human infants and adults. The metabolism studies of oligofructose in humans as well as animals consistently show that nearly all ingested oligofructose reach the colon intact where they are almost entirely fermented by the colonic bacteria. The kinetics of bacterial fermentation is inversely proportional to the degree of polymerization of the fructan. Safety studies conducted in animal models included acute toxicity

studies, 4-6 week-long toxicity studies, subchronic toxicity studies, chronic toxicity and carcinogenicity studies, reproductive and developmental toxicity studies, and genotoxicity and mutagenicity studies *in vitro*. The notifier noted that very large doses of fructans may result in cecal enlargement, which was considered to be a trophic effect and not a toxic effect. The notifier stated that no consistent, statistically significant, dose-dependent effects were reported in animal studies. The notifier further stated that the studies demonstrate that oligofructose is safe to consume, and there is no evidence of toxicity, carcinogenicity, mutagenicity or clastogenicity. In addition to this, several studies conducted in a total of 2800 human infants fed fructans alone or fructans combined with galactooligosaccharides for varying periods of time were summarized in the GRAS notice (Pfizer, 2011).

Based on the totality of the available evidence, the notifier concluded that studies conducted in human infants demonstrate that fructans are safe to consume for healthy term infants and that the addition of oligosaccharides to infant formula does not pose any likelihood of harm; thus, the intended use of the oligofructose in infant formula is GRAS. The FDA reviewed the notice and responded to the notifier that, based on the information provided in the notification, as well as other information available to the FDA, the agency has no questions at this time regarding the conclusion that oligofructose is GRAS under the intended conditions of use (FDA, 2012).

2.2.3. GRN 44

The first GRAS notice on use of small chain FOS for use in food was submitted to FDA by GTC Nutrition Company (GTC Nutrition, 2000). The notifier determined that small chain FOS manufactured by the enzymatic treatment of sucrose is GRAS for use as a bulking agent. In the initial notice, GTC Nutrition intended to use its FOS in Acidophilis Milk, Bars, Baby Foods, Beverages, Biscuits, Cakes, Confectionary, Cookies, Crackers, Flavored and Unflavored Milks, Hard Candy, Ice Cream, Jams and Jellies, Muffins, Ready-to-Eat-Cereals, Sorbet and Sherbet, Soup and Yogurt at use levels of 0.1-15.4 % (GRN 44). In this GRAS notice, GTC estimated that the 90th percentile intake of naturally occurring FOS is up to 250 mg/day and that the 90th percentile intake of FOS from GTC Nutrition's proposed uses would be 12.8 g/day. Subsequently, in 2007, GTC informed FDA that it had determined that the addition of FOS to foods in general, including infant and toddler foods but excluding infant formula, at levels resulting in intakes up to 20 g/day in the general population and up to 4.2 g/day in infants less than one year of age, is also GRAS. FDA had no questions regarding the additional uses and conclusion (FDA 2007).

In GRN 44, GTC Nutrition (2000) described FOS as a mixture composed of fructose chains with a terminal glucose unit. The number of fructose units varied from 2 to 4. The first fructose unit that is attached to glucose is joined by an alpha 1-1' glycosidic linkage. The remaining fructose units are joined to the first fructose unit in a chain by beta 2-1 glycosidic linkages. The FOS that is the subject of GRAS notice GRN 44 was manufactured from sucrose syrup by the action of the fungal enzyme β-fructofuranosidase. The enzyme acts as an invertase on sucrose, yielding fructose and glucose. The enzyme also acts as a fructosyltransferase between sucrose and a fructofuranosyl-sucrose molecule (i.e., a molecule comprised of fructose chains with a terminal glucose), yielding small chain FOS molecules such as GF2, GF3, and GF4.

Based on published studies, which were conducted with FOS or related oligosaccharides, GTC Nutrition (2000) concluded that FOS is virtually unabsorbed and undigested by endogenous enzymes, although a very small amount is hydrolyzed by stomach acid and absorbed into the body as fructose and glucose. Approximately 89% of the undigested FOS was reported to pass unchanged into the colon where it is fermented by microflora into gases and short-chain carboxylic acids (predominantly acetic acid, while propionic and butyric acids are generated in smaller amounts). GTC Nutrition described additional studies conducted with FOS. The published animal studies included acute studies in rats and mice, 6-week feeding studies in rats, a teratogenicity study in rats, and a chronic bioassay in rats. In addition to these published studies, GTC also included an unpublished 90-day feeding study in rats. Additional published studies included mutagenicity studies, studies describing physiological or systemic effects of fructooligosaccharide, and human studies.

The FDA reviewed the notice and responded to the notifier that, based on the information provided in the notification, as well as other information available to the agency, the FDA has no questions at this time regarding the conclusion that FOS is GRAS under the intended conditions of use. In a subsequent additional correspondence letter for GRN 44, the agency reiterated that on the basis of information provided by GTC Nutrition in GRN 44, the supplement dated January 26, 2007, and other information available to FDA, the agency has no questions regarding the intended uses (including use in additional food categories) of FOS as GRAS ingredient.

2.3. EFSA

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

In reviewing an application for the use of 300 mg oligofructose/100 ml in infant formula, the European Food Safety Authority (EFSA, 2004) concluded that there was an increased prevalence of adverse effects, including loose stools, in infants fed formula with added FOS; as no measures were made to demonstrate satisfactory water balance, the possibility of increased risk of dehydration cannot be excluded, raising concerns with respect to the safety of such formulas; and there is no evidence of benefits to infants from the addition of FOS to infant formula at the conditions specified by the manufacturer while there are reasons for safety concerns. These concerns raised by EFSA have been extensively reviewed and negated, including in GRN 392 and 537. While the concern expressed by EFSA regarding water balance must be addressed, two other aspects of the conclusion are less central to evaluating the safety of

the intended addition of scFOS to infant formula. The increased prevalence of loose stools is regarded as a beneficial effect of the formula supplemented with fructans in that infants receiving these formulas exhibited stooling performance more closely matching that of breastfed infants than did infants receiving control formulas without fructans. EFSA (2004) appears to have interpreted loose, poorly formed, or watery stools as reported by a parent as being equivalent to clinically diagnosed diarrhea. Other studies in infants did not reveal diarrhea. The absence of statistically significant long-term benefit in short-term studies of scFOS or other fructans does not bear upon the safety of the formula. FSANZ considered a concern raised by EFSA relating to water balance. The available evidence that oligofructose and inulin are fermented by colonic microflora in formula fed infants, reduces the concern that water balance could be adversely affected by an increase in osmotic potential due to undigested inulin-derived substances in the colon. Furthermore, as discussed below, in a more recent evaluation FSANZ did not note any safety concern of the use of short chain FOS in infant formula.

2.4. FSANZ

In 2013, the Food Standards Australia New Zealand (FSANZ) government agency reviewed the safety of the addition of FOS to infant formula and follow-on formula. Following its assessment, the agency issued a report approving the optional addition of FOS produced from sucrose by action of the enzyme β-fructofuranosidase as an alternative to inulin-derived substances to infant formula products. In granting this approval, FSANZ concluded that FOS is technologically suited to its proposed use and complies with international specifications. No public health and safety issues were identified with the proposed use of β- fructofuranosidase from *A. niger* as a processing aid, and an ADI "not specified" is considered appropriate. Results of laboratory animal studies confirmed that FOS has no identifiable hazard at concentrations likely to be encountered under Good Manufacturing Practice, and digestion of FOS was equivalent to inulin-derived substances in an *in vitro* model of human colonic fermentation, producing comparable levels of short-chain fatty acids and gas. Also, no adverse effects on growth, hydration status, nutrient intake, frequency and nature of adverse events, gastrointestinal intolerance, stool consistency and frequency, or fecal flora were observed in studies conducted in healthy infants or young children at amounts of FOS up to 3.0 g/L for periods ranging from 1 week to approximately 3 months.

2.5. Recent Safety Publications

A literature search of recent publications from scientific databases such as PubMed and Toxline was conducted on FOS to determine whether any additional or new publications appeared during the past one year since the submission of the GRAS notice GRN 537 (GTC Nutrition, 2014; FDA, 2015). The literature search did not reveal any significant new safety-related studies. Hence, all data and information used in support of this GRAS affirmation is the same as that presented in previous GRNs 44, 392, and 537, and the data made available to EFSA and FSANZ. Some of the pertinent information related to the safety, primarily from recent publications is summarized in the following sections.

2.5.1. Summary of Recent Safety Studies

2.5.1.1. Metabolism

Several non-digestible oligosaccharides and polysaccharides have been shown to act as prebiotic compounds, of which inulin, FOS and GOS are presently most widely used in food. As described in the above GRAS notices and regulatory assessments, pharmacokinetic studies of FOS demonstrate that FOS is not hydrolyzed by human salivary or pancreatic enzymes and passes undigested and unabsorbed to the colon where it is fermented by colonic microflora to short-chain fatty acids, carbon dioxide, methane and hydrogen gases. The unfermented dietary FOS is excreted in the feces.

Sivieri et al. (2014) studied the prebiotic effect of FOS in the simulator of the human intestinal microbial ecosystem (SHIME® model). The model was used to study the effect of FOS on the fermentation pattern of the colon microbiota. Initially, an inoculum prepared from human feces was introduced into the reactor vessel and stabilized over 2 weeks using a culture medium. This stabilization period was followed by a 2-week control period during which the microbiota was monitored. The microbiota was then subjected to a 4-week treatment period by adding 5 g/day FOS to vessel one (the "stomach" compartment). A significant increase in the Lactobacillus spp. and Bifidobacterium spp. populations was observed during the treatment period. Overall microbial community was changed in the ascending colon compartment of the SHIME reactor. FOS induced an increase of the SCFA concentration during the treatment period, mainly due to significant increased levels of acetic and butyric acids. However, ammonium concentrations increased during the same period. This study indicates the usefulness of *in vitro* methods that simulate the colon region as part of research towards the improvement of human health.

2.5.1.2. Animal and Other Studies

A search of the scientific databases including PubMed, ToxLine, for recent animal studies of FOS did not reveal any new publications. The available safety related animal studies with FOS described in the FDA GRAS notices and also summarized in the EFSA and FSANZ reports are included Appendix I.

2.5.1.3. Adult Human and Children Studies

The available human studies with FOS are described in the FDA GRAS notices. Additionally, these studies are also summarized in the EFSA and FSANZ reports. Some of the safety related and relevant studies conducted in healthy individuals are included Appendix II. In addition to the studies in healthy individuals, there are several studies with FOS and other fructans in compromised individuals, these studies are extensively described and summarized in GRN 537. The available FOS and other fructan studies conducted in normal and compromised children are presented in Appendix III. No new safety or toxicity related studies of FOS in adult and children were noted since the submission of GRN 537.

2.5.1.4. Infant Studies

In GRN 537, the available studies of FOS and other fructans related to infant tolerance, growth and safety have been extensively described and summarized. These studies are presented in Appendix IV. In GRN 537, it is summarized that the totality of the extensive body of research discussed in the notice that includes reports of open-label studies and other research clearly supports the conclusions that prebiotic supplementation of formula intended for consumption by preterm and term infants is safe and has physiological effects that bring the performance of formula-fed infants closer to that of breastfed infants. In the published literature 27 studies enrolling 3163 infants have appeared in which infants were given formula or infant food supplemented with fructans. In 7 studies that included a total of 637 infants, the effects of ingestion of short chain FOS were assessed. The test articles in remaining studies were oligofructose, inulin, and blends of intact and hydrolyzed inulin. These studies lasted from 1 week to 6 months with a median duration of 1 month. The use levels of fructan in these studies ranged from 150 to 800 mg/100 ml formula, with a median addition level of 300 mg prebiotic/100 ml formula. The findings from these theses studies support the notion that prebiotic addition to infant formula intended for consumption by either preterm or term infants is safe, with no indications of serious adverse effects or reduction in growth. In no study were any serious adverse events associated with ingestion of the added prebiotic, and the less serious symptoms such as flatulence or fussiness were generally reported with approximately equal frequency in both prebiotic and placebo groups. Fifteen studies measured growth and none found any difference between prebiotic and placebo groups.

As summarized in the GRN 537, the efficacy of frucants as a prebiotic supplementation were less consistently demonstrated than was the safety of the supplementation, possibly due to the great variability in both the test article and the addition level across studies. In 15 studies, fecal bifidobacteria were enumerated and lactobacilli in 5. Significant increases in both genera were seen in the majority of studies, but not in all. As regard to other colonic bacteria, little effect was generally observed. Similarly, in 5 of the 10 studies that recorded defecation frequency, a significant increase was noted and stools were significantly softer in 5 of the 7 studies that included assessment of stool consistency, bringing the performance of formula-fed infants into closer resemblance to that of human milk-fed infants. Fecal pH, however, was significantly lowered in all 5 studies that measured acidity.

In summary, the several (27) studies fructans have been administered to infants for periods as long as six months (in addition to the many studies of GOS-fructan blends). In spite of some variability in the effects seen on stooling, bifidobacteria and other colonic bacteria, the findings from these studies consistently showed the absence of prebiotic-associated adverse effects and the ability of the prebiotic-containing formula to support normal growth of both preterm and term infants. Based on these studies, the notifier in GRN 537 concluded that the addition of short chain FOS and other fructans to infant formula has been shown to be without likelihood of harm.

In a recent study that appeared after the FDA review of GRN 537, Ripoll et al. (2015) investigated the effect of FOS on digestive tolerance and growth parameters in infants up to 10 months of age. In this randomized, controlled, double blind study, 75 formula-fed healthy infants were included at the age of 4 months and received either a placebo or FOS supplemented formula for six months. Fecal poliovirus sIgA after vaccination and bifidobacteria concentration,

height, weight and digestive tolerance were monitored. No significant differences were observed between the groups for the evolution of poliovirus sIgA concentration compared to baseline after 1 and 2 months of supplementation. A significant increase in bifidobacteria count was noted after 1 month of FOS supplementation, but was no longer significant after 2 months. Tolerance and growth parameters were similar in both the groups. The results of this study show that a follow-on milk formula supplemented with FOS modulates intestinal microbiota via an increase of fecal bifidobacteria concentration, while no effect on sIgA concentrations was noted. FOS addition elicited normal digestive tolerance and normal growth suggesting it can be used safely at 5 g/L in infants after 4 months of age.

In another study, Lasekan et al. (2015) evaluated the effects of soy-based infant formulas containing supplemental FOS. A randomized, double-blind, 28 day parallel feeding trial compared gastrointestinal (GI) tolerance and hydration in healthy term newborn infants fed either a commercialized soy formula (with history of safe use) containing sucrose as 20% of total carbohydrate, no supplemental short-chain FOS (scFOS) and no mixed carotenoids (lutein, lycopene, beta-carotene) as a control (CF, n = 62 infants) or one of two experimental soy-based formulas, EF1 (n = 64) and EF2 (n = 62) containing short chain FOS (2.5 g/L) and mixed carotenoids. EF1 differed from EF2 by containing sucrose. No significant study group differences in study completion rates (CF = 81, EF1 = 86, and EF2 = 87%), growth, mean rank stool consistency, stool frequency, formula intake, spit-up/vomit, and safety measures (urine specific gravity, USG; hydration status and adverse events) were noted. The findings from this study suggested that term infants fed soy-based formulas supplemented with FOS and mixed carotenoids, with or without sucrose, in the first 35 days of infancy demonstrated good tolerance and hydration comparable to the control soy-based formula with history of safe use.

3. SUMMARY AND DISCUSSION

New Francisco Biotechnology Corporation (NFBC), China, intends to market fructooligosaccharides (FOS) as ingredients in conventional foods, including non-exempt infant formula. The products will be marketed under trade name King-Prebiotics®. The manufacturing process of FOS involves the biotransformation of sucrose by the action of a microbial derived enzyme β-D-fructofuranosidase from *Aspergillus oryzae*. The FOS are prepared using raw materials and processing aids that are food-grade and comply with applicable U.S. federal regulations. The FOS is manufactured according to cGMP and NFBC has established food grade specifications for its different grades of FOS. The FOS manufactured by NFBC is composed of sucrose molecules (glucose-fructose disaccharides, GF) to which one, two, or three additional fructose units have been added by β 2-1 glycosidic linkages to the fructose unit of sucrose.

FOS has been the subject of three GRAS notices. FDA did not question the use of FOS in a variety of foods such as Acidophilus Milk; Analogs and Substitutes for Meat, Poultry or Fish; Bars; Breakfast Cereals; Beverages and Juices; Cakes; Cheese; Cream; Confectionery; Cookies; Crackers; Dessert Toppings and Fillings; Hard candy; Ice cream; Infant Foods; Jams and Jellies; Milk, flavored and unflavored; Milk, evaporated and condensed; Muffins and Quick Bread; Sauces, Gravies, and Condiments; Snacks; Sorbet and Sherbet; Soup; Toddler Foods (12-24 months); and Yogurt at use levels of 0.4-6.7%. These food categories and use levels are described in GRN 44 and in additional correspondence related to this GRAS notice. FOS is safe when used in foods, beverages and supplements, for the general population, one year of age or greater, at levels up to 20 g/day in the general population and at levels up to 4.2 g in infants

(from uses in infant foods) less than one year of age in accordance to good manufacturing practices. In addition to above foods, FOS is also intended as a prebiotic in non-exempt infant formula at the maximum intended addition levels of 400 mg FOS/100 ml in starter formula as consumed and 500 mg FOS/100 ml in follow-on formula as consumed. Thus, NFBC intends to use FOS in the same foods and at identical levels to those mentioned in the GRN 44 (all food categories mentioned in GRN 44 and in additional correspondence of June 1, 2007 between GTC Nutrition and FDA) and also those in GRN 537 described for non-exempt infant formula.

