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July 29, 2014

Paulette Gaynor, Ph.D. ATTN: Richard Bonnette
Senior Regulatory Project Manager
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Gaynor:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Ingredion, Inc., through me as its agent, hereby provides notice of a claim that the use of short-chain fructooligosaccharides (scFOS) in infant formula, as described in the enclosed notification document, is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Ingredion has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, one signed copy of the notification is provided, which also includes the conclusion signed by the four members of the GRAS expert panel. Additionally, I have enclosed a virus-free CD-ROM with the GRAS notice and copies of the signature pages.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5548 or jh@jheimbach.com.

Sincerely,

(b) (6)

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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Determination of the GRAS Status of the Addition of Short-Chain Fructooligosaccharides to Infant Formula as a Nutritional Supplement

Prepared for
Ingredion Incorporated
Bridgewater NJ

Prepared by
JHeimbach LLC
Port Royal VA

July
2014

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1. GRAS EXEMPTION CLAIM

Ingredion Incorporated (Ingredion), through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration that the use of NutraFlora® short-chain fructooligo-saccharides in infant formula as described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Ingredion has determined through scientific procedures that this use is generally recognized as safe (GRAS).

_____ _____

James T. Heimbach, Ph.D., F.A.C.N. Date
President, JHEIMBACH LLC

1.1. Name and Address of Notifiers

Contact: Richard L. Barndt, Ph.D.
Telephone: 908-685--7035
Facsimile: 908-707-3688
E-mail: richard.barndt@ingredion.com

1.2. Name of GRAS Substance

The common name of the substance that is the subject of this GRAS notice is short-chain fructooligosaccharide (scFOS); it is also commonly called short-chain fructan. This substance is chemically identical to other fructan products on the market, consisting of a linear chain of β(2-1)-linked fructose residues with a glucose termination. It is sold by Ingredion under the trade name NutraFlora®. The systematic name of all fructans is {α-D-glucopyranoside-(1-2)-}β-D-fructofuranosyl-$[(1\text{-}2)\text{-}\beta\text{-D-fructofuranosyl}]_n$. The brace around the terminal glucopyranoside is placed there to recognize that the presence of this residue is not invariable in fructans, although it is always present in scFOS. While fructans can have degrees of polymerization (DP; the number of fructose or glucose residues) ranging from 2 to more than 60, scFOS consists entirely of molecules with DP between 3 and 5, consisting of 2 to 4 fructose residues and a single terminal glucose residue. These molecules may also be regarded as consisting of a single sucrose molecule with 1-3 fructose units linked by β(2-1) linkages.

1.3. Intended Use and Consumer Exposure

Ingredion intends the use of NutraFlora® scFOS to be as a prebiotic in non-exempt infant formula. The maximum intended addition levels of scFOS are 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed.

According to tables of daily energy intake by formula-fed infants provided by Fomon (1993), the subpopulation of infants with the highest intake per kg body weight is boys age 14–27 days. The U.S. Food and Drug Administration (FDA) typically uses the 90th percentile of the intake distribution to represent extreme intakes. The 90th percentile energy intake by this group is 141.3 kcal/kg bw/day. Among girls, the highest energy intake is found in the same age group, 14–27 days, and is nearly as high as boys: 138.9 kcal/kg bw/day[1]. Most standard infant formulas

[1] These estimates are corroborated by data from the 2008 Feeding Infants and Toddlers Study (Butte et al. 2010).

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(b) (6)

James T. Heimbach, Ph.D., F.A.C.N.
President, JHEIMBACH LLC

7/30/14
Date

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[1] These estimates are corroborated by data from the 2008 Feeding Infants and Toddlers Study (Butte et al. 2010).

provide 0.676 kcal/ml. Therefore, in order to obtain 141.3 or 138.9 kcal energy/kg bw, an infant must consume 209.0 or 205.5 ml/kg bw of formula, respectively. The 90th percentile formula intake for both sexes combined is 207 ml/kg bw/day.

The 90th percentile daily intake of scFOS, added at a maximum concentration of 500 mg/100 ml, is thus estimated to be 1.035 g/kg bw/day.

