ORIGINAL SUBMISSION

GRAS Notice (GRN) No. 605
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm

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#605

GRN 000605

October 22, 2015

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

Subject: GRAS Notification for Fructo-oligosaccharides

Dear Sir/Madam:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Tata Chemicals Limited, India, through Soni & Associates Inc. as its agent, hereby provides notice of a claim that the food ingredient fructo-oligosaccharide preparation 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.

Enclosure: Three copies of GRAS notification

Soni & Associates Inc.

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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)

Tata Chemicals Limited, India has determined that fructo-oligosaccharides derived from a consortium of microorganisms is Generally Recognized As Safe and, therefore, exempt from the requirement of premarket approval, under the conditions of its intended use. This determination is based on scientific procedures as described in the following sections, under the conditions of fructo-oligosaccharides's intended use in food, among experts qualified by scientific training and expertise.

Signed, (b) (6)
(b) (6)

Date: 10/22/15

Madhu G. Soni, PhD, FACN, FATS

Agent for:

Tata Chemicals Limited,
INDIA

B. Name and Address of Notifier:

Mr. Ramesh Kumar Verma
Manager - IPR
Tata Chemicals Limited - Innovation Centre
Survey No 315, Hissa No 1-14,
Ambedveth (V), Paud road, Mulshi
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INDIA

Phone No: 020-66549710
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Email: rkverma@tatachemicals.com

C. Common or usual name of the GRAS substance:

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

D. Conditions of use:

Fructo-oligosaccharides (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 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 (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%. FOS is not intended for use in meat and poultry products that come under USDA jurisdiction and in infant formula.

E. Basis for GRAS Determination:

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. 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 indented uses discussed in this dossier, FOS is safe.

F. Availability of Information:

The data and information that forms the basis of FOS GRAS determination will be available at the above mentioned Tata Chemicals Limited address in India 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 a process that involves the biotransformation of sucrose (cane sugar) by the action of the microbial, cell bound enzyme β-fructofuranosidase/fructosyltransferase from a wild strain of dimorphic fungus, *Aureobasidium pullulans*. Additionally, *Pachysolen tannophilus* (yeast) is also used in the production process to remove the reducing sugars to improve the purity.

A. Synonyms and Trade Name:

FOS; Oligofructose; short-chain fructo-oligosaccharide (scFOS or FOS), also referred to as 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 FOSSENCE™.

B. Physical Characteristics

Colorless to light yellow syrup and white color powder

C. Chemical Abstract Registry (CAS) Number

The CAS Registry Number for fructo-oligosaccharide 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 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-C30H52O26. The molecular weight (MW) of scFOS is 700, representing the average of the molecular weights of its 3 components (505, 666, and 828, respectively), which are present at approximately 44%, 42%, and 8%, respectively. The structural formulas of 1-kestose, nystose, and fructofuranosylnystose are shown in Figure 1.

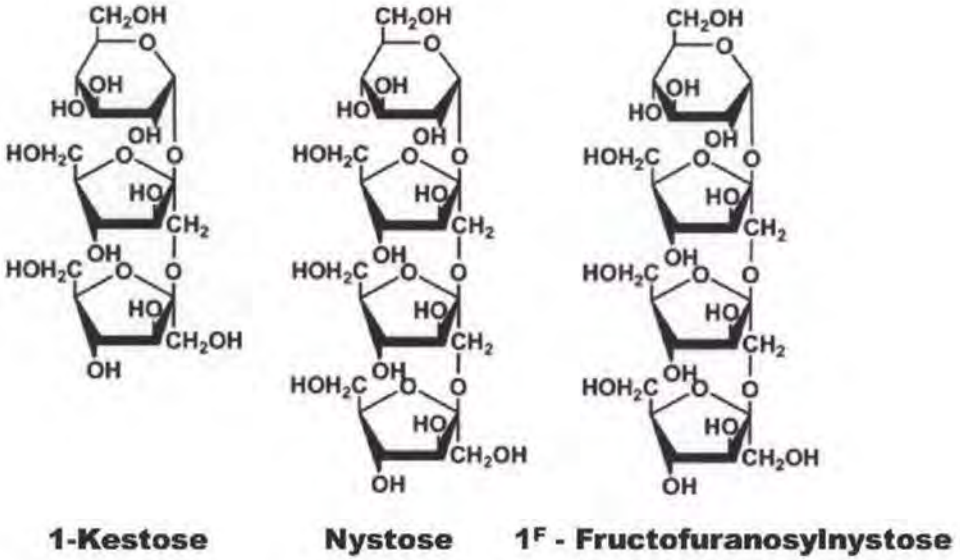

Figure 1. Structural Formulas of FOS components 1-Kestose, Nystose, and Fructofuranosylnystose

F. Food Grade Specifications

Food grade specifications of FOS have been established by Tata Chemical Limited. FOS will be marketed in the U.S. in the form of syrup and powder. The specifications for FOS are presented in Table 1. To demonstrate conformance with the food-grade specifications, Tata Chemicals analyzed several batches of FOS. Analytical results from five non-consecutive lots (Table 1) suggest that FOS is consistently manufactured to meet the standard specifications. In addition to this four additional lots were analyzed at an independent lab and the findings support the standard specifications established. The specification parameters comprise physical appearance, purity, and FOS distribution, as well as limits for potential chemical and microbiological impurities, and contaminants. Aflatoxins- B1, B2, G1 and G2 were analyzed from four batches and the levels were found to be below limits of detection of 1 µg/kg for each of the specific aflatoxins. The subject of this GRAS determination, FOS, is substantially equivalent to the FOS that was the subject of GRAS notified substances reviewed by the FDA without any questions [including GRN 537 (Ingredion, 2014) and GRN 44 (GTC Nutrition, 2000)].