There is sufficient qualitative and quantitative scientific evidence to determine the safety-in-use of FOS in the above mentioned food applications, including infant formula. FOS products have been used in food for over 15 years with no evidence of adverse effects related to the safety of its use. FOS has been the subject of three GRAS notices to FDA. In the GRN 44, use of FOS in specified food categories was determined to as safe at levels of up to 20 g/person/day. The daily intake of FOS from its uses in infant formula is estimated as 1035 mg/kg bw/day based on consumption of follow-on formula, which is intended to contain FOS at a higher concentration than starter formula. In response to three separate GRAS notifications on FOS (GRN 44; GRN 392; and GRN 537), FDA did not question the safety of FOS for the specified food uses, including infant formula. The subject of this present GRAS determination is substantially equivalent to the FOS that has been the subject of the FDA GRAS notified substances. The use of a similar manufacturing process in the preparation of the FOS that is the subject of this GRAS assessment and those that has been the subject of FDA notifications suggests that the differences between various FOS products would be limited to minor variations in the compositional distribution of the FOS oligomers, and to differences in the residual levels of other sugars. These observations also suggest that the safety information on FOS products can be interchangeably used.

The available metabolism related information of FOS demonstrate that FOS is not digested by human gastric juice or by pancreatic enzymes and passes undigested and unabsorbed to the colon where it is fermented by colonic microflora to short-chain fatty acids, carbon dioxide, methane and hydrogen gases. Any unfermented dietary FOS will be excreted in the feces. Several published studies of FOS are described in the GRAS notices submitted to FDA. In safety studies such as acute, subacute, subchronic, chronic, and developmental toxicological, conducted in multiple animal models (including neonatal piglets) with FOS and other fructans no treatment-related adverse effects at the highest exposures were noted. In subchronic and chronic feeding studies conducted in rats, the highest overall exposures of FOS at 4680 mg/kg bw/day, and at 2170 mg/kg bw/day, respectively, did not reveal adverse effects. In published studies conducted with infants that were given formulas or infant foods supplemented with FOS or other fructans, no significant adverse effects were reported.

The FDA responses to GRAS notifications on FOS indicate that the agency is satisfied with the safety-in-use of FOS at use levels up to 20 g/person/day and in infants at use levels of 400 mg FOS/100 ml in starter formula as consumed and 500 mg FOS/100 ml in follow-on formula as consumed. Additionally, FSANZ also completed a safety evaluation of FOS and did not raise any safety concerns for the uses of FOS in infant formula. Recent studies that appeared subsequent to the most recent FDA GRAS notification (GRN 538) also did not reveal any significant findings that affect the safety conclusion from the GRAS notices. The safety determination of FOS is based on the totality of available evidence, including current approved

uses, *in vitro* and *in vivo* metabolism studies, a variety of animal studies and human and infant studies that supports the safety-in-use of FOS.

In summary, on the basis of scientific procedures[3], exposure from diet and current uses, the consumption of FOS derived from sucrose as a food ingredient at use levels ranging from 0.4 to 6.7% in specified foods and in infants consuming infant foods, and from its uses at 400 mg FOS/100 ml in starter formula as consumed and 500 mg FOS/100 ml in follow-on formula as consumed is considered as safe. The proposed uses are compatible with current regulations, *i.e.*, FOS is used in Acidophilus Milk; Analogs and Substitutes for Meat, Poultry or Fish; Bars; Breakfast Cereals; Beverages and Juices; Cakes; Cheese; Cream; Confectionery; Cookies; Crackers; Dessert Toppings and Fillings; Hard candy; Ice cream; Infant Follow-On formula (not in infant formula); Jams and Jellies; Milk, flavored and unflavored; Milk, evaporated and condensed; Muffins and Quick Bread; Sauces, Gravies, and Condiments; Snacks; Sorbet and Sherbet; Soup; Toddler Foods (12-24 months); and Yogurt at use levels of 0.4-6.7% (all food categories mentioned in GRN 44 and additional correspondence) at levels ranging from 0.4 to 6.7% and in infant formula at use levels of 400 mg FOS/100 ml in starter formula as consumed and 500 mg FOS/100 ml in follow-on formula when not otherwise precluded by a Standard of Identity, and is produced according to current good manufacturing practices (cGMP).

[3] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that fructo-oligosaccharides (FOS), meeting the specifications cited above, and when used as a food ingredient in selected food products [Acidophilus Milk; Analogs and Substitutes for Meat, Poultry or Fish; Bars; Breakfast Cereals; Beverages and Juices; Cakes; Cheese; Cream; Confectionery; Cookies; Crackers; Dessert Toppings and Fillings; Hard candy; Ice cream; Infant Food; Jams and Jellies; Milk, flavored and unflavored; Milk, evaporated and condensed; Muffins and Quick Bread; Sauces, Gravies, and Condiments; Snacks; Sorbet and Sherbet; Soup; Toddler Foods (12-24 months); and Yogurt at use levels of 0.4-6.7%], when not otherwise precluded by a Standard of Identity as described in this dossier and in infant formula at use levels of 400 mg FOS/100 ml in starter formula as consumed and 500 mg FOS/100 ml in follow-on formula is Generally Recognized As Safe (GRAS).

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

Signatures

(b) (6)

Douglas Archer, Ph.D. — January 2, 2016 — Date

(b) (6)

Robert L. Martin, Ph.D. — Dec. 30, 2015 — Date

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. — January 4, 2016 — Date

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5. REFERENCES

Abbott Laboratories. 2000. Fructooligosaccharides and laxation in children. [Unpublished report submitted in support of Application A1055 from GTC Nutrition to Food Standards Australia New Zealand to permit the optional addition of scFOS to Infant Formula Products, Foods for Infants, and Formulated Supplementary Foods for Young Children; available online at http://www.foodstandards.gov.au/foodstandards/applications/applicationa1055shor4991.cfm]

Abbott Laboratories. 1996. Effects of a milk-based beverage with short-chain fructooligosaccharides on tolerance and incidence of diarrhea in toddlers. [Unpublished report submitted in support of Application A1055 from GTC Nutrition to Food Standards Australia New Zealand to permit the optional addition of scFOS to Infant Formula Products, Foods for Infants, and Formulated Supplementary Foods for Young Children; available online at http://www.foodstandards.gov.au/foodstandards/applications/applicationa1055shor4991.cfm]

Abbott Laboratories. 1993. Growth, tolerance, and stool characteristics of term infants consuming short-chain fructooligosaccharides in infant formula. [Unpublished report submitted in support of Application A1055 from GTC Nutrition to Food Standards Australia New Zealand to permit the optional addition of scFOS to Infant Formula Products, Foods for Infants, and Formulated Supplementary Foods for Young Children; available online at http://www.foodstandards.gov.au/foodstandards/applications/applicationa1055shor4991.cfm]

Abbott Laboratories. 1992. Growth, tolerance, and stool characteristics of term infants consuming short-chain fructooligosaccharides in infant formula. [Unpublished report submitted in support of Application A1055 from GTC Nutrition to Food Standards Australia New Zealand to permit the optional addition of scFOS to Infant Formula Products, Foods for Infants, and Formulated Supplementary Foods for Young Children; available online at http://www.foodstandards.gov.au/foodstandards/applications/applicationa1055shor4991.cfm]

Abrams, S.A., Griffin, I.J., Hawthorne, K.M., Ellis, K.J. 2007. Effect of prebiotic supplementation and calcium intake on body mass index. *J Pediatr* 151:293-298.

Abrams, S.A., Griffin, I.J., Hawthorne, K.M., Liang, L., Gunn, S.K., Darlington, G., Ellis, K.J.,2005. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *Am J Clin Nutr* 82:471-476.

Alles, M.S., Hautvast, G.J.A., Nagengast, F.M., Hartemink, R., Van Laere, K.M.J., Jansen, J.B.M.J., 1996. Fate of fructo-oligosaccharides in the human intestine. *Br J Nutr* 76:211-221.

Aunstrup, K. 1979. Production, Isolation, and Economics of Extracellular Enzymes in Applied Biochemistry and Bioengineering, Vol. 2, Enzyme Technology, eds. L. B. Wingard, E. Katchalski-Katzir, and L. Goldstein, pp. 28-68.

000030

Aunstrup, K., Anderson, O., Falch, E.A., Nielsen, T.K. 1979. Production of Microbial Enzymes in Microbial Technology, 2nd ed., Vol. 1, eds H.J. Peppler and D. Perlman. Ch. 9, pp. 282-309.

Bartosch S, EJ Woodmansey, JCM Paterson, MET McMurdo, GT Macfarlane. 2005. Microbiological effects of consuming a synbiotic containing *Bifidobacterium bifidum*, *Bifidobacterium lactis*, and oligofructose in elderly persons, determined by real-time polymerase chain reaction and counting of viable bacteria. *Clin Infect Dis* 40:28-37.

Bettler J and MJ Kullen. 2007. Infant formula enriched with alpha-lactalbumin has a prebiotic effect in healthy term infants *J Pediatr Gastroenterol Nutr* 44 (Suppl 1):E197. [Abstract]

Bettler J and AR Euler. 2006. An evaluation of the growth of term infants fed formula supplemented with fructooligosaccharide. *Int J Probiot Prebiot* 1:19-26.

Bettler J, DK Mitchell, MJ Kullen. 2006. Administration of *Bifidobacterium lactis* with fructo-oligosaccharide to toddlers is safe and results in transient colonization. *Int J Probiot Prebiot* 1:193-202.

Bornet, F.R., Brouns, F., Tashiro, Y., Duvillier, V. 2002. Nutritional aspects of short-chain fructooligosaccharides: natural occurrence, chemistry, physiology and health implications. *Dig. Liver Dis.* 34(Suppl 2):S111-120.

Bouhnik Y, L Raskine, G Simoneau, D Paineau, F Bornet. 2006. The capacity of short-chain fructo-oligosaccharides to stimulate faecal bifidobacteria: a dose-response relationship study in healthy humans. *Nutr J* 5:8-13.

Bouhnik Y, L Raskine, G Simoneau, E Vicaut, C Neut, B Flourie, F Brouns, FR Bornet. 2004. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *Am J Clin Nutr* 80:1658-1664.

Bouhnik Y, K Vahedi, L Achout, A Attar, J Salfati, P Pochart, P Marteau, B Flourie, F Bornet, J-C Rambaud. 1999. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. *J Nutr* 129:113-116.

Bouhnik Y, B Flourie, M Riottot, N Bisetti, M-F Gailing, A Guibert, F Bornet, J-C Rambaud. 1996. Effects of fructo-oligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. *Nutr Cancer* 26:21-29.

Boyle FG, JM Wrenn, BB Marsh, WI Anderson, FA Angelosanto, AL McCartney, EL Lien. 2008. Safety evaluation of oligofructose: 13 week rat study and *in vitro* mutagenicity. *Food Chem Toxicol* 46:3132-3139.

Brighenti F, MC Casiraghi, E Canzi, A Ferrari. 1999. Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. *Eur J Clin Nutr* 53:726-733.

Brunser O, M Gotteland, S Cruchet, G Figueroa, D Garrido, P Steenhout. 2006a. Effect of a milk formula with prebiotics on the intestinal microbiota of infants after an antibiotic treatment. *Pediatr Res* 59:451-456.

Brunser O, G Figueroa, M Gotteland, E Haschke-Becher, C Magliola, F Rochat, S Cruchet, R Palframan, G Gibson, F Chauffard, F Haschke. 2006b. Effects of probiotic or prebiotic

supplemented milk formulas on fecal microbiota composition of infants. *Asia Pac J Clin Nutr* 15:368-376.

Buddington RK, CH Williams, S-C Chen, SA Witherly. 1996. Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. *Am J Clin Nutr* 63:709-716.

Campbell, J.M., Bauer, L.L., Fahey, G.C., Hogarth, A.J.C., Wolf, B.W., Hunter, D.E. 1997a. Selected fructooligosaccharide (1-kestose, nystose, and 1F-ß-fructofuranosylnystose) composition of foods and feeds. *J. Agric. Food Sci.* 45(8):3076-3082.

Campbell, J.M., Fahey, G.C. Jr., Wolf, B.W. 1997b. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *J. Nutr.* 127(1):130-136.

Campoy C, MT Miranda, A Santamaria, M Riyero, G Colome, JA Molina-Font, R Bayes. 2005. New dietary management of infant colic. *Proceedings of the 18th International Congress of Nutrition.* Durban, South Africa, September.

Cani PD, E Joly, Y Horsmans, NM Delzenne. 2006. Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr* 60:567-572.

Castiglia-Delavaud C, E Verdier, JM Besle, J Vernet, Y Boirie, B Beaufrere, RD Baynast, M Vermorel. 1998. Net energy value of non-starch polysaccharide isolates (sugarbeet fibre and commercial inulin) and their impact on nutrient digestive utilization in healthy human subjects. *Br J Nutr* 80:343-352.

Clausen MR, J Jorgensen, PB Mortensen. 1998. Comparison of diarrhea induced by ingestion of fructooligosaccharide Idolax and disaccharide lactulose: role of osmolarity versus fermentation of malabsorbed carbohydrate. *Digest Dis Sci* 43:2696-2707.

Clevenger MA, D Turnbull, H Inoue, M Enomoto, JA Allen, LM Henderson, E Jones. 1988. Toxicological evaluation of neosugar: genotoxicity, carcinogenicity, and chronic toxicity. *J Am Coll Toxicol* 7:643-662.

Coudray C, J Bellanger, C Castiglia-Delavaud, C Remesy, M Vermorel, C Demigne. 1997. Effect of soluble and partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *Eur J Clin Nutr* 51:375-380.

De Preter V, T Vanhoutte, G Huys, J Swings, L De Vuyst, P Rutgeerts, K Verbeke. 2007. Effects of *Lactobacillus casei* Shirota, *Bifidobacterium breve*, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans. *Am J Physiol Gastrointest Liver Physiol* 292:G358-G368.

De Preter V, H Raemen, L Cloetens, E Houben, P Rutgeerts, K Verbeke. 2008. Effect of dietary intervention with different pre- and probiotics on intestinal bacterial enzyme activities. *Eur J Clin Nutr* 62:225-231.

Ducros V, J Arnaud, M Tahiri, C Coudray, F Bornet, C Bouteloup-Demange, F Brouns, Y Rayssiguier, AM Roussel. 2005. Influence of short-chain fructo-oligosaccharides (sc-FOS) on absorption of Cu, Zn, and Se in healthy postmenopausal women. *J Amer Coll Nutr* 24:30-37.

000032

Duggan C, ME Penny, P Hibberd, A Gil, A Huapaya, A Cooper, F Coletta, C Emenhiser, RE Kleinman. 2003. Oligofructose-supplemented infant cereal: 2 randomized, blinded, community-based trials in Peruvian infants. *Am J Clin Nutr* 77:937-942.

Enzyme Applications. 1994. In Encyclopedia of Chemical Technology, 4th ed., edt. J. I. Kroschwitz, Vol. 9, pp. 597-620.

Euler AR, DK Mitchell, R Kline, LK Pickering. 2005. Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. *J Pediatr Gastroenterol Nutr* 40:157-164.

FDA. 2015. Agency Response Letter GRAS Notice No. GRN 000537 (Fructooligosaccharide). February 5, 2015. Available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm440653. Site visited on October 30, 2015.

FDA. 2012. Agency Response Letter GRAS Notice No. GRN 000392 (Oligofructose). May 7, 2012. Available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm307720.htm. Site visited on October 30, 2015.

FDA. 2007. Agency Additional Correspondence Letter: GRAS Notice No. GRN 000044 (Fructooligosaccharide). June 1, 2007. Available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154400.htm. Site visited on October 30, 2015.

FDA. 2000. Agency Response Letter GRAS Notice No. GRN 000044 (Fructooligosaccharide). November 22, 2000. Available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154122.htm. Site visited on October 30, 2015.

Firmansyah A, GD Pramita, AL Carrie Fassler, F Haschke, H Link-Amster. 2000. Improved humoral immune response to measles vaccine in infants receiving infant cereal with fructooligosaccharides. *J Paediatr Gastroenterol Nutr* 31, A521. [Abstract]

Fisberg M, I Maulen, E Vasquez, J Garcia, G Comer, P Alarcon. 2000. Effect of oral supplementation with and without synbiotics on catch-up growth in preschool children. *J Pediatr Gastroenterol Nutr* 31:A987. [Abstract]

Food Standards Australia New Zealand (FSANZ). 2013. Approval report- application A1055, short chain fructo-oligosaccharides. May 23, 2013.Forcheron F and M Beylot. 2007. Long-term administration of inulin-type fructans has no significant lipid-lowering effect in normolipidemic humans. *Metab Clin Exp* 56:1093-1098.

FSANZ. 2008. Addition of Inulin/FOS & GOS to Food: Final Assessment Report: Proposal P306. Food Standards Australia New Zealand (FSANZ); Canberra, Australia. Available at: http://www.foodstandards.gov.au/code/proposals/documents/P306%20FOS%20_%20GOS%20FAR%20FINALv2.pdf. Site visited on October 30, 2015.

Fukushima Y, CJ Jun, K Kegai, A Ohta, K Sakai, K Uenishi, Y Kagawa. 2002. Calcium absorption of malt drinks containing fructooligosaccharides and safety in humans. *J Nutr Food* 5:49-60.

Garleb KA, JT Snook, MJ Marcon, BW Wolf, WA Johnson. 1996. Effect of fructooligosaccharide containing enteral formulas on subjective tolerance factors, serum chemistry profiles, and faecal bifidobacteria in healthy adult male subjects. *Microb Ecol Health Dis* 9:279-285.