There are no other sources of fructans in the diets of formula-fed infants and, therefore, the above estimated daily intake constitutes the total daily exposure of the infant to fructans.

1.4. Basis for GRAS Determination

Ingredion's GRAS determination for the intended use of NutraFlora® scFOS is based on scientific procedures as described under 21 CFR §170.30(b). A comprehensive search of the literature through April 2014 conducted by JHeimbach LLC served as the basis for preparation of a monograph summarizing the totality of the information available germane to determining the safety of the intended use of NutraFlora® scFOS. Some of the information in the monograph is unpublished research which corroborates the published information that alone is the basis for the GRAS determination.

It may be concluded that NutraFlora® scFOS is safe under its intended conditions of use because the total exposure to scFOS resulting from this use is within levels shown by extensive animal research and human studies of scFOS and other fructans, including studies in infants, to be tolerable and safe. An Expert Panel determined the intended use of NutraFlora® scFOS to be safe, and also GRAS, by concluding that the safety of this level of intake is based on publicly available and accepted information and is generally recognized by experts qualified by scientific training and experience to evaluate the safety of substances added to food.

Therefore, the intended use of NutraFlora® scFOS in infant formula is determined to be safe and GRAS. Determination of the safety and GRAS status of the addition of NutraFlora® scFOS to infant formula was made through the deliberations of an Expert Panel consisting of George C. Fahey, Jr., Ph.D., Ronald E. Kleinman, M.D., Berthold V. Koletzko, M.D., and John A. Thomas, Ph.D., who reviewed information in this monograph as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of infant formula and infant formula ingredients. They critically reviewed and evaluated the publicly available information, including the potential intake of NutraFlora® scFOS by infants, and individually and collectively concluded that the available information on NutraFlora® scFOS, other fructans, and other oligosaccharides contains no evidence that demonstrates or suggests reasonable grounds to suspect a hazard to the public health under the intended conditions of use of NutraFlora® scFOS.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion. Therefore, the addition of NutraFlora® scFOS to infant formula under the conditions of use described is GRAS by scientific procedures.

1.5. Availability of Information

The data and information that serve as the basis for the GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times

at the office of James T. Heimbach, Ph.D., President, JHEIMBACH LLC, 923 Water Street, P.O. Box 66, Port Royal VA 22535, telephone 804-742-5548 and e-mail jh@jheimbach.com.

1.6. Abbreviations Used in This Document

AE = adverse event
ALP = alkaline phosphatase
ALT = alanine aminotransferase
AMY = amylase
AST = aspartate aminotransferase
BUN = blood urea nitrogen
bw = bodyweight
CFR = Code of Federal Regulations
cfu = colony forming units
cGMP = current Good Manufacturing Practice
CMC = carboxymethylcellulose
DP = degree of polymerization
EDI = estimated daily intake
EDTA = ethylenediaminetetraacetic acid
FOS = fructooligosaccharide
GF_2 = 1-kestose
GF_3 = nystose
GF_4 = fructofuranosylnystose
GGT = γ-glutamyltransferase
GI = gastrointestinal
GLS = glucooligosaccharide
GOS = galactooligosaccharide
GPT = glutamic pyruvic transaminase
GRAS = generally recognized as safe
HDL = high-density lipoprotein
HFI = hereditary fructose intolerance
HMO = human milk oligosaccharide
IBS = irritable bowel syndrome
Ig = immunoglobulin
IL = interleukin
IMO = isomaltooligosaccharide
INF-γ = interferon-γ
LC-PUFA = long-chain polyunsaturated fatty acids
LDL = low-density lipoprotein
Lp(a) = lipoprotein(a)
MCC = microcrystalline cellulose
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
MCV = mean corpuscular volume
NOAEL = no observed adverse effect level
NO_x = nitrogen oxides (NO and NO_2)

OF = oligofructose
PCR = polymerase chain reaction
PUFA = polyunsaturated fatty acids
SCFA = short-chain fatty acids
scFOS = short-chain fructooligosaccharide (from sucrose)
TAG = triacylglycerol
TC = total cholesterol
XOS = xylooligosaccharide