Table 1. Physical and Chemical Specifications of FOS

Parameters	Specification	Batch No.				
		(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Description	Colorless to sunshine yellow color syrupy liquid.	Colorless syrupy liquid.	Light yellow color syrupy liquid.	Sunshine yellow color syrupy liquid.	Light yellow color syrupy liquid.	Light yellow color syrupy liquid.
Moisture Content	≤ 25%	21.6	21.6	21.6	22.6	23.4
BRIX	> 75°	78	77	78	77	77
Ash	≤ 0.1%	0.02	< 0.01	< 0.01	< 0.01	< 0.01
Composition (Dry basis)						
FOS	95.0 ± 2.0%	94.41	95.03	94.13	94.61	93.29
GF2	Informative	43.52	35.03	37.71	37.16	42.16
GF3	Informative	41.72	48.55	45.77	46.32	44.89
GF4	Informative	7.88	11.09	10.84	10.76	6.2
Other sugars	5.0 ± 2.0%	5.59	4.97	5.87	5.39	6.71
Heavy metals*						
Pb	≤ 1ppm	BDL	BDL	BDL	BDL	BDL
As	≤ 0.2ppm	BDL	BDL	BDL	BDL	BDL
Hg	≤ 1ppm	BDL	BDL	BDL	BDL	BDL
Cd	≤ 1ppm	BDL	BDL	BDL	BDL	BDL
Microbial Analysis						
Standard plate count	≤ 300 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
Yeast Count	≤ 20 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
Mould Count	≤ 20 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
Enterobacteriaceae	≤ 3 MPN/g	≤ 3 MPN/g	≤ 3 MPN/g	≤ 3 MPN/g	≤ 3 MPN/g	≤ 3 MPN/g
Escherichia coli	Should be Absent	Absent/10g	Absent/10g	Absent/10g	Absent/10g	Absent/10g
Salmonella sp	Should be Absent	Absent/100g	Absent/100g	Absent/100g	Absent/100g	Absent/100g
Shigella sp	Should be Absent	Absent/10g	Absent/10g	Absent/10g	Absent/10g	Absent/10g

BDL = Below detection limits- *Detection limit for individual heavy metals is <0.005 mg/kg.

G. Production Process:

Tata Chemicals Limited developed a novel and patented process for the manufacture of ≥95% purity fructo-oligosaccharides (FOS) or oligofructose, by using a consortium of microorganisms (*Aureobasidium pullulans* and *Pachysolen tannophilus*). The process comprises the biotransformation of sucrose (cane sugar) by the action of a microbial, cell bound enzyme β-fructofuranosidase/fructosyltransferase from a wild strain of dimorphic fungus, *A. pullulans*.

Another microorganism, a yeast culture *P. tannophilus*, is used to remove reducing sugars, that are produced during the conversion of sucrose to FOS.

The production process of FOS is carried out in five major steps:

1. **Upstream Process**: Microbial fermentation for microbial biomass generation.
2. **Biotransformation**: Conversion of sucrose to oligofructose/fructo-oligosaccharides.
3. **Downstream process:** Purification of oligofructose/fructo-oligosaccharides.
4. **Spray drying:** For the preparation of powder form, pasteurized FOS is subjected to spray drying.
5. **Pasteurization and Packaging:** Pasteurization to kill microbial contaminants and filling of final concentrated FOS solution in containers.

1. **Upstream Process:** Microbial fermentations are carried out for the generation of microbial biomass: In the first step of FOS production both the microbial cultures; *Aureobasidium pullulans* and *Pachysolen tannophilus* are generated by a fermentation technique as described below.

   a. **Generation of *Aureobasidium pullulans* biomass:** For the production of *Aureobasidium pullulans* biomass, the following food grade media components are used and a seed culture is inoculated to produce a large amount of biomass, which holds the enzyme Fructosyltransferase in its cell membrane. At the end of the fermentation, cells are harvested by asimple filtration technique and stored at -20 ^{0}C as a source of the enzyme.

Growth media for *Aureobasidium pullulans* fermentation:

S.N	Media Components
1	Sucrose
2	Yeast Extract
3	Di-Potassium Hydrogen Phosphate (K2HPO4)
4	Potassium Di-hydrogen Phosphate (KH2PO4)
5	Sodium Chloride (NaCl)
6	Magnesium Sulfate Hepta-Hydrate. (MgSO4.7H2O)

   b. **Generation of *Pachysolen tannophilus* biomass:** *Pachysolen tannophilus* biomass is similarly generated by fermentation using thefood grade media composition below. At the end of the fermentation, cells are separated by centrifugation and used immediately in the biotransformation step for the online removal of generated reducing sugars; Glucose and Fructose.