Genta SB, WM Cabrera, A Grau, SS Sanchez. 2005. Subchronic 4-month oral toxicity study of dried *Smallanthus sonchifolius* (yacon) roots as a diet supplement in rats. *Food Chem Toxicol* 43:1657-1665.

Gibson, G. R., Roberfroid, M. B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125:1401-1412.

Gibson GR, ER Beatty, X Wang, JH Cummings. 1995. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterol* 108:975–982.

Grasten S, K-H Liukkonen, A Chrevatidis, H El-Nezami, K Poutanen, H Mykkanen. 2003. Effects of wheat pentosan and inulin on the metabolic activity of fecal microbiota and on bowel function in healthy humans. *Nutr Res* 23:1503-1514.

Griffin IJ, PMD Hicks, RP Heaney, SA Abrams. 2003. Enriched chicory inulin increases calcium absorption mainly in girls with lower calcium absorption. *Nutr Res* 23:901-909.

Griffin IJ, PM Davila, SA Abrams. 2002. Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes. *Br J Nutr* 87 (Suppl):S187-S191.

GTC Nutrition, 2000. Fructo-oligosaccharides. GRN No. 44. Available online: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm261587 Site visited on October 30, 2015.

Guesry PR, H Bodanski, E Tomsit, JM Aeschimann. 2000. Effect of 3 doses of fructooligosaccharides in infants. *J Pediatr Gastroenterol Nutr* 31(Suppl 2):S252. [Abstract]

Hawkes, M., Rennie, R., Sand, C., Vaudry, W. 2005. *Aureobasidium pullulans* infection: fungemia in an infant and a review of human case. *Diagn. Microbiol. Infect. Dis.* 51:209-213.

Henquin JC. 1988. Reproduction toxicity: study on the influence of fructooligosaccharides on the development of foetal and postnatal rat. Raffinerie Tirlemontoise internal report. [Unpublished report presented in Carabin and Flamm 1999]

Hess JR, AM Birkett, W Thomas, JL Slavin. 2011. Effects of short-chain fructooligosaccharides on satiety responses in healthy men and women. *Appetite* 56:128-134.

Hidaka H, T Eida, T Takizawa, T Tokunaga, Y Tashiro. 1986. Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora* 5:37-50.

Hidaka, H., Eida, T., Takizawa, T., Tokanaga, T., Tashiro, Y. 1986. Effects of fructoligosaccharides on intestinal flora and human health. Bifidobact. Microflora 5:37-50.

Ingredion Corporation. 2014. Fructo-oligosaccharides. GRN No. 537. Available online: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM422895. Site visited on October 30, 2015.

Juffrie M. 2002. Fructooligosaccharide and diarrhea. *Biosci Microflor* 21:31-34.

000034

Kapiki A, C Costalos, C Oikonomidou, A Triantafyllidou, E Loukatou, V Pertrohilou. 2007. The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. *Early Hum Devel* 83:335-339.

Kim S-H, DH Lee, D Meyer. 2007. Supplementation of infant formula with native inulin has a prebiotic effect in formula-fed babies. *Asia Pac J Clin Nutr* 16:172-177.

Kruse H-P, B Kleessen, M Blaut. 1999. Effects of inulin on faecal bifidobacteria in human subjects. *Br J Nutr* 82:375-382.

Langlands SJ, MJ Hopkins, N Coleman, JH Cummings. 2004. Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. *Gut* 53:1610-1616.

Lasekan, J., Baggs, G., Acosta, S., Mackey, A. 2015. Soy protein-based infant formulas with supplemental fructooligosaccharides: Gastrointestinal tolerance and hydration status in newborn infants. *Nutrients* 7(4):3022-3037.

Lasekan JB, S Acosta, D Albrecht, G Baggs. 2010. Gastrointestinal (GI) tolerance and hydration status of newborn infants fed soy-based infant formulas with supplemental fructooligosaccharides (FOS). Poster presented at the 9^{th} *International Symposium on the Role of Soy in Health Promotion and Chronic Disease Prevention and Treatment*, Washington DC, October.

Letexier D, F Diraison, M Beylot. 2003. Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in humans. *Am J Clin Nutr* 77:559-564.

Lugonja NM, OB Martinov, MR Rasovic, SD Spasic, GD Gojgic, MM Vrvic. 2010. A comparative investigation of an *in vitro* and clinical test of the bifidogenic effect of an infant formula. *J Clin Biochem Nutr* 47:208-216.

Luo J, SW Rizkalla, C Alamowitch, A Boussairi, A Blayo, J-L Barry, A Laffitte, F Guyon, FRJ Bornet, G Slama. 1996. Chronic consumption of short-chain fructooligosaccharides by healthy subjects decreased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism. Am J Clin Nutr 63:939-945.

Menne E, N Guggenbuhl, M Roberfroid. 2000. Fn-type chicory inulin hydrolysate has a prebiotic effect in humans. *J Nutr* 130:1197-1199.

McGinnis, M., 1980, Laboratory Handbook of Medical Mycology. Academic Press Inc., N.Y., USA.

Mitsuoka, T., Hidaka, H., Eida T. 1987, Effects of fructo-oligosaccharides on intestinal microflora. *Die Nahrung* 31:427-436.

Molis C, B Flourie, F Ouarne, M-F Gailing, S Lartigue, A Guibert, F Bornet, J-P Galmiche. 1996. Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. *Am J Clin Nutr* 64:324-328.

Moore N, C Chao, L-P Yang, H Storm, M Oliva-Hemker, JM Saavedra. 2003. Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. *Br J Nutr* 90:581-587.

Nakamura S, SA Sarker, MA Wahed, Y Wagatsuma, T Oku, K Moji. 2006. Prebiotic effect of daily fructooligosaccharide intake on weight gain and reduction of acute diarrhea among children in a Bangladesh urban slum: A randomized double-masked placebo-controlled study. *Trop Med Health* 34:125-131.

Ohta A, K Sakai, M Takasaki, T Tokunaga. 1999. The advantages of calcium supplement tablet (candy) containing fructooligosaccharides for the healthy human being. J Nutr Food 2:37-43.

Ohta A, K Sakai, M Takasaki, M Uehara, T Tokunaga, T Adachi. 1999. Dietary heme iron does not prevent postgastrectomy anemia but fructooligosaccharides improve bioavailability of heme iron in rats. *Int J Vitam Nutr Res* 69:348-355.

Oku T and S Nakamura. 2003. Comparison of digestibility and breath hydrogen gas excretion of fructo-oligosaccharide, galactosylsucrose, and isomalto-oligosaccharide in healthy human subjects. *Eur J Clin Nutr* 57:1150-1156.

Paineau D, F Respondek, V Menet, R Sauvage, F Bornet, A Wagner. 2014. Effects of short-chain fructooligosaccharides on faecal bifidobacteria and specific immune response in formula-fed infants: a randomized, double-blind, placebo-controlled trial. *J Nutr Sci Vitaminol* in press.

Panigrahi P, S Parida, L Pradhan, SS Mohapatra, PR Misra, JA Johnson, R Chaudhry, S Taylor, NI Hansen, IH Gewolb. 2008. Long-term colonization of a *Lactobacillus plantarum* synbiotic preparation in the neonatal gut. *J Pediatr Gastroenterol Nutr* 47:45-53.

Pedersen A, B Sandstrom, JMM Van Amelsvoort. 1997. The effect of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. *Br J Nutr* 78:215-222.

Pfizer Nutrition, 2011. Oligofructise. GRN No. 392. Available online: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm277112. Site visited on October 30, 2015.

Rao VA. 2001. The prebiotic properties of oligofructose at low intake levels. *Nutr Res* 21:843-848.

Ripoll, C., Chappuis, E., Respondek, F., Wanger, A., Gottarand, F. 2015. scFOS supplemented follow-on formula in healthy infants: Impact on vaccine specific faecal secretory IGA response, faecal bifidobacteria, growth and digestive tolerance. *Biactive Carb. Dietary Fiber* 5(2): 169-178.

Ripoll C, F Respondek, A Wagner, F Gottrand. 2014. Effects of scFOS on immune parameters, faecal bifidobacteria and digestive tolerance of follow-on formula-fed infants. Unpublished.

Rumessen JJ, and E Gudmand-Hoyer. 1998. Fructans of chicory: intestinal transport and fermentation of different chain lengths and relation to fructose and sorbitol malabsorption. *Am J Clin Nutr* 68:357-364.

Rumessen JJ, S Bode, O Hamberg, E Gudmand-Hoyer. 1990. Fructans of Jerusalem artichokes: intestinal transport, absorption, fermentation, and influence on blood-glucose, insulin, and C-peptide responses in healthy-subjects. *Am J Clin Nutr* 52:675-681.

Russo F, G Chimienti, G Riezzo, G Pepe, G Petrosillo, M Chiloiro, E Marconi. 2008. Inulin-enriched pasta affects lipid profile and Lp(a) concentrations in Italian young healthy male volunteers. *Eur J Nutr* 47:453-459.

Sabater-Molina, M., Larqué, E., Torrella, F., Zamora, S. 2009. Dietary fructooligosaccharides and potential benefits on health. *J. Physiol. Biochem.* 65(3):315-28.

Salkin, I. F., Martinez, J. A., & Kemna, M. E. 1986. Opportunistic infection of the spleen caused by *Aureobasidium pullulans*. *J. Clin. Microbiol.* 23(5):828-831.

SCF. 2003. Report of the Scientific Committee on Food (SCF) on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae (adopted on 4 April 2003). SCF/CS/NUT/IF/65 Final. Available at: http://ec.europa.eu/food/fs/sc/scf/out199_en.pdf. Site visited on April 30, 2015.

Scholtens PAMJ, MS Alles, LEM Willemsen, C van den Braak, JG Bindels, G Boehm, MJAP Govers. 2006a. Dietary fructo-oligosaccharides in healthy adults do not negatively affect faecal cytotoxicity: a randomised, double-blind, placebo-controlled crossover trial. *Br J Nutr* 95:1143-1149.

Sivieri, K., Morales, M.L., Saad, S.M., Adorno, M.A., Sakamoto, I.K., Rossi, E.A. 2014. Prebiotic effect of fructooligosaccharide in the simulator of the human intestinal microbial ecosystem (SHIME® model). *J. Med. Food.* 17(8):894-901.

Sleet R and J Brightwell. 1990. FS-teratology study in rats. Raffinerie Tirlemontoise Internal Report. [Unpublished report presented in Carabin and Flamm 1999]

Slevin MM, PJ Allsopp, PJ Magee, MP Bonham, VR Naughton, JJ Strain, ME Duffy, JM Wallace, EM McSorley. 2014. Supplementation with calcium and short-chain fructo-oligosaccharides affects markers of bone turnover but not bone mineral density in postmenopausal women. *J Nutr* 144:297-304.

Spiegel, J. E., Rose, R., Karabell, P., Frankos, V. H., Schmitt D. F., 1994. Safety and benefits of fructooligosaccharides as food ingredients. *Food Technol.* 48:85-89.

Stone-Dorshow T and MD Levitt. 1987. Gaseous response to ingestion of a poorly absorbed fructooligosaccharide sweetener. *Am J Clin Nutr* 46:61-65.

Tahiri M, JC Tressol, J Arnaud, FRJ Bornet, C Bouteloup-Demange, C Feillet-Coudray, M Brandolini, V Ducrose, D Pepin, F Brouns, AM Roussel, Y Rayssiguier, C Coudray. 2003. Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. *Am J Clin Nutr* 77:449- 457.

Tahiri M, JC Tressol, J Arnaud, F Bornet, C Bouteloup-Demange, C Feillet-Coudray, V Ducros, D Pepin, F Brouns, AM Roussel, Y Rayssiguier, C Coudray. 2001. Five-week intake of short-chain fructo-oligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. *J Bone Miner Res* 16:2152-2160.

Takeda U and T Niizato. 1982. Acute and subacute safety tests. Proceedings of the 1st Neosugar Research Conference. Tokyo: Meija-Seika Publications. [Presented in Carabin and Flamm 1999]

Tashiro, Y., Eida, T., Hidaka, H. 1992. Distribution and quantification of fructooligosaccharides in food materials. *Sci. Rep. Meiji Seika Kaisha* 31:35-40.

Ten Bruggencate SJM, IMJ Bovee-Oudenhoven, MLG Lettink-Wissink, MB Katan, R van der Meer. 2006. Dietary fructooligosaccharides affect intestinal barrier function in healthy men. *J Nutr* 136:70-74.

Tokunaga T, Y Nakada, Y Tashiro, M Hirayama, H Hidaka. 1993. Effects of fructooligosaccharide intake on the intestinal flora and defecation in healthy volunteers. *Bifidus-Flores Fructus Semina* 6:143-150.

Tokunaga T, T Oku, N Hosoya. 1986. Influence of chronic intake of new sweetener fructooligosaccharide (Neosugar) on growth and gastrointestinal function of the rat. *J Nutr Sci Vitaminol* 32:111-121.

Tomomatsu, H. 1994. Health effects of oligosaccharides. *Food Technol.* 48:61-65.

Tschernia A, N Moore, A Abi-Hanna, R Yolken, F Coletta, C Emenhiser, J Saavedra. 1999. Effects of long-term consumption of a weaning food supplemented with oligofructose, a prebiotic, on general infant health status. *J Pediatr Gastroenterol Nutr* 29:503. [Abstract]

Tuohy KM, S Kolida, AM Lustenberger, GR Gibson. 2001. The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides—a human volunteer study. *Br J Nutr* 86:341-348.

Uenishi K, A Ohta, Y Fukushima, Y Kagawa. 2002. Effects of malt drink containing fructo-oligosaccharides on calcium absorption and safety of long-term administration. *Jap J Nutr Dietet* 60:11-18.

Underwood MA, NH Salzman, SH Bennett, M Barman, DA Mills, A Marcobal, DJ Tancredi, CL Bevins, MP Sherman. 2009. A randomized placebo-controlled comparison of 2 prebiotic/probiotic combinations in preterm infants: impact on weight gain, intestinal microbiota, and fecal SCFA. *J Pediatr Gastroenterol Nutr* 48:216-225.

van den Heuvel EGHM, T Muijs, F Brouns, HFJ Hendriks. 2009. Short-chain fructo-oligosaccharides improve magnesium absorption in adolescent girls with a low calcium intake. *Nutr Res* 29:229-237.

van den Heuvel EGHM, T Muys, W van Dokkum, G Schaafsma. 1999. Oligofructose stimulates calcium absorption in adolescents. *Am J Clin Nutr* 69:544-548.

van den Heuvel EGHM, G Schaafsma, T Muys, W van Dokkum. 1998. Nondigestible oligosaccharides do not interfere with calcium and non-heme-iron absorption in young, healthy men. *Am J Clin Nutr* 67:445-451.

van Dokkum W, B Wezendonk, TS Srikumar, EGHM van den Heuvel. 1999. Effect of nondigestible oligosaccharides on large-bowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. *Eur J Clin Nutr* 53:1-7.

Veereman-Wauters G, S Staelens, H Van de Broek, K Plaskie, F Wesling, LC Roger, AL McCartney, P Assam. 2011. Physiological and bifidogenic effects of prebiotic supplements in infant formulae. *J Pediatr Gastroenterol Nutr* 52:763-771.

Waligora-Dupriet A-J, F Campeotto, I Nicolis, A Bonet, P Soulaines, C Dupont, M-J Butel. 2007. Effect of oligofructose supplementation on gut microflora and well-being in young children attending a day care centre. *Int J Food Microbiol* 113:108-113.

Williams CH, SA Witherly, RK Buddington. 1994. Influence of dietary Neosugar on selected bacterial groups of the human faecal microbiota. *Microb Ecol Health Dis* 7:91-97.

000038

Yao M, R Capeding, M Fitzgerald, K Ramanujam, S Yachetti, P DeRusso. 2010. High 2-palmitate and oligofructose in lower protein alpha-lactalbumin-enriched term infant formula: effects on stool characteristics and stool composition. *J Pediatr Gastroenterol Nutr* 50 (Suppl 2):E207-208.

Yap KW, S Mohamed, MH Jamal, M Diederick, YA Manap. 2008. Changes in infants faecal characteristics and microbiota by inulin supplementation. *J Clin Biochem Nutr* 43:159- 166.

Yap KW, S Mohamed, AM Yazid, I Maznah, DM Meyer. 2005. Dose-response effects of inulin on the faecal SCFA content and mineral absorption of formula-fed infants. *Nutr Food Sci* 35:208-219.

Xia Q, T Williams, D Hustead, P Price, M Morrison, Z Yu. 2012. Quantitative analysis of intestinal bacterial populations from term infants fed formula supplemented with fructo-oligosaccharides. *J Pediatr Gastroenterol Nutr* 55:314-320.