2. IDENTITY OF THE SUBSTANCE

2.1. Chemical Name

The substance that is the subject of this GRAS notification is NutraFlora® short-chain fructooligosaccharide (scFOS); it is also commonly called short-chain fructan. Short-chain FOS is one member of a group of β(2-1) oligo- and polysaccharides designated fructans. The common feature of the fructan oligosaccharides is that they comprise essentially linear chains of fructose units linked by β(2-1) fructosyl-fructose linkages, sometimes with a single terminal glucose molecule; this terminal glucose residue is invariably present in scFOS. The systematic name of all fructans, including scFOS, is {α-D-glucopyranoside-(1-2)-}β-D-fructofuranosyl-[(1-2)-β-D-fructofuranosyl]$_n$. The brace around the terminal glucopyranoside is placed there to recognize that the presence of this residue is not invariable in fructans other than scFOS. Since the β(2-1) linkage joining the glucose residue with the neighboring fructose residue is the same as is found in the disaccharide sucrose, the configuration may also be viewed as a sucrose molecule linked to a chain of fructose units by β(2-1) fructosyl-fructose linkages.

NutraFlora® scFOS consists of 3 different molecules, each containing a terminal glucose residue and 2, 3, or 4 fructose residues, designated GF_2, GF_3, and GF_4, with the respective names 1-kestose, nystose, and fructofuranosylnystose.

2.2. Trade Names

The trade name of the product is NutraFlora®. It is also sold as Actilight® while similar products are trade named Meioligo® and Neosugar.

2.3. CAS Registry Numbers

Short-chain FOS (scFOS) derived from sucrose has Chemical Abstracts Service (CAS) registry number 308066-66-2.

2.4. Molecular and Structural Formulas

The molecular formula of NutraFlora® scFOS is the same as that of all fructans: $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$. The formulas of its three components are: 1-kestose—$C_{18}H_{32}O_{16}$, nystose—$C_{24}H_{42}O_{21}$, and fructofuranosylnystose—$C_{30}H_{52}O_{26}$. The molecular weight (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 37%, 53%, and 10%, respectively. The structural formulas of the 3 components are shown in Figure 1.

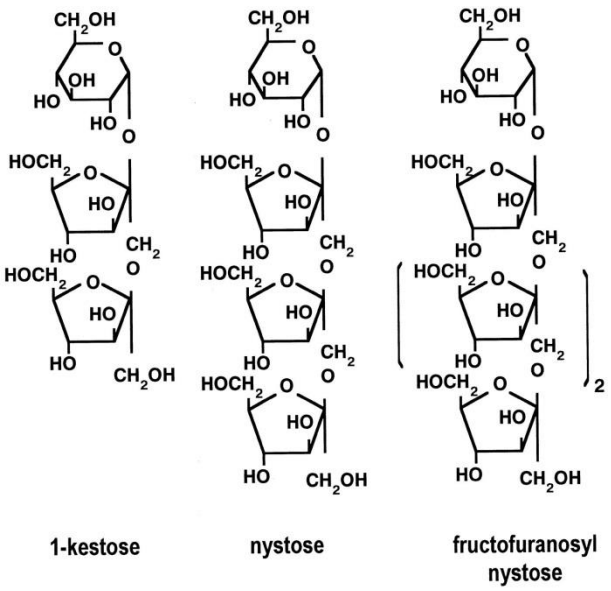

Figure 1. Structural Formulas of 1-Kestose, Nystose, and Fructofuranosylnystose.

2.5. Physical and Chemical Properties

NutraFlora® scFOS is available in both liquid (syrup) and powder forms. A brief summary of the physical and chemical properties of both forms of the product is provided in Table 1.

Table 1. Physical and Chemical Properties of NutraFlora® scFOS.