Growth media for *Pachysolen tannophilus* fermentation:

S.N	Media
1	Glucose
2	Malt Extract
3	Yeast Extract
4	Peptone
5	Antifoam

2. **Biotransformation**: Conversion of sucrose to fructo-oligosaccharides: In the second step, the generated biomass of microbial cultures; *Aureobasidium pullulans* and *Pachysolen tannophilus* are mixed in a suitable proportion with pasteurized sucrose solution in a bioreactor and the reaction is carried out at a suitable pH, temperature, aeration & agitation conditions. The progress of the reaction is monitored by monitoring the product formation by analysis with HPLC. The reaction is terminated after complete conversion of sucrose to FOS and at the same time reducing sugars are maintained in the range of 2-5% of total FOS. At the end of the reaction, 50% of the product is separated from the biomass with the help of membrane filtration system. The product is collected in the separate tank and the separated cell mass are pumped back to the bioreactor. The reactor is again supplied with 50 % of fresh sucrose solution and the reaction is again initiated for the second round of biotransformation.

   All the operations of biotransformation are carried out under the sterile conditions. By this way, 10-15 biotransformation cycles are carried out with the help of same microbial biomass.

3. **Downstream process:** Purification of fructo-oligosaccharides: Collected dilute FOS solution in the biotransformation step is further subjected to various downstream processing steps for the purification and concentration purpose as mentioned below:

   a) Activated carbon treatment: Activated carbon treatment is carried out to remove color and organic impurities generated during the biotransformation process. In this process activated carbon is added in to the FOS solution and the mixture is stirred for the 3-4 hours and then the activated carbon is separated from the FOS solution by the help of filtration, with candle type filters.

   b) Ion exchange resin treatment: Resin treatment is carried out by using approved ion exchange resins for the removal of color and ash components. There are two types of the resins, Cation and Anion that are used for the treatment. Cation and Anion resins are filled in separate columns and regenerated with acid and alkali treatment, respectively. The FOS solution collected after carbon treatment is passed from the column in a tandem manner.

   c) Filtration and Clarification: Filtration and Clarification is carried out to remove unwanted precipitates and fines of the resins generated during the resin treatment and particles carried form the activated carbon treatment step. The collected FOS solution after resin treatment is passed through 0.2 micron filter and clarified.

   d) Evaporation and concentration: Evaporation and concentration is carried out to remove the water content of the dilute FOS solution collected after the filtration and clarification steps. The concentration is mainly carried out with the help of forced circular evaporator (FCE) at low temperature to make a FOS solution of 75-80 brix.

   e) Pasteurization: The concentrated; 75-80 brix FOS solution is finally pasteurized at 80^0C and packaged.

4. **Spray drying:** For the preparation of powder form, pasteurized FOS is subjected to spray drying.

5. **Packaging:** Filling of final concentrated FOS solution in containers: The concentrated pasteurized FOS solution is finally filtered through 50-100 micron filter and packaged aseptically in clean HDP containers and sealed.

All the processing aids used in the manufacturing process for FOS such as hydrochloric acid, sodium hydroxide, and activated carbon, are suitable, food grade, and are used in accordance with current good manufacturing practices.. Hydrochloric acid and sodium hydroxide are GRAS for use in food production, limited only by current Good Manufacturing Practice (21 CFR §182.1057 and §184.1631, respectively). Food-grade activated carbon is an unlisted GRAS substance with a long history of safe use in food processing. The resins and microfiltration used are in compliance with FDA guidelines.

In the food industry, both microorganisms are used in the production of food ingredients. *Aureobasidium pullulan* is used in the production of pullulan, while *Pachysolen tannophilus* (yeast) has been reported for the production of ethanol. *Aureobasidium pullulan,* used in the production of FOS is registered with the Microbial Type Culture Collection and Gene Bank (MTCC) under the number MTCC 5490, while *Pachysolen tannophilus* is 99% aligned to *P. tannophilus* strain NRRL Y- 2460.