6. APPENDIX I

Animal Toxicity Studies of Frucro-oligosaccharides

Reference	Study Design & Objective	Animal Model	Source & Description of Test Article	Dose & Duration of Feeding	Findings
Acute Oral Toxicity					
Takeda and Niizato (1982) [acute mouse study]	Acute oral toxicity study of scFOS	48 4-week-old male and female JcL-IcR mice (6 mice/sex/dose)	scFOS	Single gavage doses of 0, 3, 6, or 9 g scFOS/ kg bw	No deaths occurred and there were no differences in body weight gain between the test and the control animals. No abnormalities were seen in either sex. The LD50 for oral administration of scFOS to rats in this study was > 9000 mg/kg bw.
Takeda and Niizato (1982) [acute rat study]	Acute oral toxicity study of scFOS	48 6-week-old male and 10-week-old female Sprague Dawley rats (6 rats/sex/ dose)	scFOS	Single gavage doses of 0, 3, 6, or 9 g scFOS/ kg bw	There were no deaths and no abnormalities or changes in body weight of animals of either sex. The LD50 for oral administration of scFOS to rats in this study was > 9000 mg/kg bw.
Subacute Oral Toxicity					

Tokunaga et al. (1986)	Safety and metabolic handling of scFOS or gluco-mannan	24 male Wistar rats (40-50 g (6 rats/dose)	scFOS	0, 10, or 20% dietary concentration for 4-6 weeks	Feed intake was similar in all feeding groups. No difference in weight gain between control rats and those receiving diets containing 10% scFOS. The diets with 20% scFOS or glucomannan resulted in significantly reduced weight gain. Diet had no effect on absolute weights of the liver or kidneys. Ingestion of scFOS or glucomannan produced dose-dependent significant increases in the weights of the cecum and colon. Wet weight of the small intestine was also significantly increased in the rats receiving 20% scFOS or glucomannan. Significant dose-dependent effects of prebiotic ingestion were seen in increased fecal weight and shortened GI transit time. Glucomannan was more potent than scFOS in producing these effects. Ingestion of prebiotics had no effect on cholesterol but significant dose-dependent decreases in TAG concentration. The excretion of both neutral and acidic sterols was significantly enhanced by prebiotic ingestion, as was that of SCFA, especially acetic and propionic acids. Ingestion of both scFOS and glucomannan resulted in diarrhea during the first few days of feeding, but there was no evidence of toxicity seen from ingestion of scFOS at 10% or 20% (equivalent to approximately 7500 and 15000 mg/kg bw/day). The reduction in body weight gain seen with 20% scFOS was due to its not being fully utilized as an energy source. NOAEL not indicated.
Takeda and Niizato (1982)	Subacute oral toxicity study of scFOS	Seventy-two 6-7-week-old male Wistar rats (18 rats/dose)	scFOS	0, 1.5, 3, or 4.5 g/kg bw/ day for six weeks	There were no deaths or abnormalities and no consistent treatment-related differences were observed in blood chemistries. Animals receiving 3 or 4.5 g scFOS/kg bw/day showed a slight increase in BW compared to the controls. A slight swelling of the appendix was observed in the rats receiving scFOS; not seen in the other groups. Histopathology revealed no abnormalities. No treatment-related toxicity occurred in any of the scFOS-treated groups. The NOAEL for orally administered scFOS was 4500

Takeda and Niizato (1982) [2nd sub-acute rat study]	Subacute oral toxicity study of scFOS and sorbitol	108 6-7-week-old male Wistar rats (18 rats/treat-ment/dose)	scFOS	5 or 10% dietary concentration for 6 weeks	No deaths occurred and no treatment-related abnormalities were observed. The sorbitol and scFOS groups had lower body weights in weeks 1-5. Body weight gains in the latter part of the study were the same as those of the control groups. Animals in the sorbitol group exhibited diarrhea on day 2 and those in the scFOS group on day 10. There was a reduction in cholesterol in animals in the scFOS groups. Slight hepatic necrosis and infiltration of round cells as well as renal changes and degeneration of the proximal tubular epithelial cells were seen in isolated specimens from all groups. Isolated cases of Ca deposits were seen in all groups. Oral administration of scFOS resulted in decreased body weight, lowered cholesterol, and swelling of the appendix. Adverse changes in the kidneys and liver and Ca deposits in the cortex were observed in isolated cases; however, these were also prevalent in the control groups. The effects observed in the proximal renal tubules were not as severe in the scFOS-treated animals as in those receiving sucrose. The lower caloric content of scFOS was thought to contribute to the reduction in body weight gain. There was no evidence of scFOS being of higher toxicity than the sugars used as controls even at concentrations as high as 10% of the diet. The NOAEL in this study was 10%, approximately equivalent to 7500 mg/kg bw/day.
Subchronic Oral Toxicity					
Boyle et al. (2008)	Oral toxicity (feeding) study of oligofructose	Male and female 7-week-old Sprague-Dawley CD® rats (male weights 191-287 g; female weights 155-197 g); 20 rats/sex/dose	Oligofructose	0, 0.55, 1.65, 4.96, or 9.91% dietary concentration (high doses = 4680 and 5720 mg/kg bw/day for M&F, respectively) for 13 weeks	Feed intake was significantly lower in rats fed higher levels of oligofructose, especially during the first half of the study. Body weights were lower, reaching statistical significance only for male rats receiving the highest dose. No significant clinical observations and no significant test-article-related differences in hematology, biochemistry, or coagulation. A significant dose-related increase was seen in both absolute and relative cecal weights of both sexes, but there was no associated histopathology. There was no other significant organ-weight or histopathological differences. Total fecal bacteria populations were slightly but statistically significantly higher in rats fed the two higher doses of oligofructose. Bifidobacteria population changes were statistically significant. The researchers concluded that the NOAEL of oligofructose was 9.91% dietary concentration, the highest level tested, equivalent to 4680 and 5720 mg/kg bw/day in males and females, respectively.

Genta et al. (2005)	Subchronic oral toxicity (feeding) of flour from yacon root containing oligofructose	60 3-month-old male and female Wistar rats (10 rats/sex/ dose)	Yacon root flour containing 55.3% oligofructose	0, 340, or 6800 mg flour/kg bw/ day providing 0, 188, or 3760 mg oligofructose/ kg bw/day for 4 months	No mortality and no signs of toxicity were observed during the study. There were no signs of GI effects. No effects on body weight gain in male or female rats, nor in feed consumption. The three groups did not differ significantly in their responses to the glucose tolerance test in either latency or maximum glucose concentration. No differences were seen in hematological or coagulation outcomes across the test and control groups, nor were any effects attributable to treatment observed in the clinical chemistries. A significant difference was noted in the postprandial state, in which the rats receiving oligofructose exhibited decreased serum TAG, but no differences in cholesterol or glucose. Urinalysis revealed no significant differences among the groups. Necropsy revealed no treatment-related abnormalities except that both absolute and relative weights of the GI tract were increased in both sexes in the high-dose groups due to enlargement of the cecum. Histopathological examination showed no effects besides increased cecal hypertrophy and increased size of epithelial cells. There was no sign of cell tumefaction, in the high-dose oligofructose group. These effects were regarded as trophic effects of the extremely high dose level rather than evidence of toxicity. The NOAEL for yacon flour oral toxicity was 6800 mg/kg bw/day, equivalent to 3760 mg oligofructose/kg bw/day.
Chronic Oral Toxicity					

Clevenger et al. (1988)	Combined 104-week chronic toxicity and carcino-genicity study of scFOS	100 4-week-old male and female Fischer 344 rats (12-13 rats/ sex/dose)	scFOS	0, 0.8, 2.0%, and 5.0% dietary concentration (equivalent to 0, 341, 854, and 2170 mg/kg bw/day for male rats and 0, 419, 1045, and 2664 mg/kg bw/day for female rats for 2 years	Some mortality was observed in all groups of males and females but was not considered treatment-related. scFOS did not affect feed intake, body weight gain, feed conversion efficiency, absolute organ weights, or any hematology outcomes. There were slight elevations of Na and Cl in male rats. Male rats in the mid-dose scFOS group exhibited slightly elevated levels of blood glucose and creatinine but creatinine levels in males in the high-dose group decreased. Other outcomes did not significantly differ between test groups and controls. In females, all blood chemistry outcomes were similar to those of the controls except for a slight elevation of uric acid in the low- and mid-dose groups. No test-article-related macro- or microscopic changes were found in either males or females. The NOAEL was 50,000 ppm, the highest concentration tested, equivalent to 2170 mg/kg bw/day for males and 2664 mg/kg bw/day for females. Similar numbers of neoplastic lesions (e.g., pheochromocytomas, thyroid C- cell adenomas, leukemias, and pituitary adenomas) occurred in scFOS- treated animals and controls, with the exception of pituitary adenomas. In male rats, the incidence of pituitary adenomas for the 0-, 8000-, 20,000-, and 50,000-ppm dose groups was 20, 26, 38, and 44%, respectively. The historic incidence of pituitary adenomas in F-344 male rats from the test laboratory ranges from 1 to 49%. While the incidence of this tumor was well within historical range for all male rats, the incidence in the two highest dose groups (20,000 and 50,000 ppm) was significantly greater than the incidence in controls. Cochran–Armitage chi-square indicated a dose-response trend ($p = 0.007$), but logistic regression analysis showed no trend ($p = 0.51$), giving an overall equivocal result. In the female rats, a negative trend in the incidence of pituitary adenomas was recorded. It was concluded that higher incidence of pituitary adenomas in males was not treatment related. The results indicate that scFOS is not carcinogenic.
Developmental Toxicity					

Henquin (1988)	Develop-mental toxicity study of oligo-fructose	19 female Wistar rats with copula- tion plugs (12 test, 17 control)	Oligofructose	20% dietary concentration from day 1 to day 21 of gestation	Oligofructose had no effect on the number of pregnancies but produced a reduction in body weight gain of the pregnant rats, perhaps because oligofructose has a lower caloric value, decreased intake of food for this group, or diarrhea observed in the first week and softer stools in the second and third weeks. Fetus and newborn weights were not affected despite the reduction in body weight gain in pregnant rats.During the nursing period, a growth delay was observed for the male pups in the test group, which may be indicative of the restricted nutritional status of the lactating mothers. The researchers concluded that a diet containing 20% oligofructose has no significant effects on the course of pregnancy in rats and on the development of their fetuses and newborns.
Sleet and Brightwel l (1990)	Study of maternal and develop-mental toxicity of oligofructose	~100 pregnant female Crl CD (SD) BR Sprague Dawley rats (~24 rats/dose)	Oligofructose	4.75% dietary concentration from post coitum day 0 to 6, then 0, 5, 10, or 20% dietary concentration to day 15	No treatment-related effects were observed during Day 0 - 6 or during Day 6 - 15. There was no mortality and no diarrhea observed in any of the test animals. Oligofructose administered at a dietary concentration of 4.75% during Day 0-6 did not affect body weights. Beginning on Day 8, body weight was reduced dose-dependently in all three oligofructose groups relative to the controls. The 5% group had a lower weight gain than the controls while the 10 and 20% groups lost weight. From Day 11 to the end of the study, the body weight and body weight change were similar for all groups except the 20%-oligofructose group, which remained below the controls. At necropsy, the number of pups per litter, sex ratio, and viability of both embryo and fetus were not affected by oligofructose. Litter and fetal weights were not significantly different except that the fetal weight of the 20% group was significantly greater than that of the controls. Structural development of the fetuses was unremarkable. The only treatment-related effect was slightly lowered body weights of the dams, only seen in the 20% oligofructose-group. The researchers concluded that oligofructose at dietary concentrations up to 20% does not cause adverse effects or adversely affect the pregnancy outcome or *in utero* development of the rat.

Adapted from GRN 537 and other available studies

7. APPENDIX II

Summary of Available Studies of FOS in Healthy Adults.

Reference	Study Design & Objective	Subjects	Source & Characteristics of Test Article	Dose & Duration	Results
scFOS					
Bouhnik et al. (1996)	Prospective, randomized, double-blind, placebo-controlled study of the effects of scFOS on micro- biota and stooling	20 healthy adults (10 of each sex) aged 22-39 years	scFOS	0 or12.5 g/day for 12 days	Intake of scFOS had no effect on fecal pH or total anaerobic counts, but bifidobacteria numbers increased during scFOS feeding, returning to baseline levels within 12 days after cessation. β-fructosidase activity increased during scFOS ingestion, returning to baseline within 12 days, but other enzyme activity levels and bile acids were not affected. There were no differences observed in stool weights or in lipid content. The researchers concluded that ingestion of 12.5 scFOS/day was well tolerated and produced significant bifidogenesis but no other significant effects.
Bouhnik et al. (1999)	Open-label dose-response study of bifidogenesis to scFOS	40 healthy adults (18 males and 22 females aged 18 to 47 years; mean age = 29.6 years)	scFOS	0, 2.5, 5, 10, or 20 g/day for 7 days	There was no increase in total anaerobes observed in any group. Bifidobacteria did not increase in groups receiving 0 or 2.5 g scFOS/day, but increased in all 3 groups receiving larger doses of scFOS; the correlation was r = 0.53. Flatus was reported frequently by those receiving 20 g scFOS/day than the other groups, but no other GI symptom differed among groups. The researchers concluded that "10 g/day [is] the optimal and well-tolerated dose of scFOS which leads to a significant increase in colonic bifidobacteria in healthy volunteers consuming their usual diet."

Bouhnik et al. (2004) Part 1	Prospective, randomized, parallel-group, placebo-controlled, double-blind dose-response study of bifidogenic activity of long-chain inulin and scFOS	64 healthy men and women (aged 18-54 years with a mean age of 30 years)	scFOS, soyoligo-saccharides, GOS, long-chain inulin from chicory, or type III resistant starch, isomalto-oligo-saccharides, or lactulose	0 or 10 g/day for 7 days	There were no differences seen in any of the groups in fecal pH or counts of total anaerobic bacteria, *Bacteroides* spp., *Lactobacillus* spp., or enterobacteria, nor in stool frequency. Increased bifidobacteria counts were seen in the groups receiving scFOS, soy oligosaccharides, type III resistant starch, or GOS. Long-chain inulin, isomaltooligosaccharides, and lactulose were not found to be bifidogenic. All groups had increases in flatus, bloating, and abdominal pain; however, there were no differences among the different treatments. Diarrhea was not reported in any of the groups.
Bouhnik et al. (2004) Part 2	Prospective, randomized, parallel-group, placebo-controlled, double-blind dose-response study of bifidogenic activity of scFOS	136 healthy men and women (aged 18-54 years with a mean age of 30 years)	scFOS, soyoligo-saccharides, type III resistant starch, or GOS	0, 2.5, 5.0, 7.5, or 10 g/day for 7 days	As in phase 1, no significant differences were seen in stool frequency, fecal pH, or counts of total anaerobic bacteria, *Bacteroides* spp., *Lactobacillus* spp., or enterobacteria. All treatments were significantly bifidogenic, but a dose-response effect was significant only for scFOS. All treatments produced GI side effects; however, there were no differences across treatments. Diarrhea was not reported in any of the groups.
Bouhnik et al. (2006)	Randomized, double-blind, placebo-controlled trial to study the dose-response relationship between scFOS ingestion and bifidogenesis	40 apparently healthy adults (18M and 22F) aged 20±1.3 years	scFOS	0, 2.5, 5.0, 7.5, or 10.0 g/day for 7 days	Bifidobacteria counts increased at all doses; r=0.307 between dose and response. There were no differences found in fecal pH or in the numbers of lactobacilli, bacteroides, or enterobacteria. The frequency of symptoms of intolerance did not differ between the control group and any scFOS group. The severity of bloating was significantly higher at doses of 2.5 and 5.0 g/day, but not at the higher doses of 7.5 or 10.0 g/day. No diarrhea was reported by any volunteer. The researchers concluded that "scFOS is bifidogenic and well tolerated in healthy volunteers . . . and a dose-response relationship was demonstrated from 2.5 to 10 g/day."

Buddington et al. (1996)	Open-label study of the effect of scFOS on intestinal microbiota and reductive enzymes	12 apparently healthy adults (6 of each sex) aged 20-34 years	scFOS	4 g/day for 25 days	Counts of both total anaerobes and bifidobacteria increased significantly with ingestion of scFOS, but counts of aerobes did not change. Nitroreductase activity remained constant through the period of scFOS intake, but both β-glucuronidase and glycocholic acid hydroxylase activities decreased significantly. The researchers concluded that "4 g [scFOS] alters the fecal flora in a manner perceived as beneficial by decreasing activities of some reductive enzymes." There were no reports of digestive or health problems.
Ducros et al. (2005)	Prospective, randomized, double-blind, placebo-controlled crossover study of the effect of scFOS on mineral absorp- tion in postmeno- pausal women	11 women aged 53-70 years (mean = 59 years)	scFOS	0 or 5 g/day for 4 days, then 0 or 10 g/day for the rest of 5 weeks	Intake of scFOS significantly improved absorption of copper but had no effect on absorption of zinc or selenium. The ingestion of 10 g scFOS/day was well tolerated and no adverse effects were noted.
Fukushima et al. (2002) Part1	2 randomized, double-blind, placebo-controlled, crossover trials of the effect of scFOS on calcium absorption	8 apparently healthy females aged 20.9±0.6 years in the 1st study; 5 apparently healthy females aged 21.4±0.9 years in the 2nd study	scFOS	0 or 1.9 g single dose in the 1st study and 0 or 1.5 g single dose in the 2nd study	Urinary calcium was significantly increased at 4, 6, and 8 hours after ingestion with both scFOS-containing drinks (canned liquid malt drink in the 1st study and a dehydrated powder malt drink in the 2nd study). No adverse effects were reported.