Physical/Chemical Property	Value	
	Syrup	Powder
Appearance	Slightly viscous fluid	Colorless powder
Appearance in solution	Colorless	Colorless
Taste	Slightly sweet	Slightly sweet
Odor	None	None
pH (10% solution)	5.0 – 7.5	5.0 – 7.0
Solubility in water	High	High
Solubility in ethanol	Insoluble	Insoluble
Viscosity (cP, 70% solution at 30ºC)	1007 - 1759	1000

2.6. Production Process

NutraFlora® scFOS is produced by the treatment of sucrose with a food-grade preparation of β-fructofuranosidase derived from *Aspergillus japonicus* (ATCC 20611). *A. japonicus* is a member of the *niger* group and enzymes derived from it are regarded as GRAS (Pariza and Johnson 2001). With an optimum temperature of 50ºC and pH of 5.5, but stable up to 55ºC and within a pH range of 4 to 8 (Quang et al. 2005), the enzyme acts as an invertase on sucrose molecules and a fructosyltransferase between sucrose molecules and fructofuranosyl-sucrose molecules. The invertase activity cleaves sucrose into glucose and fructose units while the fructofuranosidase activity transfers the fructose unit from sucrose to a growing fructose chain to yield 1-kestose (GF2), nystose (GF3), and fructofuranosylnystose (GF4) molecules. One glucose residue is released as each fructose unit is added to the chain.

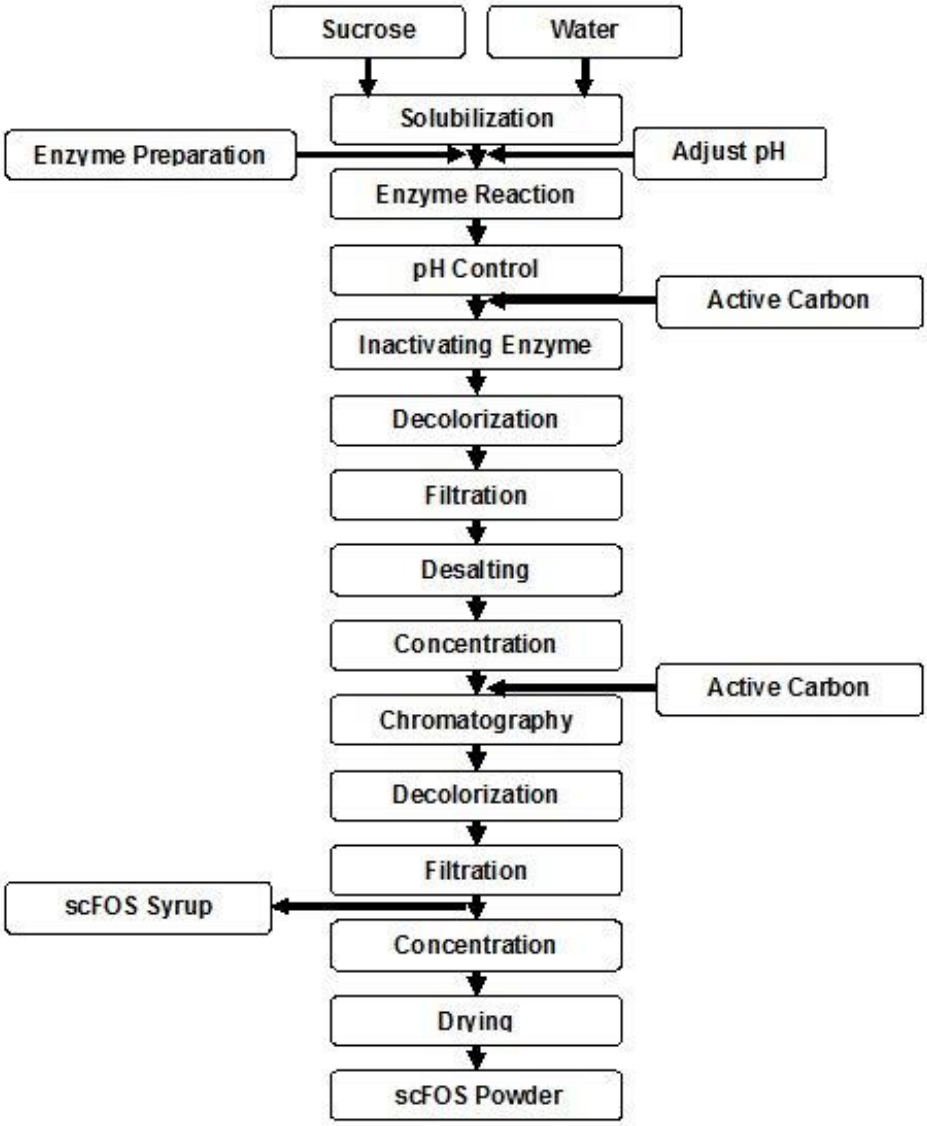

Figure 2. Production of NutraFlora® scFOS.