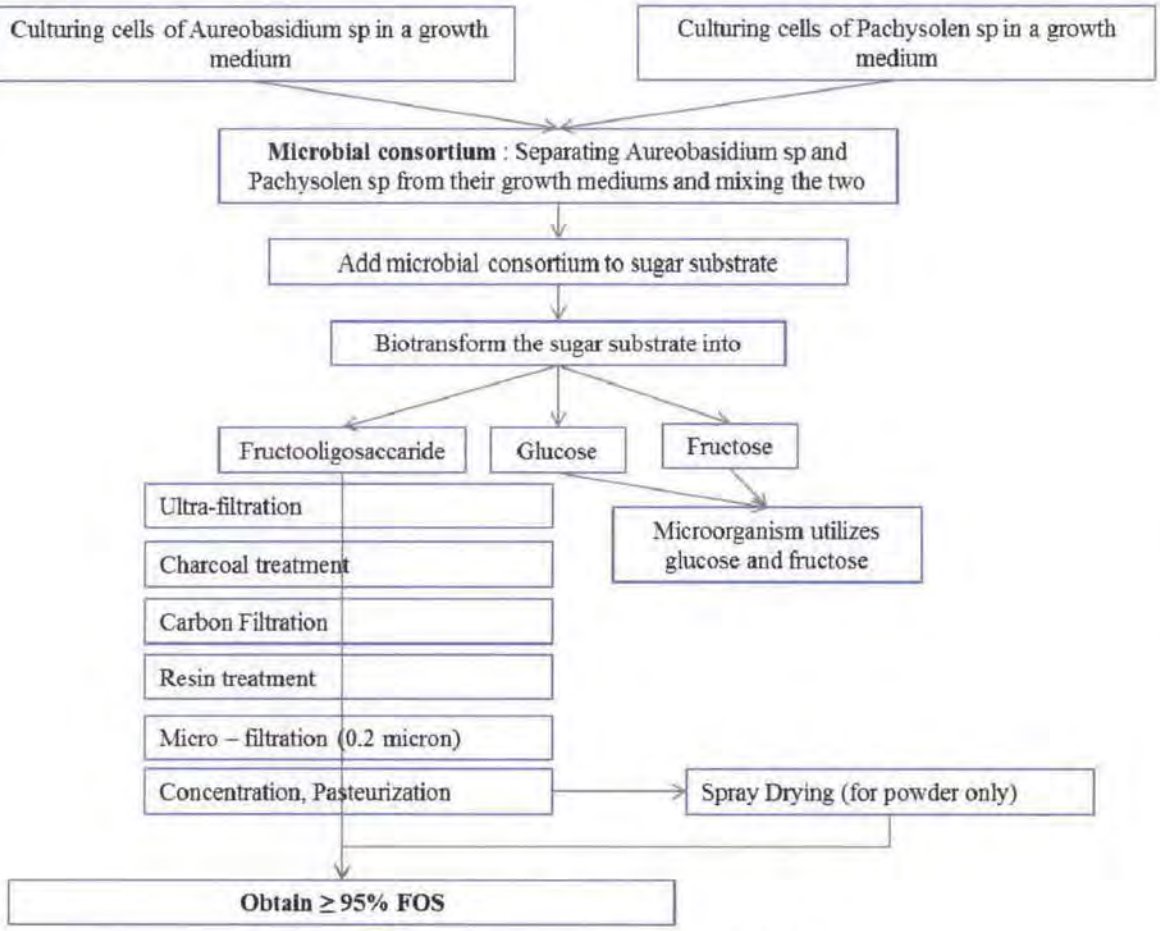

Figure 2. Manufacturing Process of FOS

In an extensive analysis of FOS by employing Quadrupole-Time of Flight mass spectrophotometry, Tata Chemicals identified the FOS along with mono and disaccharide in the

product manufactured as described above. The findings from these experiments demonstrate the composition of product and support the specifications.

III. Summary of the Basis for the Notifier'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 Tata Chemicals Limited 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 microorganisms, *Aureobasidium pullulans* and *Pachysolen tannophilus* (yeast).

A comprehensive search of the scientific databases for safety and toxicity information on FOS was conducted through August 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 in FDA's public inventory.

Based on a critical evaluation of the pertinent data and information summarized herein, and employing scientific procedures, the Expert Panel members have individually and collectively determined that the addition of 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 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 (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%], 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, 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. The safety information and other relevant information are hereby incorporated by reference into this document and was considered in evaluating the GRAS status of Tata Chemicals Limited's proposed use of FOS. 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 FRUCTO-OLIGOSACCHARIDES AS FOOD INGREDIENT

1. EXECUTIVE SUMMARY

At the request of Tata Chemicals Limited, a comprehensive search of the scientific literature for safety and toxicity information on FOS was conducted through by Soni & Associates Inc., to determine the Generally Recognized As Safe (GRAS) status of FOS as a food ingredient. Tata Chemicals Limited 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 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 (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 exposure from added FOS in the proposed food uses 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 proposed uses in infant follow-on 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, fructo-oligosaccharides (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, Tata Chemical Limited 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 colorless to sunshine yellow color syrupy liquid completely soluble in water, practically odorless with a slight sweet taste. It is produced by enzymatic biotransformation of sucrose by the action of microbial, cell bound enzyme β-fructofuranosidase/fructosyltransferase from *Aureobasidium pullulans*. Another yeast culture *Pachysolen tannophilus* is used to remove reducing sugars, that

is produced during the conversion of sucrose to fructo-oligosaccharides. 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 GF2, GF3, and GF4. Both the microorganisms used in the production of FOS are 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 Table 1. The manufacturing process is summarized in Figure 2.