Fukushima et al. (2002) Part 2	Open-label studies of the 2 drinks used in Part 1 to assess safety	9 apparently healthy adults (6 males and 3 females) consuming the canned drink; 10 adults (7 males and 3 females) consuming the powder-type drink	scFOS	5.7 and 4.5 g/day for 7 days	No abnormal changes were observed in clinical chemistry or hematology (total protein, AST, ALT, ALP, GGT, AMY, uric acid, BUN, creatinine, calcium, phosphorus, iron, TC, HDL-cholesterol, neutral lipid, glucose, red and white blood cell counts, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count), or in urinalysis (specific gravity, pH, urobilinogen, bilirubin, ketone bodies, protein, glucose, occult blood), or in the clinical interview. Stooling was normal with no indication of diarrhea.
Garleb et al. (1996)	Randomized, double-blind, controlled study of the effect of the addition of scFOS to enteral feeding formulas	27 apparently healthy male college students	scFOS	0, 5, or 10 g/L for 14 days	The low and high scFOS groups had daily intakes of about 15 and 30 g scFOS. There was no change in body weight or deviations from the normal range of blood chemistry values (glucose, BUN, creatinine, bilirubin, TC, TAG, protein, albumin, globulin, ALP, lactate dehydrogenase, ALT, AST, calcium, sodium, potassium, chloride, iron, phosphorus, and GGT). Fecal acetate, isobutyrate, and isovalerate concentrations were higher among volunteers ingesting scFOS, but there were no differences in propionate or butyrate, fecal pH, or fecal percent dry matter. Consumption of scFOS increased fecal bifidobacteria levels. The tolerance of the scFOS-containing formula was good. Complaints of nausea, cramping, distension, vomiting, diarrhea, and regurgitation were similar across all groups and were present on fewer than 5% of participant-days. Flatus was reported more frequently by those consuming 30 g scFOS/day, but most complaints occurred during the first 4 days. The researchers concluded that "these results indicate that [scFOS] does not compromise serum chemistry profiles, is well tolerated particularly at an intake of 15 g/d and would serve as a bifidogenic factor when incorporated into a liquid enteral product."

Hess et al. (2011)	Randomized, double-blind, controlled crossover study of the effect of scFOS on hunger and satiety	20 apparently healthy adults aged 18-64 years (10 men aged 28±2 years and 10 women aged 28±4 years)	scFOS	0, 10, or 16 g/day taken in 2 doses over 24 hours	Breath H_2 measured at 0 and 4 hours after scFOS ingestion increased dose-dependently, indicating that scFOS undergoes fermentation within 4 hours. There were no symptoms of intolerance and no consistent effects on hunger or satiety. No adverse effects were reported.
Hidaka et al. (1986) Part 1	Open-label study of the effect of scFOS ingestion on blood glucose and insulin	No information was given regarding the number, sex, or age of subjects	scFOS	25 g in a single dose	scFOS ingestion did not have an effect on blood glucose or insulin, indicating the nondigestibility of the substance. No adverse effects were reported.
Hidaka et al. (1986) Part 2	Open-label study of the effect of scFOS ingestion on microbiota	Healthy adults (no further information was reported)	scFOS	8 g/day for 2 months	Fecal bifidobacteria counts increased significantly, as well as fecal SCFA and urinary phenol and p-cresol. No adverse effects were reported.

Luo et al. (1996)	Prospective, randomized, double-blind crossover study of the effects of scFOS on hepatic glucose production, insulin- mediated glucose metabolism, erythrocyte insulin binding, and blood lipids	Twelve 19-32-year- old males (mean age = 24)	scFOS	0 or 20 g/day for 4 weeks	None of the participants dropped out or complained of any adverse effects. Energy intakes, proximates, or body weight were not affected by the diet. Dietary interventions did not cause significant changes in fasting plasma glucose or insulin, total or HDL cholesterol, apolipoprotein A-1 or B, lipoprotein$_a$, or TAG concentrations or change the mean insulin binding to erythrocytes or apparent receptor affinity. Mean basal hepatic glucose production was significantly lower after scFOS than after sucrose. The researchers concluded that 4 weeks of 20 g scFOS/ day "had no detectable adverse metabolic effect" on healthy adults."
Molis et al. (1996)	Open-label study of the digestion and excretion of scFOS	Six healthy adults, 3 of each sex, aged 20-27 years	scFOS	5 g/day at first, in-creased over 3 days to 20.1 g/day for 8 days	None of the ingested scFOS was excreted in stools, and less than 11% was absorbed in the small intestine, indicating that the portion reaching the colon was completely fermented by colonic microbiota. Only 0.12% of scFOS was recovered in the urine. scFOS appeared in the distal ileum within 30-60 minutes after ingestion and continued to be recovered for 8 hours. The estimated energy value of the tested scFOS was 2.3 kcal/g. No GI symptoms were reported during either the placebo or scFOS periods.
Ohta et al. (1999) Part 1	Open-label study of the safety of tablets containing scFOS	10 apparently healthy men aged 37±10 years	scFOS	9 g in a single dose	Two men reported increased flatus, but no other side effects were reported. The authors concluded that "This study provides strong evidence that our tablets are . . . safe."

Ohta et al. (1999) Part 2	Randomized, double-blind, placebo-controlled crossover study of the effect of scFOS on calcium absorption and excretion	10 apparently healthy men aged 37±10 years	scFOS	0 or 3 g in a single dose	Calcium excretion was significantly higher at 2, 6, and 8 hours after ingestion when it was accompanied by scFOS rather than sucrose and the calcium/creatinine ratio was significantly higher at all time points. There were no significant differences in urine volume or levels of hydroxyproline, pyridinoline, or deoxypyridinoline relative to creatinine. The researchers concluded that "Calcium absorption was stimulated and the calcium supplementary effects of the calcium tablets were improved." No adverse effects were reported.
Oku and Nakamura (2003)	Open-label crossover study of differences in digestibility of scFOS and other oligosaccharides	38 apparently healthy adults (9 men and 29 women) aged 23.7±6.6 years	scFOS (and GOS and IMO)	10 or 20 g in a single dose	No H_2 was detected in breath after administration of either 10 or 20 g IMO. H_2 was seen in greater quantity and more quickly after ingestion of scFOS than GOS (at 100 minutes vs. 180 minutes). Breath H_2 was twice as abundant after ingestion of 20 g scFOS or GOS than after 10 g. The reseachers concluded that "scFOS is not hydrolyzed by intestinal enzymes and . . . almost all scFOS reached the large intestine where they were fermented by intestinal microbes." This conclusion was supported by the fact that many of the volunteers showed abdominal symptoms such as flatus, distention, and borborygmi after ingestion of 10 or 20 g scFOS and after 20 g, but not 10 g, GOS. No GI symptoms were reported after ingestion of 10 or 20 g IMO.
Slevin et al. (2014)	Randomized, double-blind, placebo-controlled trial of effects of Ca supplementation w/ or w/o scFOS	300 non-osteoporotic postmenopausal women	scFOS	3.6 g/day for 24 months	While the calcium supplement had a beneficial effect on markers of bone turnover, scFOS had no significant additional effect. No adverse effects were reported.
Stone-Dorshow and Levitt (1987)	Randomized double-blind, placebo-controlled study to test the effect of scFOS on breath hydrogen excretion	15 healthy adults aged 21-65	scFOS	0 or 15 g/day for 12 days	Peak excretion of H_2 following 10 g scFOS occurred at 3 hours; neither the peak nor the total area under the curve was affected by 12 days consumption of scFOS or sucrose. The breath tests of scFOS and lactulose were similar, suggesting that no absorption was occurring. The 10 volunteers who consumed 15 g scFOS/day had significantly greater flatulence than did the five taking sucrose, but no other adverse effects were reported.

Tahiri et al. (2001)	Randomized, double-blind, placebo-controlled crossover trial of the effect of scFOS on magnesium absorption and excretion	11 apparently healthy postmenopausal women age 59±6 years	scFOS	0 or 5 g/day for the first 4 days, then 0 or 10 g/day for 31 days	The women consumed a mean of 9.8±0.2 g/day of both scFOS and sucrose. scFOS ingestion increased magnesium absorption by 12.3%, which resulted in higher levels of both plasma and urine magnesium. The researchers concluded that "ingestion of moderate doses of scFOS did improve intestinal magnesium absorption and status in postmenopausal women." No adverse effects were reported.
Tahiri et al. (2003)	Prospective, randomized, double-blind, crossover study of the effect of scFOS on calcium absorption in postmenopausal women.	12 healthy postmenopausal women aged 50-70 years (mean = 59.8 years)	scFOS	0 or 10 g/day for 5 weeks	The mean calcium absorption and urinary excretion with scFOS treatment and placebo were not significantly different, nor were plasma parathyroid hormone or 1,25-dihydroxyvitamin D concentrations. The researchers suggested that scFOS intake may improve calcium absorption by women in late post-menopause. No adverse effects were reported.
Tokunaga et al. (1993)	Open-label study of the effect of scFOS on the intestinal microbiota	27 apparently healthy adults (21 males aged 36.8±9.0 years and 6 females aged 25.2±3.3 years)	scFOS	1, 3, or 5 g/day for 2 weeks	All three groups showed statistically significant increases in bifidobacteria, with 2.5-, 3.2, and 4-fold increases among those receiving 1, 3, or 5 g scFOS/day, respectively. The increased counts of bifidobacteria disappeared over the 2-week follow-up. All three groups also had statistically significantly increased frequency of defecation and softening of the stool, with no apparent dose effect. No adverse effects reported.
Uenishi et al. (2002) Part 1	Randomized, double-blind crossover study of the effect of scFOS on calcium absorption in young women	8 women aged 20-22 years (mean = 20.5±0.8 years)	scFOS	0 or 3.0 g in a single dose	Consumption of scFOS significantly raised total urinary calcium and $^{44}Ca/^{43}Ca$ ratio measured 4 hours and longer after ingestion. The researchers concluded that "addition of scFOS enhances absorption of calcium." No adverse effects were reported.

Uenishi et al. (2002) Part 2	Open-label study of the safety of scFOS in adults and children	26 apparently healthy adults aged 31.8±7.5 years and 15 children aged 6.0±2.6 years	scFOS	3.0 g/day for 13 weeks	No change was observed in mean values for biochemistry (total protein, AST, ALT, GPT, ALP, GGT, AMY, uric acid, BUN, creatinine, calcium, phosphorus, iron, TC, HDL cholesterol, neutral fat, and blood glucose), hematology (leucocyte count, erythrocyte count, hemoglobin, hematocrit value, MCV, MCH, MCHC, and platelet count), or urinalysis (specific gravity, pH, urobilinogen, bilirubin, ketone bodies, protein, sugar, and occult blood). No adverse events were reported and there was no diarrhea. The researchers concluded, "Adverse changes in the health status of subjects were not observed in subjective symptoms, inquiry by the doctor and blood examination for either children or adults, who ingested the test drink for 13 successive weeks. The test drink used thus did not have adverse effects within the range of condition of this study."
van den Heuvel et al. (2009)	Randomized, double-blind, placebo-controlled crossover trial of the effect of scFOS on calcium absorption	14 healthy 12-14-year-old girls (mean age = 13±1 years) with chronically low calcium intake	scFOS	0 or 10 g/day for 36 days	No short-term (8 days) or long-term (36 days) increase in calcium absorption was observed in response to scFOS intake. Long-term magnesium absorption increased significantly. The authors stated that "consumption of scFOS did not lead to an increased number of adverse events as compared to the control treatment."

Williams et al. (1994)	Open-label study of the effect of scFOS on the intestinal microbiota	10 apparently healthy adults, 5 of each sex, aged 20-40 years	scFOS	4 g/day for 14 days	Nine of the 10 individuals showed increased bacterial counts; the largest change was the increase in total anaerobes. Smaller increases in total aerobes, bifidobacteria, and lactobacilli were statistically significant, while a nonsignificant decrease was seen in *Enterobacteriaceae*. The researchers concluded that the findings "demonstrate that supplementing the diet with [scFOS] selectively encourages the proliferation of bacterial groups perceived as being beneficial (e.g., bifidobacteria and lactobacilli)." No adverse effects were reported.
Other Fructans					
Alles et al. (1996)	Balanced multiple crossover trial using an orthogonal Latin-square design to investigate the metabolic fate of two different levels of oligofructose	24 non-smoking non-overweight healthy males aged 19-28 years (mean = 22.1 years)	Oligofructose from chicory	5 or 15 g/day for 7 days each	Ingestion of 15 g oligofructose/day resulted in increased flatulence. No other differences in GI effects, defecation frequency, stool pH, stool form, or stool wet/dry weight were reported. Oligofructose increased breath H_2 in a dose-dependent manner, reaching statistical significance only at a daily dose of 15 g. No traces of the ingested oligofructose were detected in any of the stool samples and no effects were observed on concentrations of SCFA. The researchers concluded that oligofructose added to the diet is fully metabolized in the large intestine. No adverse effects were reported other than increased flatulence at the high dose.

Bartosch et al. (2005)	Randomized double-blind, placebo-controlled trial of the effect of a synbiotic on gut bacteria in healthy elderly people	18 healthy elderly women aged 63-90 years (mean age = 72 years)	1:1 combination of long-chain inulin and oligofructose from chicory and $3.5x10^{10}$ cfu each of *B. bifidum* BB-02 and *B. lactis* BL-01	0 or 6 g/day for 4 weeks	The synbiotic intervention had no significant effect on total bacterial counts, but both bifidobacteria and lactobacilli were increased significantly. The increase in bifidobacteria included both the species administered in the synbiotic and other bifidobacterial species. The researchers concluded that administration of the synbiotic provided significant beneficial modification of the gut microbiome of elderly individuals with no apparent adverse effects.
Brighenti et al. 1999	Randomized single-blind, placebo-controlled crossover study of the effect of inulin on blood lipids and the colonic ecosystem	12 healthy, normolipidemic young men with a mean age of 23.3 years	Chicory-derived inulin	0 or 9 g/day for 4 weeks	No changes in body weight, dietary habits, fecal pH, bile acid output, or SCFA were observed. The glucose tolerance tests obtained the same incremental areas under the curve. Plasma TC and TAG were lowered by ingestion of inulin, while fecal concentration of lactic acid and breath H_2 increased. Inulin lowered counts of total facultative anaerobes and increased the proportion of bifidobacteria. No adverse effects were reported.
Cani et al. 2006	Randomized single-blinded, placebo-controlled, crossover study to assess the effects of oligofructose on satiety and energy intake in humans	10 healthy individuals (5M and 5F aged 21– 39 years with a mean of 27.2 years)	Oligofructose from chicory	0 or 16 g/day for 2 weeks	Ingestion of 16 g oligofructose/ day significantly increased satiety and reduced breakfast, lunch, and total energy intake as compared to the placebo. There were few tolerance problems. Minor GI effects reported disappeared after Day 3.
Castiglia-Delavaud et al. (1998)	Randomized parallel-group, Latin-square design to study fermentation of inulin	9 healthy young men (average age of 21.5 years)	Chicory inulin	Increasing dose for 14 days, then 50 g/day for 12 days	Inulin induced significant increases in defecation frequency and stool weight resulting from increases in excretion of water, dry mass and microbial mass. After deduction of microbial nitrogen, differences in fecal nitrogen excretion between diets were not significantly different. The fermentability of inulin approached 100%. The calculated energy content of inulin was 1.2 kcal/g. No adverse effects were reported.

Clausen et al. (1998)	Randomized cross-over study to assess the induction of diarrhea by ingestion of oligofructose	12 healthy adults (4M, 8F) aged 27-56 years	Oligofructose from chicory	Escal-ating dose of 20, 40, 80, and 160 g for 3 days each	Oligofructose produced significant dose-dependent increases in stool weights. Fecal pH decreased. Na concentration increased while K decreased, but there was no change in fecal osmolarity. Little oligofructose appeared in the feces until intake reached 160 g/day, when about 20% of the ingested dose was excreted, about 40% as the free monosaccharide fructose. The researchers concluded that a laxation effect was shown, reaching the level of diarrhea induction at large doses. Furthermore, "Fecal volume in carbohydrate-induced diarrhea is proportional to the osmotic force of the malabsorbed saccharide, even though all or the majority of the saccharide is degraded by colonic bacteria." No adverse effects were reported other than the laxation effects.
Coudray et al. (1997)	Randomized cross-over study to investigate the effect of inulin on mineral absorption	9 healthy young men, average age of 21.5 years	Inulin derived from chicory	In- creasing dose for 14 days, then 40 g/day for 12 days	Ingestion of inulin significantly increased the absorption and balance of calcium. Absorption and balance of magnesium, iron, and zinc were not significantly altered. No adverse effects were reported.
De Preter et al. (2007)	Randomized double-blind, placebo-controlled crossover trial to study prebiotic, probiotic, and synbiotic effects on colonic nitrogen- protein metabolism in healthy humans	20 healthy young adults (10 of each sex, mean age = 21 years)	1:1 blend of long-chain inulin and oligofructose from chicory; *B. breve* and *L. casei*	0 or 10 g/day for 4 weeks with and 4 weeks without probiotics	Oligofructose-enriched inulin significantly reduced proteolytic activity in the colon, while both probiotics had smaller but still statistically significant effects. Both of these effects were temporary and disappeared over the 2-week washout periods. No adverse effects were reported due to the interventions.