1.3. Natural Occurrence

Fructo-oligosaccharides (FOS) occur naturally in plants and is commonly consumed by humans in foods. It occurs in a number of plants such as onions, Jerusalem artichokes, bananas, lettuce, asparagus, rye, garlic and wheat (rough and bran forms) (GTC Nutrition, 2000; Bornet et al., 2002). 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

[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.

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 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). 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 Tata Chemicals for FOS. The substance mentioned in GRN 44 (GTC Nutrition, 2000) has been reported to contain ≥ 95% FOS, which is similar to the subject of this GRAS determination. Thus, Tata Chemicals Limited 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 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 (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 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 less than one year of age (from the proposed uses in infant follow-on formula) in accordance to good manufacturing practices.

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

Table 2. Typical Use Levels of Fructo-oligosaccharides*

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.

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 3.1 to 12.8 g/person/day at the 90th percentile consumption level. 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 and infant formula, 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 proposed uses in infant follow-on formula). The revised dietary analysis with the inclusion of additional food categories was not questioned by FDA in its response letter of June 1, 2007 (FDA, 2007).

2. DATA PERTAINING TO SAFETY

2.1. Preamble

In a series of safety evaluations by national and international regulatory agencies such as FDA, EFSA (SCF) and FSANZ, the FOS have been reviewed and demonstrated to be safe for use as ingredient in food, including infant formula. In several published experimental studies and review articles, the toxicity potentials of FOS have been summarized. These studies include metabolic (*in vitro* and *in vivo*) experiments, short- and long-term toxicity in experimental animals, as well as human clinical studies. The currently marketed FOS products are manufactured using sucrose as a starting material that is converted to FOS using β-fructofuranosidase enzymes obtained from different non-toxigenic strains of bacteria. 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 new safety-related data and additional uses for FOS have been requested, the regulatory agencies, particularly FDA, have also updated their evaluations. The majority of these studies are described 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 and replied to all these notifications the agency received with recognition of the notifiers request and a statement that they had no questions regarding the conclusions that the FOS is GRAS for the intended applications. Given the similarity between the FDA notices and the subject of 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 described in this dossier.

2.2. GRAS Notices on FOS

2.2.1. GRN 44 – November 22, 2000

In 2000, GTC Nutrition Company (GTC Nutrition, 2000) determined that small chain FOS manufactured by the enzymatic treatment of sucrose is GRAS for use as a bulking agent. In the initial notice to FDA, 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 the 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 Nutritions' intended 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 (from the proposed uses in infant follow-on formula), is also GRAS. FDA had no questions regarding this conclusion (FDA 2007b).

In this GRAS notice, GTC Nutrition (2000) described FOS as a mixture composed of fructose chains with a terminal glucose unit. The number of fructose units varyed from 2-4. 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 dossier 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 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 fructo-oligosaccharide, 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 FDA, the agency 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 000044, 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.

2.2.2. GRN 392- May 7, 2012

This GRAS notice by Pfizer Nutrition and BENEO-Orafti relates to 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 (Pfizer, 2011; FDA 2012). 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 000392 has a DP generally in the range of 2 to 8 and predominantly (90%) in the range of 3 to 6 (Pfizer, 2011).

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. Additionally, 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 (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 537- February 5, 2015

In this recent GRAS notice, Ingredion Incorporated informed FDA that FOS is GRAS, through scientific procedures, for use as an ingredient in term infant formulas at use levels up to 400 mg/100 ml in starter formula (as consumed) and 500 mg/100 ml in follow-on formula (as

consumed) (Ingredion, 2014). The FOS that is the subject of GRAS notice is derived from sucrose and is a fructan oligosaccharide. In particular, this FOS is 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. This FOS is manufactured by dissolving granular sucrose in water, then adding β-fructofuranosidase enzyme preparation produced by *Aspergillus japonicus*. The specification and compositional analysis for this 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).

The notifier discussed 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 microbiota, and is not absorbed. Additional studies such as acute, subacute, subchronic, chronic, and developmental toxicological studies conducted in multiple animal models (including neonatal piglets) with FOS and other fructans 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 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. Ingredion (2014) also discussed 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, Ingredion (2014) concluded that the intended use of FOS in term infant formulas is GRAS. 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).

2.3. 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 (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

Food Standards Australia New Zealand (FSANZ, 2013) also 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. Human 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 a recent study, 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, & 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.

2.6. Identity and Safety of Microorganisms

As mentioned earlier, FOS, the subject of present GRAS assessment, is produced by using a consortium of microorganisms. The process involves the biotransformation of sucrose by the action of a microbial, cell bound enzyme β-fructofuranosidase/fructosyltransferase from a wild strain of dimorphic fungus, *Aureobasidium pullulans*. Another microorganism, a yeast culture *Pachysolen tannophilus*, is used to remove reducing sugars that are produced during the conversion of sucrose to FOS.