De Preter et al. (2008)	Randomized double-blind, placebo-controlled crossover trial of pre-, pro-, and synbiotic effects on intestinal bacterial enzyme activity	53 healthy adults (28M and 25F aged 19-26 years, mean = 22 years)	1:1 blend of long-chain inulin and oligofructose from chicory; *S. boulardi, B. breve* and *L. casei*	20 g/day for 4 weeks with and 4 weeks without probiotics	Oligofructose-enriched inulin significantly reduced activity of β-glucuronidase, but not that of β-glucosidase. None of the interventions affected total fecal output or fecal dry mass. The researchers concluded that the reduction in β-glucuronidase activity is beneficial and may have important health implications. No adverse effects were reported.
Forcheron and Beylot (2007)	Randomized double-blind, placebo-controlled investigation of fructans and lipid-lowering in adults	17 healthy adults	1:1 blend of long-chain inulin and oligofructose from chicory	0 or 10 g/day for 6 months	There were no differences in dietary intakes, body weights, plasma glucose, insulin, glucagon, nonesterified fatty acids, or TAG between the oligofructose-enriched inulin and placebo groups. There was a nonsignificant reduction in TC and LDL. Lipid and cholesterol synthesis rates were not different between groups and no differences were seen in messenger RNA concentrations of key regulatory genes of cholesterol synthesis. No adverse effects were observed from ingestion of 10 g long-chain inulin+FOS/day for 6 months.
Gibson et al. (1995)	Randomized single-blind, parallel-group study of the effects of oligofructose or inulin on colonic microbiota and colonic function	8 healthy volunteers (7M, 1F aged 21 to 48 years; mean age = 33.6 years)	Oligofructose or inulin from chicory	0 or 15 g/day for 15 days	Both oligofructose and inulin increased bifidobacteria concentrations in stools, while bacteroides, clostridia, and fusobacteria decreased when volunteers were fed oligofructose. Gram-positive cocci decreased when volunteerss were fed inulin; however, total bacterial counts were not different. Fecal wet and dry matter, nitrogen, energy excretion, and breath H_2 increased significantly with both substrates, but SCFA and breath CH_4 were not affected. No adverse effects were reported due to ingestion of 15 g/day of either inulin or oligofructose.
Grasten et al. (2003)	Randomized double-blind, parallel-group study of the effects of inulin on the metabolic activity of intestinal microbiota	14 healthy adults (3M and 11F) with mean age = 34 years	Chicory inulin	0 or 15 g/day for 4 days	There was no effect on fecal phenol or *p*-cresol or affected enzyme activity when measured as change from baseline, but β-glucuronidase was significantly lower in the inulin group at termination. Inulin increased concentrations of SCFA, especially acetate and propionate. Non-significant increases were seen in defecation frequency, stool softness, and GI discomfort. No increase was seen in diarrhea.

Kruse et al. (1999)	Non-randomized cross-over study of the effects of inulin on bifidogenesis	11 healthy volunteers (6M and 5F) aged 26-53 years	Inulin from chicory roots	0 or 22-34 g/day for 64 days	No effect was seen on TC, HDL or LDL, or TAG. Bifidobacteria counts increased during the inulin phase and decreased again during the control phase, but total bacteria counts were not changed. Concentrations of SCFA were unaffected. Mild flatulence and bloating lessened over time. There were no complaints about nausea or diarrhea.
Langlands et al. (2004)	Open-label study of the effect of inulin+FOS on the bacterial micro- biome and epithelial cell proliferation markers	14 healthy but high-cancer risk pre-colonoscopy patients (8M and 6F aged 35-72 years; mean age = 59 years)	1:1 combination of oligofructose and long-chain inulin from chicory	15 g/day for 2 weeks	Increases were noted in bifidobacteria, lactobacilli, and eubacteria in both the proximal and distal colon. There were no differences observed in total aerobes or anaerobes, bacteroides, coliforms, or clostridia. The mucosa was macroscopically normal in all participants and prebiotic supplementation had no effect on markers of epithelial cell proliferation. 15 g/day of prebiotic supplement was well tolerated, but all patients reported an increase in flatulence and some reported mild bloating or increased laxation.
Letexier et al. (2003)	Randomized double-blind, placebo-controlled crossover study of the effect of inulin on hepatic lipo- genesis and TAG levels	8 healthy 23-32-year-old volunteers, 4 of each sex	Inulin from chicory	0 or 10 g/day for 6 weeks	There were no significant differences in intake of total energy intake, fat, carbohydrate, protein, fiber, fructose, or cholesterol. No differences in blood levels of glucose, insulin, glucagon, TC, or HDL and LDL cholesterol. TAG concentrations were significantly lowered by consumption of inulin. Hepatic lipogenesis was also significantly lower after inulin. Cholesterol synthesis was not different and none of the adipose tissue messenger RNA concentrations changed significantly after inulin ingestion. Ingestion of 10 g inulin/day did not produce any reported adverse effects.
Menne et al. (2000)	Randomized single-blinded, crossover study to assess the bifidogenic effect of oligofructose	8 healthy adults (5F and 3M) aged 20-50 years	Chicory-derived oligofructose	0 or 8 g/day for up to 5 weeks	Oligofructose ingestion resulted in a significant increase in bifidobacteria with no change in the numbers of total anaerobes, lactobacilli, bacteroides, coliforms, or *C. perfringens*. Bacterial counts at 2 and 5 weeks did not differ significantly from each other. Fecal pH levels decreased significantly, defecation frequency increased 12%, and reported side-effects were infrequent and mild. The researchers concluded that oligofructose functions as a bifidogenic prebiotic and is unlikely to cause significant intestinal discomfort. No adverse effects were reported.

Pedersen et al. (1997)	Randomized double-blind crossover study of the effect of inulin on blood lipids and GI discomfort	72 healthy normolipidemic women aged 20-36 years	Inulin from chicory	0 or 14.4 g/day for 4 weeks	No significant differences were observed in dietary intakes, nor in plasma TC, HDL- or LDL-cholesterol, or TAG concentrations. During the inulin-ingestion period, there was significant GI discomfort from flatulence and other symptoms was reported, but no serious adverse effects.
Rao (2001)	Non-randomized placebo-controlled crossover study of the effect of oligofructose on the fecal microbiota	4 healthy men and 4 women aged 24-48 years (mean age = 28 years)	Oligofructose from chicory	0 or 5 g/day for 3 weeks	Ingestion of 5 g oligofructose/day significantly increased bifidobacteria numbers over those present at pretest or after sucrose ingestion. Bifidobacteria numbers reached a maximum after 11 days of ingestion and declined to near baseline within 2 weeks after termination of ingestion of oligofructose. Increases in numbers of bacteroides and total anaerobic bacteria and decreases in coliforms were also observed. The researchers concluded that oligofructose has a prebiotic effect at low doses. No adverse effects were reported.
Rumessen et al. (1990)	Open-label study of intestinal handling of inulin with a high proportion of FOS and its effects on blood glucose, insulin, and C- peptide	8 healthy adults (6M and 2F) aged 23-33 years	Inulin with a high proportion of FOS from Jerusalem artichokes	5, 10, or 20 g in single doses	Inulin was apparently completely unabsorbed at any dose. Traces (less than 1% of the administered dose) were detected in the urine of only one participant after a 20-g dose. Extensive fermentation was indicated by significantly increased dose-dependent breath H_2. Orocecal transit times ranged from 145 minutes after a 20-g dose to 270 minutes after a 5-g dose. Inulin, even at 20 g, had little effect on blood glucose and insulin. There was no apparent interference of oligofructose with starch absorption. Only mild flatulence was reported. No diarrhea or abdominal pain was reported.
Rumessen and Gudmand-Hoyer (1998)	Randomized single-blind crossover study of the intestinal transport and fermentation of long-chain inulin and oligofructose	5 healthy men and 5 women aged 18-25 years	Long-chain chicory inulin or oligofructose	10, 20, or 30 g in single-doses	All participants showed a significant rise in H_2 after lactulose challenge. Average orocecal transit times for both shorter- and longer-chain fructans ranged from 30 to 105 minutes, with long-chain inulin having a significantly longer transit time than oligofructose. Breath H_2 and venous acetate production increased in proportion to increasing fructan dose. Abdominal symptoms after fructan ingestion increased with increasing dose and decreasing chain length, but no other adverse effects were reported.

Russo et al. (2008)	Randomized double-blind, placebo-controlled cross-over study of the effect of long- chain inulin on lipid profile and lipoprotein(a)	22 healthy men with a mean age of 18.8 ± 0.7	Long-chain inulin from chicory	0 or 11 g/day for 5 weeks	The period of ingestion of long-chain inulin showed significant improvement in levels of HDL, TAG, Lp(a), and TC/HDL ratio compared to baseline, but only cholesterol/HDL ratio differed significantly from placebo. The researchers concluded that the intervention exerts "slight but significant effects on the lipid profile and Lp(a) concentration." No change in bowel habits was recorded and no GI side effects related to administration of long-chain inulin were reported.
Scholtens et al. (2006a)	Randomized double-blind, placebo controlled, crossover trial to evaluate the effect of oligofructose on fecal water cytotoxicity in healthy adults with adequate calcium status	12 volunteers (6 men and 6 women) aged 18-35 years; mean age = 21.4 years	Oligofructose from chicory	0 or 25-30 g/day for 2 weeks	Oligofructose increased defecation frequency but had no effect on consistency. Oligofructose intake resulted in no overall change in SCFA level, but acetate increased and butyrate decreased. Fecal water cytotoxicity was lower during oligofructose intake than during the control period. No differences were seen in fecal ALP activity or O-linked oligosaccharide production, indicating no difference in fecal mucin content. Consumption of 30 g oligofructose/day had no effect on the cytotoxicity of fecal water, fecal ALP activity, or fecal concentration of mucin-type oligosaccharides in volunteers consuming a normal diet unrestricted in calcium. There was no indication of intestinal epitheliolysis. Side effects except flatulence did not differ between the oligofructose and placebo periods.
Ten Bruggencate et al. (2006)	Randomized double-blind, placebo-controlled, cross-over study of the effect of oligofructose on gut barrier function in healthy men	34 healthy men aged 18-55 years	Oligofructose from chicory	0 or 20 g/day for 2 weeks	Flatulence and intestinal bloating were reportedly more common during oligofructose consumption. Oligofructose ingestion increased fecal wet weight and excretion of mucin and lactic acid and increased counts of bifidobacteria and lactobacilli. Oligofructose did not affect the cytotoxicity of fecal water and had no effect on intestinal permeability. The researchers speculated that the increased excretion of mucin reflected oligofructose-induced mucosal irritation in humans caused by the rapid production of organic acids in the proximal colon. They did not recognize that the artificial low-calcium environment created by the dietary calcium restriction led to poor buffering capacity that may have resulted in mucosal irritation.

Tuohy et al. 2001	Randomized double-blind, placebo-controlled crossover study of the prebiotic effects of inulin and guar gum	31 healthy adults (14M and 17F aged 18-50 years)	Inulin (not further described; identified as FOS in the article) + partially hydrolyzed guar gum	0 or 6.6 g inulin/day and 3.4 g guar gum/day for 21 days	No significant differences were found in the numbers of total bacteria, *Bacteroides* spp, *Clostridium* spp, or *Lactobacillus* spp, but *Bifidobacterium* spp increased significantly during ingestion of inulin+guar gum. Bifidobacteria returned to pre-treatment levels within 7 days of cessation of treatment. No changes were observed in fecal pH or in stool frequency or consistency. Reports of GI effects such as flatulence, abdominal pain, and bloating increased during prebiotic ingestion. No other adverse effects were reported.
van den Heuvel et al. (1998)	Randomized double-blind, placebo-controlled, crossover study of the effect of inulin, oligofructose, and GOS on absorption of iron and calcium in healthy men	12 healthy men aged 20-30 years	Chicory inulin or oligofructose	0 or 15 g/day for 3 weeks	There were no significant differences in either iron or calcium absorption. The researchers concluded that 15 g/day inulin, oligofructose, or GOS had no effect on iron and calcium absorption in young healthy men. All participants completed the study with no reported difficulties and with no reported adverse effects.
van den Heuvel et al. (1999)	Randomized double-blind, placebo-controlled, crossover study of the effect of oligofructose on calcium absorption in healthy male adolescents	12 healthy male adolescents aged 14–16 years (mean age = 15.3 years)	Oligofructose from chicory	0 or 15 g/day for 9 days	Calcium absorption was significantly higher during the oligofructose treatment than during the control period. All volunteers completed the study with no apparent tolerance issues. Reports of GI complaints did not differ between treatments.

van Dokkum et al. 1999	Randomized double-blind, diet- controlled study of the effect of inulin, oligofructose, or GOS on large-bowel function, blood lipid concentrations, and glucose absorption	12 healthy men (mean age = 23 years)	Chicory inulin or oligofructose	0 or 15 g/day for 3 weeks	The treatments had no effect on fecal weight, transit time, colonic function, or concentrations of SCFA other than acetic acid, which was significantly higher during ingestion of inulin and GOS, and valeric acid, higher with inulin ingestion. Breath H_2 was significantly higher on the oligofructose diet than the control. Inulin and oligofructose significantly lowered concentration of fecal deoxycholic acid and inulin and GOS lowered β-glucuronidase activity. There were no significant differences in blood lipids. All volunteers completed the study with no significant differences between treatments in GI tolerance issues.

Adapted from GRN 537 and other available studies

8. APPENDIX III

Studies of Fructans in Healthy and Compromised Children

Reference	Dose & Duration	Study Design & Objective	Subjects	Source & Characteristics of Test Article	Results
Studies in Healthy Children					
scFOS					
Nakamura et al. (2006)	0 or 1.9 g/day for 6 months	Randomized, double-blind, placebo-controlled study of the effect of scFOS on diarrhea and weight gain in urban-slum children	133 children aged 25-59 months (mean age = 46.4±9.7 months)	scFOS	There was no significant difference in growth (bodyweight, height, or arm circumference) between the scFOS and control groups. Children receiving scFOS did not experience fewer incidences of diarrhea than did controls, but the mean duration was significantly shorter. There were no differences in side effects or antibiotic treatment between the groups.
Abbott (2000) [unpublished; submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula]	0 or 200 mg/kg bw/day, increasing by 0 or 200 mg/kg bw/day every 2nd day up to 800 mg/kg bw/day	Randomized, double-blind, placebo-controlled, multi-center, acute-dosage study of the dose of scFOS needed to ameliorate constipation in children	55 male and female children aged 2-5 years (mean = 3.96 years) with a history of constipation but otherwise healthy	scFOS	Children's stool was significantly softened at an average dose of 600 mg/kg bw/day, although administration of scFOS up to the maximum dose did not alter the frequency of bowel movements. The authors concluded that, "Results from this study indicate that scFOS has good potential for improving the treatment of childhood constipation." There were no differences between groups in reported adverse events, and none was serious in either group.

Abbott (1996) [unpublished; submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula]	0 or 2.5 g/day for 16 weeks	Randomized, double-blind, placebo-controlled study of the effect of scFOS on the incidence of diarrhea in toddlers	283 apparently healthy children aged 10-24 months attending daycare	scFOS	No differences were reported in daycare attendance, mean number of feedings, growth, or incidence of stomach cramps or vomiting. The children ingesting scFOS had softer or runnier stools and increased bifidobacteria, but no difference was reported for lactobacilli. There was no difference between groups on the incidence of diarrhea, but the scFOS group had shorter duration of diarrheal episodes as well as a reduced incidence of otitis media. No adverse effects due to scFOS treatment were reported.
Other Fructans					
Abrams et al. (2005, 2007)	0 or 8 g/day for 1 year	Randomized, double-blind, placebo-controlled study of the long-term effect of fructan intake on calcium absorption	50 healthy girls and 50 healthy boys aged 9 to 13 years	1:1 mixture of long-chain inulin and oligofructose from chicory	Drop-outs did not differ by feeding group, and none were for reasons related to treatment. Calcium absorption was greater in the fructan group at 8 weeks and 1 year. After 1 year, the fructan group had a greater increment in both whole-body bone mineral content and whole-body bone mineral density. No adverse effects on growth or other measures were reported. A follow-up analysis (Abrams et al. 2007) revealed that the adolescents receiving the fructan prebiotic had a significantly smaller increase in body mass index (BMI) than the controls, remaining within the
Griffin et al. (2003)	0 or 8 g/day for 3 weeks	Randomized, double-blind, crossover study of the effect of fructans on calcium absorption in premenarche girls	54 healthy premenarche girls aged 10 to 15 years (mean age = 12.4 years)	1:1 mixture of long-chain inulin and oligofructose from chicory	8 g/day of oligofructose-enriched inulin significantly increased calcium absorption with no reported adverse effects.
Griffin et al. (2002)	0 or 8 g/day for 3 weeks	Randomized, double-blind, crossover study of the effect of fructans on calcium absorption in girls near menarche	59 healthy girls aged 11 to 14 years (mean age = 12.0 years), with adequate intake of calcium	Oligofructose from chicory or a 1:1 mixture of long-chain inulin and oligofructose	Calcium absorption was significantly higher in the group receiving the oligofructose-enriched inulin mixture than in the placebo group but no significant difference was seen between the oligofructose group and the placebo group. The groups did not differ in compliance or in calcium intake, and no tolerance problems were observed.

Tschernia et al. (1999)	0 or 1.19 g/day for 6 months	Randomized, double-blind, placebo-controlled study of the effects of FOS on health status	123 infants and toddlers aged 4-24 months (mean age = 11.7 months)	FOS (not further described)	There were no significant differences in growth, but the FOS group of toddlers had significantly fewer febrile events, respiratory-tract symptoms, periods of antibiotic use, and daycare absences than the controls. No adverse effects were reported.