The characteristics of *A. pullulans*, as well as the development, safety, and identity of the production strain has been established. *A. pullulans* the "black yeast," is a ubiquitous polymorphic fungus that can be found in lake water, on leaves and wood, as well as in used cosmetics and on foods such as fruits, cereals, tomatoes, and cheese. The fungus contains multiple life forms (polymorphic) including blastospores, hyphae, chlamydospores, and swollen cells. The chlamydospores and swollen cells are considered resting forms. The fungus produces a green melanin which turns black over time. The fungus also produces the polysaccharide pullulan, which is currently used in industrial applications such as a coating for pill tablets, cosmetics, foods, and dissolvable breath fresheners.

The production strain, *A. pullulans* MTCC 5490, was subjected to genetic identification by 16S ribosomal RNA gene, partial sequence for confirmation. *A. pullulans* strain MTCC 5490 is maintained in the Microbial Type Culture Collection and Gene Bank. The phylogenetic tree based on 16S rRNA and as compared to other related species and designates was developed for *A. pullulan*.

As a member of the phyllosphere community and a common biofilm member on painted surfaces and shower curtains, *A. pullulans* is inhaled and ingested with fresh fruits and vegetables on a daily basis. Prior to the mid- 1980's, the species was associated occasionally with superficial infections in humans, but many of these reports have been considered questionable (McGinnis, 1980). Early clinical studies either failed to establish a pathogenic association or the taxonomic procedures failed to distinguish their isolates from *Exophialia* spp. In the past several decades there have been a few additional reports (Salkin et al., 1986) on the pathogenicity of *A.*

pullulans for seriously immunocompromised patients, a phenomenon that is considered possible for most fungi including the baker's yeast *Saccharomyces cerevisiae*. The available information reveals far more reports associating this beneficial and safe industrial yeast with various disease syndromes than the rare associations indicated for *A. pullulans*. Host debilitation is by far the primary factor in the opportunistic or adventitious involvement of saprobic fungi with humans. Nevertheless, the available evidence for the past three decades with yeasts and moulds in environmental, industrial and clinical settings, the involvement of *A. pullulans* with any adverse human health related problems is extremely rare.

The published literature revealed two case reports in which *A. pullulans* has been described as an opportunistic organism. However, both of reports concluded that severe host debilitation is by far the primary factor in the opportunistic or adventitious involvement of *A. pullulans* with humans. The first case report, described opportunistic infection of the spleen caused by *A. pullulans* (Salkin et al., 1986). The mold *A. pullulans* was isolated on several nutrient media from a splenic abscess in a patient with disseminated lymphoma. Examination of stained smears and paraffin sections revealed fungal structures characteristic of this organism. This is the first reported association of *A. pullulans* with an opportunistic visceral infection. In another case report, Hawkes et al. (2005) reported a case of *A. pullulans* fungemia with invasive infection in an infant. The authors reviewed the previously reported 23 cases of human infection from the literature. This infant case is also, the first case of documented invasive pulmonary infection and the first patient with a recently repaired cardiac lesion as the identified risk factor.

The other microorganism used in the production of FOS, *P. tannophilus* is a rare yeast isolated by Boidin and Adzet in 1957. *P. tannophilus* was the first yeast shown to convert xylose directly to ethanol. It ferments glucose, mannose, xylose, galactose, and even glycerol to ethanol. On the basis of colony characteristics, morphological and biochemical characteristics, the microorganism used in the production of FOS has been confirmed as *P. tannophilus*. The *P. tannophilus* used in the FOS production was also subjected to genotypic analysis using consensus/ universal oligos, the ~1.8 kb 18S rDNA fragment from isolated gDNA, was amplified using Taq DNA Polymerase and sequence data was aligned and analyzed for finding the closest homologous microbes. It was found to be 99% aligned to *P. tannophilus* strain NRRL Y- 2460. The carbohydrate utilization as well as β-galactosidase activity of this strain has been confirmed. There are no reports in the medical literature of *P. tannophilus* causing harm in humans as a pathogen or opportunist.

The manufacture of FOS involves extensive purification steps such as activated carbon filtration, ion-exchange and chromatography separation stages that are intended to remove any potential metabolic impurities produced during fermentation.

3. SUMMARY AND DISCUSSION

Tata Chemicals Limited intends to market fructo-oligosaccharides (FOS) as ingredients for use in food and beverages. The products will be marketed under trade name xxx. The manufacturing process of FOS involves the biotransformation of sucrose by the action of a microbial, cell bound enzyme β-fructofuranosidase/fructosyltransferase from *Aureobasidium pullulans*, while another microorganism, *Pachysolen tannophilus*, is used to remove reducing sugars, produced during the conversion of sucrose to FOS. 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 Tata Chemicals has established food grade specifications for its FOS. Tata chemicals' FOS 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.

At present FOS is used 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 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%. These food categories and use levels are described in GRN 44 additional correspondence. The maximum daily intake of FOS, when used in foods, beverages and supplements, for the general population, one year of age or greater, was determined as 20g and 4.2 g for infants less than one year of age (from the proposed uses in infant follow-on formula) in accordance to good manufacturing practices. Tata Chemicals Limited intends to use FOS in the same foods and at identical levels to those mentioned in the GRN 44.