Studies in Compromised Children

Reference	Dose & Duration	Study Design & Objective	Subjects	Source & Characteristics of Test Article	Results
scFOS					
Juffrie (2002)	0 or 2.5-5 g/day	Randomized, placebo-controlled trial of the effect of scFOS on children with acute diarrhea	93 children aged 1-14 years with acute diarrhea	scFOS	The duration of diarrhea was significantly shortened among the children receiving scFOS compared with controls and the author reported that "No side effects such as an increase of diarrhea or prolonged diarrhea were found."
Other Fructans					
Brunser et al. (2006a)	0 or 4.5 g/L formula for 3 weeks	Randomized, double-blind, placebo-controlled trial of prebiotic mitigation of the adverse effects of antibiotic treatment on the intestinal microbiota of young children	140 children aged 1-2 years who had received 1-week amoxicillin treatment for acute bronchitis	Blend of chicory oligofructose and inulin in a 70:30 ratio	Antibiotics caused a drop in total bacterial counts, but they regained their previous level by day 7 of the feeding period in both feeding groups. Bifidobacteria were more prevalent on day 7 in the prebiotic group than in the control group, and lactobacilli and enterococci were non-significantly more prevalent. These differences were maintained through day 21. No withdrawals were for reasons related to treatment. There were no differences between the groups in feeding habits or volume, or in GI symptoms or stool frequency or consistency. Diarrhea occurred in 4 children in the prebiotic group and 7 controls, all of short duration.

Fisberg et al. (2000)	Dose not reported; duration = 4 months	Randomized, double-blind, parallel-design, multi-country study to study the effect on growth recovery of the addition of a synbiotic to nutritional supplements	668 malnourished children aged 1-6 years	FOS (not further described) along with *B. infantis* and *L. acidophilus*	Both feeding groups experienced catch-up growth with no significant differences between the groups. However, the children aged 3-5 years receiving the synbiotic had significantly fewer sick days than the controls and experienced less constipation. Both nutritional supplements were reported to be well tolerated.

Adapted from GRN 537 and other available studies

9. APPENDIX IV

Studies of Fructans in Infants

Reference	Dose & Duration	Study Design & Objective	Subjects	Source and Characteristics of Test Article	Results
scFOS					
Paineau et al. (2014)	4 g/L to age 4 months	Prospective, randomized, double-blind, placebo controlled, multicenter trial of effect of scFOS on bifidogenesis and antipoliovirus IgA	61 healthy term infants aged 0-7 days (mean age = 4.1±0.8 days)	scFOS	Formula consumption and growth did not differ between the group receiving scFOS and a control group that received maltodextrin. There was no difference in incidence or severity of adverse effects between groups. Fecal bifidobacteria counts were significantly higher among infants receiving scFOS than those receiving maltodextrins, but no significant difference was seen in poliovirus-specific IgA. The authors concluded that, "This study demonstrates that a milk-based infant formula supplemented with scFOS at 4 g/L will increase the faecal content of Bifidobacteria in healthy term infants in comparison to a placebo formula without inducing any problem of digestive tolerance."
Ripoll et al. (2014); unpublished	5 g/L for 6 months	Prospective, randomized, double-blind, placebo-controlled, multicenter study of the effect of scFOS on growth, digestive tolerance, fecal bifidobacteria count, and specific poliovirus secretory IgA	75 healthy 4-month-old infants	scFOS	81% of the infants suffered adverse events, but there were no significant differences between groups receiving scFOS or maltodextrin placebo; few were regarded as feeding-related and these did not differ between groups. No differences were observed between groups in the incidence or severity of intolerance symptoms, growth (weight and height), or secretory IgA levels. A significantly greater number of fecal bifidobacteria was noted in the scFOS group as compared to controls after one month of feeding, but the difference was no longer significant after 2 months. The authors concluded that, "The overall digestive tolerance of the scFOS supplemented follow-on milk formula is very good and confirms that scFOS can be used safely at 5 g/L in infants older than 4 months."

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Xia et al. (2012)	0, 2.4, or 3.4 g/L formula for 4 weeks	Randomized, double-blind, placebo-controlled, multi-center study of the effects of feeding on the intestinal microbiota	97 healthy term infants aged ≤6 days (mean = 2.3±0.3 days)	scFOS	Dropouts from each group were: Control group—10 drop-outs, 1 due to parental report of intolerance; 2.4-g scFOS group—11 drop-outs, 3 due to parental report of intolerance, 2 withdrawn by investigators due to non-test-article related adverse events; 3.4 g scFOS group—6 drop-outs, 1 due to parental report of intolerance. No differences were reported among groups in stool frequency or consistency, frequency of feedings with spit-ups or vomit, or total bacterial loads. The highest abundance of bifidobacteria was in the high-scFOS group, but differences among groups were not significant. Lactobacilli, bacteroides, *E. coli*, and *C. difficile* levels were not significantly different across groups. The authors concluded that infant formula is similar to human milk in its ability to support bifidobacteria and lactobacilli, but suggested that "future improvement of infant formula should be directed to reduce the abundance of potentially harmful bacteria including *E. coli* and *C. difficile*."
Lasekan et al. (2010)	0 or 2.5 g/L formula until 35 days of age	Randomized, double-blind, placebo-controlled, multi-center study of tolerance to soy-based infant formulas with scFOS and mixed carotenoids	186 healthy term infants aged 0- 8 days	scFOS	There were no significant differences between formula groups in completion rates, formula intake, growth, stool frequency or consistency, feeding-associated spit-up or vomit, urine specific gravity, hydration status, adverse events, or serious adverse events. Two serious adverse events were reported in each formula group, but all were considered not study related. The authors concluded that, "This study demonstrated that the addition of FOS at 2.5 g/L and mixed carotenoids to soy protein-based formulas, with or without sucrose, was safe and well tolerated in healthy term newborn infants."
Guesry et al. (2000)	200, 400, or 600 mg/day for 2 weeks	Prospective, randomized double-blind study comparing the effects of 3 concentration levels of scFOS in infant formula	53 infants aged 7-20 days	scFOS	Drop-out rates did not differ by group. Stooling frequency increased dose-dependently with scFOS intake. There were no differences in fecal pH, bifidobacteria counts, or adverse effects.

Abbott (1993) [unpublished; submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula	0 or 3 g/L formula for about 16 weeks (to 112 days of age)	Randomized, double-blind, placebo-controlled, multicenter study of the safety and bifidogenic effect of scFOS in infant formula	102 healthy term infants aged 1-8 days (and 25 healthy breast-fed infants aged 0-9 days as a human-milk reference group)	scFOS	Six infants were withdrawn from the non-scFOS formula group and 8 from the scFOS group due to adverse events: symptoms of milk intolerance (2 and 4 infants, respectively), diarrhea or watery stools (2 and 1 infants), constipation (2 and 1 infants), and colic or gassiness (1 scFOS-group infant each). Differences between groups were not statistically significant. There were no differences between groups in measures of weight, length, head circumference, feeding frequency or intake, feedings with spit-up or vomit, stool frequency, or stool consistency, although the human-milk-fed infants had significantly softer and more frequent stools than the 2 formula groups. Levels of AST and ALT were similar in all groups. No blood samples from any infant had detectible scFOS trimers or tetramers. No urine sample contained detectible ketones. No differences were seen between the groups in populations of *Bifidobacteria, Bacteroides,* or *Clostridia* spp., or *C. difficile*, but counts of *Lactobacillus* spp. were significantly higher among infants receiving the scFOS-supplemented formula. The authors concluded that "infant formulas containing added FOS at … up to 3 g/L are well tolerated and support normal growth in term infants."

Abbott (1992) [unpublished; submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula]	0, 1.5, or 3.1 g/L formula for 2 weeks	Randomized, double-blind, placebo-controlled study of the safety and bifidogenic effect of scFOS in infant formula	63 healthy term infants aged 4-10 weeks with a mean age of 43±4 days	scFOS	One infant from the control group, 5 from the low-scFOS group, and 4 from the high-scFOS group failed to complete the study; withdrawal of the single control-group infant, 2 of the low-scFOS infants, and 3 of the high-FOS infants was due to intolerance, while the remainder were attributed to protocol failures. Intolerance withdrawals were based on vomiting or spit-up, diarrhea or watery stools, fussiness, increased stool frequency, or weight loss; there were no differences in reported adverse events among feeding groups. No significant differences among groups were reported in formula intake, growth, stooling patterns, tolerance, or in any of the outcomes measured in blood or urine. No kestose or nystose was detected in the blood of any infant. Infants receiving scFOS had significantly reduced *Clostridia* spp. as compared to the control group. The authors concluded that "Infant formulas containing added FOS at the levels provided … are well tolerated and support normal growth in term infants."
Other Fructans					

Veereman- Wauters et al. (2011)	0, 4, or 8 g/L formula for 28 days	Prospective, randomized, double-blind, placebo-controlled, multi-center study of tolerance and bifidogenic effect of a blend of long-chain inulin and oligofructose or a blend of GOS and long-chain inulin; also included a non-randomized breastfed refer-ence group	81 healthy term neonates <5 days of age	1:1 blend of oligofructose and long-chain inulin from chicory	Neither drop-out rates nor reasons for drop-out differed among groups. There were no differences among groups in weight, length, or head circumference. The 4 formula-fed groups showed no differences in food intake. The breastfed reference infants exhibited a higher frequency of defecation than the 4 formula-fed groups, which did not differ significantly from each other. The breastfed infants' stools were softer than the formula-fed infants', and the 3 groups receiving prebiotic-supplemented formula had softer stools than did those receiving the control formula; the 3 prebiotic-supplemented groups did not differ from each other. The 3 prebiotic groups, but not the control group, showed increases in total fecal bacteria counts over time, but there were no differences among groups. There were trends for increases in bacteroides, clostridia, and lactobacilli. Bifido-bacteria increased over time in all groups, but the increase was slower in the control group. Fecal bifidobacteria counts for infants receiving 800 mg/100 ml of oligofructose-enriched inulin or GOS+long-chain inulin were higher than the controls or those receiving 400 mg oligofructose-enriched inulin/100 ml. There were no differences in crying frequency, regurgitation, or vomiting among the 4 feeding groups. No serious adverse events were observed, and there were no differences among groups in non-serious events.

Lugonja et al. (2010)	0 or 4 g/L formula for 28 days	Non-randomized, non-blinded, non-placebo-controlled study comparing the bifidogenic effects of breast milk and prebiotic-supplemented infant formula	21 healthy infants aged 5 to 16 weeks (mean = 8.6 weeks)	Blend of inulin and oligofructose from chicory	The number of daily feeds was significantly higher in the breastfed group. Counts of bifidobacteria increased significantly over the 28 days in both groups. Lactobacilli increased in both groups while aerobes, anaerobes, and fungi and yeasts decreased, but there were no significant differences between the formula and breastfed groups. Total organic acids increased and pH decreased over time in both groups. Most stools from infants in both groups were of normal consistency. The mean water content of the stools of infants receiving formula containing inulin+oligofructose was 77.9%, non-significantly lower than the mean water content of breastfed infants' stools (81.2%). All infants grew at normal rates and there was no difference between formula-fed and breastfed infants. There were no significant differences between groups in measures of intolerance, stool frequency, or stool consistency.
Yao et al. (2010)	0, 3, or 5 g/L formula for 8 weeks	Prospective, randomized, double-blind, parallel-group study of the effects of infant-formula composition (high sn-2 palmitate w/ or w/o oligofructose) on stool characteristics and composition	300 healthy formula-fed term infants aged 7-14 days; n = 75 human-milk- fed reference group	Oligofructose from chicory	Withdrawals: 2 each from the human-milk reference group and the high sn-2 group, 1 each from the control group and the 3.0-g oligofructose group, and 0 from the 5.0-g oligofructose group. The infants receiving the high sn-2 formula, whether with or without oligofructose, had significantly less stool palmitate soaps and higher bifidobacteria counts than control infants, resembling the human-milk reference group; there was no difference in stool frequency. The high sn-2 group also had significantly softer stools than did the control infants, and addition of oligofructose resulted in a further dose-dependent increase in stool softness; the 5.0 g oligofructose group was not significantly different from the human-milk reference infants. Similarly, the addition of oligofructose significantly decreased stool calcium in a dose-dependent manner. Physician-reported GI events were few and not different among the 4 formula groups and the human-milk reference group; parental reports indicated no increase in the incidence of watery stools, gassiness, or other symptoms of intolerance with the addition of oligofructose. Addition of up to 5.0 g oligofructose/L to formula had no effect on growth (weight, length, head circumference).

Underwood et al. (2009)	Intake was not reported; duration = 28 days	Prospective, randomized, double-blind, placebo-controlled study of the effects of combinations of probiotics and prebiotics on weight gain, fecal SCFA, and microbiota of preterm infants	90 healthy infants <7 days old with gestational age at birth <35 weeks	Inulin (source and characterization not reported); with $5x10^8$ cfu each of *L. acidophilus, B. longum, B. bifidum,* and *B. infantis or* $5x10^8$ cfu *L. rhamnosus* strain GG	The only infants lost to the study were ones transferred out of the ICU, none for study-related reasons. There were no significant differences between groups in fecal SCFA. The only difference in fecal bacteria was a significant increase in numbers of bifidobacteria among premature infants receiving the multi-strain probiotic+prebiotic formula. There were no differences in growth. All formulas were well tolerated; symptoms such as emesis, gastric distention, or excessive gastric residuals were minor.
Panigrahi et al. (2008)	150 mg/day for 7 days	Prospective, randomized, double-blind, placebo-controlled study of long-term colonization of *L. plantarum* when administered to breastfed neonates along with FOS	31 healthy term infants aged 1-3 days	FOS (source and characterization not reported) and 10^9 cfu *L. plantarum*	*L. plantarum* was cultured from 84% of the infants in the synbiotic group on day 3 and from 95% on day 28; 100%, 88%, 56%, and 32% remained colonized at months 2, 4, 5, and 6, respectively. *L. plantarum* was not detected in the feces of any infants in the placebo group. The infants receiving the synbiotic exhibited a significantly greater number and variety of bacterial species than did the control infants. No serious adverse events were reported in either group, and there were no differences in non-serious events.
Yap et al. (2008)	0, 750, 1000, or 1250 mg for 14 days	Prospective, randomized, double-blind, placebo-controlled trial of the effects of inulin on infants' fecal characteristics and microbial composition	36 healthy formula-fed term infants aged 5-12 months (mean age = 7.7 months)	Inulin from chicory	Inulin supplementation decreased fecal pH dose-dependently, but effects on defecation frequency or fecal consistency were not significant. Total fecal anaerobe populations were significantly decreased in the high-inulin dose group as compared with the other groups, but this difference disappeared after feeding ceased. All levels of inulin supplementation significantly decreased numbers of clostridia and the 2 higher doses resulted in significant reductions in Gram+ cocci and total coliforms. No effect was seen in lactobacilli, but bifidobacteria increased dose-dependently with inulin supplementation, reaching statistical significance compared to controls only at the high dose. No adverse events were reported.

Bettler and Kullen (2007)	0 or 3 g/L formula for 8 weeks	Prospective, randomized, double-blind, placebo-controlled, multi-center trial of oligofructose effects on bifidogenesis, the incidence of adverse events, normal growth, fecal concentrations of other bacteria, level of fecal calprotectin, stool frequency and consistency, and anti-rotaviral activity of feces.	145 healthy term infants aged 1-13 days	Oligofructose from chicory	Differences in the incidence of and reasons for withdrawal and numbers of reported adverse events and serious adverse events were not significant between the groups. Treatment-related adverse events were significantly less prevalent in the breastfed reference group than in the formula-fed groups, but the 2 formula-fed groups did not differ from each other. There were no differences in intake of formula between those receiving oligofructose-supplemented formula and the control group. No difference was observed in bifidobacterial numbers between the 2 formula groups or between the formula groups and breastfed reference group at any time during the study, although all 3 groups showed increases in bifidobacteria from baselin e to study completion. The groups did not differ in numbers of bacteroides, but clostridia were consistently more numerous in the feces of the formula-fed infants than the breastfed reference group until week 8, when there was no difference; the 2 formula groups did not differ from each other. Lactobacilli were higher in the oligofructose-fed group than in the control-formula group, which in turn had significantly higher counts than did the breastfed infants, at all time points, including baseline. There were no differences between the groups in changes in numbers of clostridia or lactobacilli over time. Similarly, no differences were seen between groups in fecal calprotectin concentration of anti-rotaviral activity. The breastfed infants had significantly higher frequency of defecation and significantly softer stools than did the formula-fed infants, who did not differ significantly from each other. There were no differences between groups in body weight at any visit, nor in hydration status.

Kapiki et al. (2007)	0 or 4 g/L formula for 14 days	Prospective, randomized, double-blind, placebo-controlled study of the bifidogenic effect of FOS in infant formula	56 bottle-fed preterm infants less than 14 days old (mean age = 7.0 days) with gestational ages < 36 weeks (mean = 33.7 weeks), and admitted to a neonatal unit but other- wise healthy	FOS described as having been produced by partial enzymatic hydrolysis of chicory inulin	Infants in the placebo group gained significantly more weight and had significantly greater arm circumference, while those in the FOS group gained non-significantly greater length. Intake of the FOS-supplemented formula produced a significantly higher frequency of defecation and softer stools as well as significantly greater concentrations of fecal bifidobacteria and bacteroides and significantly lower numbers of *E. coli* and enterococci. Both formulas were well tolerated.
Kim et al. (2007)	0 or 1.5 g/100 g powder for 3 weeks	Prospective, randomized, double-blind, crossover study of the effect of inulin on the infant's gut microbiome, frequency of defecation, and the pH and consistency of feces	14 healthy term infants averaging 12.4 weeks of age	Inulin from chicory	Stool characteristics during inulin feeding changed non-significantly toward increased frequency, softer stools, and lower pH. There were no differences between control and inulin feeding in total anaerobic bacteria or *Bacteroides* spp., but both bifidobacteria and lactobacilli increased significantly during inulin intake periods. No infants were withdrawn from the study, no formula-related adverse effects were observed, and there were no differences in growth between control and inulin feeding periods.
Waligora- Dupriet et al. (2007)	2 g/day for 21 days	Prospective, randomized, double-blind, placebo-controlled study of tolerance and bifidogenic effects of oligofructose	35 healthy term infants aged 7-19 months	Oligofructose from chicory	Because common infections such as pharyngitis, otitis, and bronchitis and consequent use of antibiotics were frequent, only 10 infants in each group completed the study; all exclusions were due to antibiotic use. Bifidobacteria populations increased while staphylococci and clostridia declined in the oligofructose-supplemented group; the difference from the placebo group was statistically significant for clostridia but not for bifidobacteria or staphylococci. No other bacterial counts differed significantly between the prebiotic and placebo groups, and all differences had disappeared by the end of the 15-day observation period. Flatulence, diarrhea, and vomiting were observed significantly less frequently in the oligofructose group than in the placebo group.