There is sufficient qualitative and quantitative scientific evidence to determine the safety-in-use of FOS in the above mentioned food applications. 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 these submissions, use of FOS in specified food categories was determined to result in levels of up to 20 g/person/day. 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. The subject of this present GRAS determination is substantially equivalent to the FOS that has been the subject of 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 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. Additionally, FSANZ also completed 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 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, and a variety of animal studies and human 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 certain specified foods resulting in a daily intake of up to 20 g/day in the general population and at levels up to 4.2 g/day in infants who use follow-on formula 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 additional correspondence) at levels ranging from 0.4 to 6.7% 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 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%], when not otherwise precluded by a Standard of Identity as described in this dossier and resulting in intake of 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 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 L. Archer, Ph.D. — Date: Sept. 17, 2015

(b) (6)

Robert L. Martin, Ph.D. — Date: Sept. 14, 2015

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. — Date: Sept. 19, 2015

5. REFERENCES

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.

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.

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 April 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 April 30, 2015.

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 April 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 April 30, 2015.

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 April 30, 2015.

Food Standards Australia New Zealand (FSANZ). 2013. Approval report- application A1055, short chain fructo-oligosaccharides. May 23, 2013.

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

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

000028

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.

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 April 30, 2015.

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.

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.

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

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.

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.

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.

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.

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

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

6. APPENDIX I

Animal Toxicity Studies of Frucro-oligosaccharides* (Complete references provided at the end of Appendix II)

Reference	Dose & Duration of Feeding	Study Design & Objective	Animal Model	Source & Description of Test Article	Findings
Acute Oral Toxicity					
Takeda and Niizato (1982) [acute rat study]	Single gavage doses of 0, 3, 6, or 9 g scFOS/ kg bw	Acute oral toxicity study of scFOS	48 6-week-old male and 10-week-old female Sprague Dawley rats (6 rats/sex/ dose)	scFOS	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.
Takeda and Niizato (1982) [acute mouse	Single gavage doses of 0, 3, 6, or 9 g scFOS/ kg bw	Acute oral toxicity study of scFOS	48 4-week-old male and female JcL-IcR mice (6 mice/sex/dose)	scFOS	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.
Subacute Oral Toxicity					
Takeda and Niizato (1982)	0, 1.5, 3, or 4.5 g/kg bw/ day for six weeks	Subacute oral toxicity study of scFOS	Seventy-two 6-7-week-old male Wistar rats (18 rats/dose)	scFOS	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 mg/kg bw/day.

Takeda and Niizato (1982) [2nd sub-acute rat study]	5 or 10% dietary concentration for 6 weeks	Subacute oral toxicity study of scFOS and sorbitol	108 6-7-week-old male Wistar rats (18 rats/treat-ment/dose)	scFOS	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.
Tokunaga et al. (1986)	0, 10, or 20% dietary concentration for 4-6 weeks	Safety and metabolic handling of scFOS or gluco-mannan	24 male Wistar rats (40-50 g (6 rats/dose)	scFOS	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.

Subchronic Oral Toxicity					
Genta et al. (2005)	0, 340, or 6800 mg flour/kg bw/ day providing 0, 188, or 3760 mg oligofructos e/ kg bw/day for 4 months	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	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.
Boyle et al. (2008)	0, 0.55, 1.65, 4.96, or 9.91% dietary concentratio n (high doses = 4680 and 5720 mg/kg bw/day for M&F, respectively) for 13 weeks	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	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.
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/dos e)	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)	20% dietary concentration from day 1 to day 21 of gestation	Developmental toxicity study of oligofructose	19 female Wistar rats with copulation plugs (12 test, 17 control)	Oligofructose	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 Brightwell (1990)	4.75% dietary concentration from post coitum day 0 to 6, then 0, 5, 10, or 20% dietary concentration to day 15	Study of maternal and developmental toxicity of oligofructose	~100 pregnant female Crl CD (SD) BR Sprague Dawley rats (~24 rats/dose)	Oligofructose	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 495 and other published studies

7. APPENDIX II

Summary of Available Studies of Fructo-oligosaccharides in Healthy Adults*

Reference	Study Design and Objective	Subjects	Source and Characteristics of Test article	Dose and Duration	Results
scFOS					
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."
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. (1999)	0, 2.5, 5, 10, or 20 g/day for 7 days	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	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. (1996)	0 or12.5 g/day for 12 days	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	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.