Bettler and Euler (2006)	0, 1.5, or 3 g/L formula for 12 weeks	Prospective, randomized, double-blind, parallel-group, multi-center trial to assess the effect of oligofructose on growth and tolerance in healthy full-term infants	297 healthy term infants aged 14 days or less who were being fed formula	Oligofructose from chicory	12 withdrawals were due to adverse events, 5 each from the control and low-dose oligofructose groups and 2 from the high-dose oligofructose group. There were no significant differences in the number of withdrawals, reason for withdrawal, or length of study participation prior to withdrawal. Mean growth in all groups was within CDC reference ranges and did not differ from each other. Adverse events were reported for 55% of the infants; the lowest incidence was in the group receiving the higher dose of oligofructose. None of the formula-related adverse events was considered to be serious. There were no differences among the groups in formula acceptance and tolerance. The authors concluded that "the experimental cow's milk-based formula supplemented with either 1.5 or 3.0 g FOS [sic]/L is safe and supports normal infant growth."
Bettler et al. (2006)	0 or 1.5 g/L formula for 28 days	Prospective, randomized, double-blind, parallel-group trial to assess the safety of addition of oligofructose and *B. animalis* ssp. *lactis* strain BI-07 in formula for healthy toddlers	318 healthy toddlers aged 12-34 months (mean = 22.1 months)	Oligofructose from chicory and *B. animalis* ssp. *lactis* strain BI-07	Both numbers of and reasons for withdrawal were similar in all feeding groups, none related to the study formulas. The amount of formula consumed was similar in all groups and gains in body weight did not differ between groups. By day 28, the proportion of toddlers with detectible fecal BI-07 had risen to 41% of those fed the probiotic formula and 50% of those receiving the synbiotic formula. By 2 weeks after feeding cessation, strain BI-07 was recovered in the feces of only 1 toddler. However, the groups did not differ significantly in fecal bifidobacteria, streptococci, or bacteroides counts. Relative to control, the probiotic and synbiotic groups had significantly increased counts of lactobacilli at day 7 and a small but statistically significant reduction in clostridia at day 28. 186 toddlers reported at least one adverse event, but there were no differences between groups in the frequency, severity, or nature of the adverse events.

Brunser et al. (2006b)	0 or 2 g/L formula for 13 weeks	Prospective, randomized, double-blind, parallel-group study of the effects of oligofructose or *L. johnsonii* NCC533 (La1) on infants' fecal microbiota	116 healthy term infants	Oligofructose from chicory	Withdrawal rates did not differ across the 3 formula groups (oligofructose, *L. johnsonii*, and control groups) and none of the withdrawals was associated with adverse reaction to the formula. At baseline, bifidobacteria counts were significantly higher in the breastfed group than among formula-fed infants, but there were no differences after 7 weeks of feeding or 2 weeks after cessation of pre- or probiotic feeding. Lactobacilli were also significantly more numerous in breastfed than formula-fed infants at baseline, and enterobacteria were less numerous. While the infants receiving probiotic formula showed a transient increase in lactobacilli, enumerations of these bacteria did not generally change significantly in the formula-fed groups. There were no differences between groups or over time in counts of *C. perfringens, Bacteroides* spp., or *Enterococcus* spp. No *Salmonella* or *Shigella* spp. were isolated, but *E. coli* and *Campylobacter jejuni* were found in a small number of samples, with no significant differences among feeding groups. The authors concluded that the study confirms a predominance of bifidobacteria in breastfed infants, and that the concentration of oligofructose used in this study had little effect on the host microbiota. All formulas were well tolerated and average formula intake was similar for all 3 groups. The number of adverse events per infant did not differ among the 3 formula groups or between the formula-fed and breastfed infants, nor were there any differences in growth measured by gain in weight and length.
Campoy et al. (2005)	Intake not reported; duration was 30 days	Prospective, randomized, prospective, double-blind, placebo-controlled study of the management of infant colic	106 colicky infants aged 15 days to 4 months (mean age = 42 days)	FOS (source & characteristics not reported)	The infants receiving the test formula had a significant reduction in colic symptoms (prolonged crying, constipation, regurgitation, and hypertonia) as compared to those receiving breast milk or standard formula. There were no significant differences in growth or any problems with feeding intolerance in any of the 3 groups.

Euler at al. (2005)	0,1.5, or 3 g/L formula for 1 week	Prospective, randomized, double-blind, crossover study of the safety and effects of different doses of oligofructose; included a non-randomized breastfed reference group	87 healthy term infants aged 2-6 weeks	Oligofructose from chicory	There were no significant differences among the groups in growth as indicated by weight, length, and head circumference. Bifidobacteria counts were higher in the oligofructose groups immediately after supplementation than among the breastfed infants, although there was no apparent dose relationship. By a week after oligofructose cessation, the counts had equalized. Oligofructose supplementation had no effect on lactobacilli. The formula-fed infants had significantly higher counts of enterococci and bacteroides at baseline than did the breastfed infants, but by the end of oligofructose supplementation, there were no differences in bacteroides counts among the 3 groups. However, the baseline difference had returned by 7 days after cessation of oligofructose feeding. Clostridia counts were similar at baseline, but oligofructose supplementation resulted in a transient significant increase in clostridia in the formula-fed groups. *C. difficile* toxin was found in no breastfed infants, in 19% of formula-fed infants at baseline, and in 11% at the end of oligofructose feeding. During the week of oligofructose supplementation, the formula-fed infants experienced significant increases in loose stools and the incidence of flatulence and spit-ups.
Yap et al. (2005)	0, 750, 1000, or 1250 mg for 14 days	Prospective, randomized, double-blind, parallel-group study of the dose effects of inulin on mineral absorption and production of SCFA in healthy term infants	36 healthy infants aged 5-12 months (mean age = 7.7 months)	Inulin from chicory	A non-significant dose-response effect was noted for increasing levels of both lactic acid and SCFA with increasing inulin dose and a significant dose-dependent decrease in pH was also observed. Ca absorption increased non-significantly with increasing inulin doses. Effects on absorption of the other minerals, while consistently in the direction of improved absorption with increased inulin intake, were generally less than those for Ca. No adverse events were reported.

Duggan et al. (2003) [Trial 1]	0 or 550 mg/15 g cereal for 6 months	Prospective, randomized, double-blind, placebo-controlled study of the effect of OS + Zn on the prevalence of infant diarrhea	282 healthy infants aged 6-12 months (mean age = 8.8 months)	Oligofructose from chicory	Daily consumption of cereal did not differ between the groups. There were no significant differences between the groups in the incidence or severity of diarrhea, the frequency with which diarrheal pathogens were isolated, constipation, blood chemistry, respiratory infections, side effects such as vomiting, or growth.
Duggan et al. (2003) [Trial 2]	0 or 550 mg/15 g cereal for 6 months	Prospective, randomized, double-blind, placebo-controlled study of the effect of OS + Zn on the prevalence of infant diarrhea	349 healthy infants aged 6-12 months (mean age = 8.6 months)	Oligofructose from chicory	The reasons for study withdrawal (10 from the prebiotic group and 21 from the control group) did not differ. No differences were seen in the incidence or severity of diarrhea, the frequency with which diarrheal pathogens were isolated, constipation, blood chemistry, respiratory infections, side effects such as vomiting, or growth.
Firmansyah et al. (2000)	0 or 600 mg/15 g infant cereal for 10 weeks	Prospective, randomized, double-blind, placebo-controlled study of the effect of oligofructose and long-chain inulin on the immune response	50 8-month- old infants	Blend of long-chain inulin and oligofructose from chicory	Post-measles-vaccination levels of IgG antibody were significantly higher among infants receiving the prebiotic and fewer side reactions were observed. The authors interpreted these findings as indicating an improved immune response to the vaccination. There were no effects on health or growth.
Moore et al. (2003)	0 or 750 mg/ serving of cereal for 28- days	Prospective, randomized, double-blind, placebo-controlled study of the GI effects of FOS-supplemented infant cereal	56 healthy term infants aged 4-11 months (mean age = 7.7 months) with demonstrated tolerance for rice cereal and milk-based formula	FOS (source and characterization not reported)	Infants receiving FOS had significantly more frequent stools with more regular and softer consistency than those receiving placebo. No serious adverse events were reported; 17 infants in the experimental group had 24 reported non-serious events as compared to 16 infants with 21 non-serious events in the placebo group. No adverse event was regarded as being related to the intake of FOS. There were no differences between the 2 groups in growth and no tolerance issues with the prebiotic cereal

10. APPENDIX V

Product specification sheets for all NFBC FOS products:

FOS-300-P

FOS-500-S

FOS-550-S

FOS-700-S

FOS-750-S

FOS-900-S

FOS-950-S

FOS-700-P

FOS-750-P

FOS-900-P

FOS-950-P

Attached separately

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King-Prebiotics
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New Francisco (Yunfu City) Biotechnology Corporation

King-Prebiotics® FOS-500-P

Fructo-oligosaccharide powder

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Moisture	≤3.5%
GF2+GF3+GF4 (on d.m.)	≥50%
Sucrose + Monosaccharides (on d.m.)	≤50%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	≤3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white powder
Taste	Slightly sweet
Behaviour	Hygroscopic
Calorie	2.90 kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	25kg multiple-layered paperbag with inner polyethylene liner
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	24 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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King-Prebiotics® FOS-500-S

Fructo-oligosaccharide syrup

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Dry matter (d.m.)	≥70%
GF2+GF3+GF4 (d.m.)	≥50%
Sucrose + Monosaccharides (d.m.)	≤50%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	<3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white light yellow syrup
Taste	Slightly sweet
Calorie	2.25kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	35KG and 1200KG Food Grade High-Density Polyethylene
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	6 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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King-Prebiotics® FOS-550-P

Fructo-oligosaccharide powder

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Moisture	≤3.5%
GF2+GF3+GF4 (on d.m.)	≥55%
Sucrose + Monosaccharides (on d.m.)	≤45%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	≤3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white powder
Taste	Slightly sweet
Behaviour	Hygroscopic
Calorie	2.80 kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	25kg multiple-layered paperbag with inner polyethylene liner
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	24 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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King-Prebiotics® FOS-550-S

Fructo-oligosaccharide syrup

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Dry matter (d.m.)	≥75%
GF2+GF3+GF4 (d.m.)	≥55%
Sucrose + Monosaccharides (d.m.)	≤45%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	<3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white light yellow syrup
Taste	Slightly sweet
Calorie	2.18kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	35KG and 1200KG Food Grade High-Density Polyethylene
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	6 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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King-Prebiotics® FOS-700-P

Fructo-oligosaccharide powder

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Moisture	≤3.5%
GF2+GF3+GF4 (on d.m.)	≥70%
Sucrose + Monosaccharides (on d.m.)	≤30%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	≤3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white powder
Taste	Slightly sweet
Behaviour	Hygroscopic
Calorie	2.51 kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	25kg multiple-layered paperbag with inner polyethylene liner
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	24 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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King-Prebiotics® FOS-700-S

Fructo-oligosaccharide syrup

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Dry matter (d.m.)	≥75%
GF2+GF3+GF4 (d.m.)	≥70%
Sucrose + Monosaccharides (d.m.)	≤30%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	<3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white light yellow syrup
Taste	Slightly sweet
Calorie	1.95kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	35KG and 1200KG Food Grade High-Density Polyethylene
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	6 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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金歐利多
King-Prebiotics
雲浮市新金山生物科技有限公司
New Francisco (Yunfu City) Biotechnology Corporation

King-Prebiotics® FOS-750-P

Fructo-oligosaccharide powder

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Moisture	≤3.5%
GF2+GF3+GF4 (on d.m.)	≥75%
Sucrose + Monosaccharides (on d.m.)	≤25%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	≤3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white powder
Taste	Slightly sweet
Behaviour	Hygroscopic
Calorie	2.41 kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	25kg multiple-layered paperbag with inner polyethylene liner
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	24 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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金歐利多
King-Prebiotics
雲浮市新金山生物科技有限公司
New Francisco (Yunfu City) Biotechnology Corporation

King-Prebiotics® FOS-750-S

Fructo-oligosaccharide syrup

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Dry matter (d.m.)	≥75%
GF2+GF3+GF4 (d.m.)	≥75%
Sucrose + Monosaccharides (d.m.)	≤25%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	<3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white light yellow syrup
Taste	Slightly sweet
Calorie	1.88kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	35KG and 1200KG Food Grade High-Density Polyethylene
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	6 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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金歐利多
King-Prebiotics
雲浮市新金山生物科技有限公司
New Francisco (Yunfu City) Biotechnology Corporation

King-Prebiotics® FOS-900-P

Fructo-oligosaccharide powder

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Moisture	≤3.5%
GF2+GF3+GF4 (on d.m.)	≥90%
Sucrose + Monosaccharides (on d.m.)	≤10%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	≤3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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New Francisco (Yunfu City) Biotechnology Corporation

Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white powder
Taste	Slightly sweet
Behaviour	Hygroscopic
Calorie	2.12 kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	25kg multiple-layered paperbag with inner polyethylene liner
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	24 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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金歐利多
King-Prebiotics
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New Francisco (Yunfu City) Biotechnology Corporation

King-Prebiotics® FOS-900-S

Fructo-oligosaccharide syrup

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Dry matter (d.m.)	≥75%
GF2+GF3+GF4 (d.m.)	≥90%
Sucrose + Monosaccharides (d.m.)	≤10%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	<3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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New Francisco (Yunfu City) Biotechnology Corporation

Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white light yellow syrup
Taste	Slightly sweet
Calorie	1.65kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	35KG and 1200KG Food Grade High-Density Polyethylene
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	6 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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金歐利多
King-Prebiotics
雲浮市新金山生物科技有限公司
New Francisco (Yunfu City) Biotechnology Corporation

King-Prebiotics® FOS-950-P

Fructo-oligosaccharide powder

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Moisture	≤3.5%
GF2+GF3+GF4 (on d.m.)	≥95%
Sucrose + Monosaccharides (on d.m.)	≤5%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	≤3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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King-Prebiotics
雲浮市新金山生物科技有限公司
New Francisco (Yunfu City) Biotechnology Corporation

Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Neglible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white powder
Taste	Slightly sweet
Behaviour	Hygroscopic
Calorie	2.03 kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	25kg multiple-layered paperbag with inner polyethylene liner
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	24 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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金歐利多
King-Prebiotics
雲浮市新金山生物科技有限公司
New Francisco (Yunfu City) Biotechnology Corporation

King-Prebiotics® FOS-950-S

Fructo-oligosaccharide syrup

Description

- It is manufactured from food grade sucrose via a transfructosylation catalyzed by an officially defined GRAS β-fructofuranosidase.
- It is a natural prebiotic that promotes the growth of bifidobacteria in the colonic microbiome.

Compositional Specification

Dry matter	≥75%
GF2+GF3+GF4 (d.m.)	≥95%
Sucrose + Monosaccharides (d.m.)	≤5%
pH (30%)	4.5~7.0
Ash	≤0.1%

Total Arsenic	≤0.05mg/kg
Total Mercury	≤0.01mg/kg
Lead	≤0.02mg/kg
Cadmium	≤0.1mg/kg
Melamine	≤0.01mg/kg

Microbiological Specification

T.B.C	≤ 1000 CFU/g
Enterobacteriaceae	<0.30 MPN/g
Staphylococcus aureus	Neg./25g
Anaerobic sulfite-reducing clostridia	<3.0 MPN/g
Yeasts and moulds	≤20 CFU/g
Bacillus cereus	<3.0 MPN/g

Coliforms	≤30 MPN/100g
E.Coli	<3.0 MPN/g
Salmonella	Neg./25g
Shigella	Neg./25g
Listeria	Neg./25g

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King-Prebiotics
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New Francisco (Yunfu City) Biotechnology Corporation

Allergens

Gluten	Absent	Enzymes	Absent
Colza	Absent	Meat/egg derivatives	Absent
Folate	Absent	Seed/soy derivatives	Absent
Vitamins and minerals	Negible	Insecticides, pesticides	Absent
Nuts, nut components	Absent	Other allergens	Absent
Protein	Absent	Fat	Absent
Milk	Absent		

Other Information

Appearance	Off white light yellow syrup
Taste	Slightly sweet
Calorie	1.58 kcal/g (EU/1169/2011)
Solubility in water	Readily soluble
Safety	Safe. Not toxic. Not dangerous. Excessive consumption may cause laxative effects. Like other fine powders, it may explode upon ignition in air.
Risk statement	None
Hazard category	Not harmful
Package	35KG and 1200KG Food Grade High-Density Polyethylene
Storage condition	Keep in clean, dry and dark conditions. Keep away from strongly odorous materials.
Shelf life	6 months after production date
Irradiation	Not irradiated
Pesticide residue	Negligible
Aflatoxin B1	≤5μg/kg
Kosher	Orthodox Union
Halal	China Islamic Association (CIA) Majelis Ulama Indonesia (MUI)
Produced by	New Francisco Biotechnology Corporation

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