Buddington et al. (1996)	4 g/day for 25 days	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	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)	0 or 5 g/day for 4 days, then 0 or 10 g/day for the rest of 5 weeks	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	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) Part 1	0 or 1.9 g single dose in the 1st study and 0 or 1.5 g single dose in the 2nd study	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	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	5.7 and 4.5 g/day for 7 days	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	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)	0, 5, or 10 g/L for 14 days	Randomized, double-blind, controlled study of the effect of the addition of scFOS to enteral feeding formulas	27 apparently healthy male college students	scFOS	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)	0, 10, or 16 g/day taken in 2 doses over 24 hours	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	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	25 g in a single dose	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	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	8 g/day for 2 months	Open-label study of the effect of scFOS ingestion on microbiota	Healthy adults (no further information was reported)	scFOS	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)	0 or 20 g/day for 4 weeks	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	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, lipoproteina, 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)	5 g/day at first, in- creased over 3 days to 20.1 g/day for 8 days	Open-label study of the digestion and excretion of scFOS	Six healthy adults, 3 of each sex, aged 20-27 years	scFOS	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	9 g in a single dose	Open-label study of the safety of tablets containing scFOS	10 apparently healthy men aged 37±10 years	scFOS	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	0 or 3 g in a single dose	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	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)	10 or 20 g in a single dose	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)	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)	3.6 g/day for 24 months	Randomized, double-blind, placebo-controlled trial of effects of Ca supplementation w/ or w/o scFOS	300 non-osteoporotic postmenopausal women	scFOS	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)	0 or 15 g/day for 12 days	Randomized double-blind, placebo-controlled study to test the effect of scFOS on breath hydrogen excretion	15 healthy adults aged 21-65	scFOS	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. (2003)	0 or 10 g/day for 5 weeks	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	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.
Tahiri et al. (2001)	0 or 5 g/day for the first 4 days, then 0 or 10 g/day for 31 days	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	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.
Tokunaga et al. (1993)	1, 3, or 5 g/day for 2 weeks	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	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	0 or 3.0 g in a single dose	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	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	3.0 g/day for 13 weeks	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	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)	0 or 10 g/day for 36 days	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	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)	4 g/day for 14 days	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	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)	5 or 15 g/day for 7 days each	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	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.

Brighenti et al. 1999	0 or 9 g/day for 4 weeks	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	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 H2 increased. Inulin lowered counts of total facultative anaerobes and increased the proportion of bifidobacteria. No adverse effects were reported.
Bartosch et al. (2005)	0 or 6 g/day for 4 weeks	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	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.
Coudray et al. (1997)	In- creasing dose for 14 days, then 40 g/day for 12 days	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	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.
Clausen et al. (1998)	Escal- ating dose of 20, 40, 80, and 160 g for 3 days each	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	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.

Castiglia-Delavaud et al. (1998)	Increasing dose for 14 days, then 50 g/day for 12 days	Randomized parallel-group, Latin-square design to study fermentation of inulin	9 healthy young men (average age of 21.5 years)	Chicory inulin	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.
Cani et al. 2006	0 or 16 g/day for 2 weeks	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)	Cligofructose from chicory	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.
De Preter et al. (2008)	20 g/day for 4 weeks with and 4 weeks without probiotics	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*	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.
De Preter et al. (2007)	0 or 10 g/day for 4 weeks with and 4 weeks without probiotics	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*	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.

Forcheron and Beylot (2007)	0 or 10 g/day for 6 months	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	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.
Grasten et al. (2003)	0 or 15 g/day for 4 days	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	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.
Gibson et al. (1995)	0 or 15 g/day for 15 days	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	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.
Kruse et al. (1999)	0 or 22-34 g/day for 64 days	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	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.

Letexier et al. (2003)	0 or 10 g/day for 6 weeks	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	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.
Langlands et al. (2004)	15 g/day for 2 weeks	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	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.
Menne et al. (2000)	0 or 8 g/day for up to 5 weeks	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	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)	0 or 14.4 g/day for 4 weeks	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	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.

Russo et al. (2008)	0 or 11 g/day for 5 weeks	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	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.
Rumessen and Gudmand-Hoyer (1998)	10, 20, or 30 g in single-doses	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	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.
Rumessen et al. (1990)	5, 10, or 20 g in single doses	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	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.
Rao (2001)	0 or 5 g/day for 3 weeks	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	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.

Scholtens et al. (2006a)	0 or 25-30 g/day for 2 weeks	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	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.
Tuohy et al. (2001)	0 or 6.6 g inulin/day and 3.4 g guar gum/day for 21 days	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	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.
Ten Bruggencate et al. (2006)	0 or 20 g/day for 2 weeks	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	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.

van Dokkum et al. (1999)	0 or 15 g/day for 3 weeks	Randomized double-blind, diet- controlled study of the effect of inulin, oligofructose, or GOS on large-bowel function, blood lipid	12 healthy men (mean age = 23 years)	Chicory inulin or oligofructose	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
van den Heuvel et al. (1999)	0 or 15 g/day for 9 days	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	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 den Heuvel et al. (1998)	0 or 15 g/day for 3 weeks	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	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.

*Adapted from GRN 495 and other published studies

References for Appendix I and II

Alles MS, JGA Hautvast, FM Nagengast, R Hartemink, KMJ Van Laere, JBMJ Jansen. 1996. Fate of fructo-oligosaccharides in the human intestine. Br J Nutr 76:211-221.

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.

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.

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, 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.

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.

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.

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.

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.

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 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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

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.

Rumessen and Gudmand- Hoyer (1998) 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.

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.

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.

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.

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]

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.

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.

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 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, T Muijs, F Brouns, HFJ Hendriks. 2009. Short-chain fructo- oligosaccharides improve magnesium absorption inadolescent girls with a low calcium intake. Nutr Res 29:229-237.

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.

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.

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