The production process of scFOS is shown schematically in Figure 2. As shown, granular sucrose is dissolved in water, held at a controlled temperature, and the β-fructofuranosidase enzyme preparation is added. The reaction is monitored and food-grade hydrochloric acid (HCl) or potassium hydroxide (KOH) is added as needed to retain the pH within the desired range. After an in-process test of sugar composition indicates that the enzyme reaction is complete, the mixture is heated to 80ºC for 30 minutes to inactivate the enzyme. Activated carbon is added to remove the enzyme and decolorize the mixture; the carbon is removed by filtration. Desalting is accomplished by chromatography, with a test for electrical conductivity used to confirm removal of electrolytes. A second round of carbon addition and filtration completes the enzyme removal and decolorization; the repeated filtration also removes any potential residues of *Aspergillus* cells.

These steps produce scFOS syrup. The scFOS powder is produced by concentrating and drying the syrup and passing the mixture through a 40-mesh sieve.

2.7. Regulatory Status of Processing Aids

In addition to the GRAS enzyme preparation of β-fructofuranosidase, processing aids used in the manufacturing process for scFOS include HCl, KOH, and activated carbon, all food grade. HCl and KOH 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.

2.8. Food-Grade Specifications for NutraFlora® scFOS

Ingredion has established the specifications displayed in Table 2 to ensure a consistent food-grade product, and analyzed five non-consecutive lots of product. Results of all analyses were within the established specifications, indicating that the production process is in control and that food-grade scFOS is consistently produced.

Table 2. Specifications for Food Grade scFOS and Results of Analyses.

Parameter	Unit	Specification	Lot				
			7643043100	7643007101	7642248100	7642226101	7642159101
Appearance		white powder	passes	passes	passes	passes	passes
Flavor		slightly sweet	passes	passes	passes	passes	passes
Odor		none	passes	passes	passes	passes	passes
Total scFOS	%	NLT[1] 95.0	96.1	95.9	96.3	96.1	96.0
1-kestose (GF2)	%	NLT 30.0	36.2	32.9	34.2	33.0	39.5
nystose (GF3)	%	NLT 45.0	48.4	50.8	49.2	50.0	47.2
fructofuranosylnystose (GF4)	%	NLT 5.0	11.5	12.2	12.9	13.1	9.3
Sugars	%	NMT[2] 5.0	3.9	4.1	3.7	3.9	4.0
Protein	%	NMT 0.01	0.00	0.00	0.00	0.00	0.00
Ash	%	NMT 0.05	0.00	0.00	0.00	0.00	0.00
Moisture	%	NMT 5.00	3.04	3.78	4.07	3.16	4.27
pH		5.0—7.0	6.6	6.5	6.5	6.3	6.4
Lead	mg/kg	NMT 1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Arsenic	mg/kg	NMT 1.0	<0.01	<0.01	<0.01	<0.01	<0.01
Standard plate count	cfu[3]/g	NMT 300	0	0	0	0	0
Yeast	cfu/g	NMT 20	0	0	0	0	0
Mold	cfu/g	NMT 20	0	0	10	0	0
Coliforms	cfu/g	NMT 10	<10	<10	<10	<10	<10
Escherichia coli	cfu/10 g	negative	negative	negative	negative	negative	negative
Anaerobic thermophilic spores	cfu/g	NMT 10	0	0	0	0	0
Aerobic thermophilic spores	cfu/g	NMT 10	0	0	0	0	5
Anaerobic mesophilic spores	cfu/g	NMT 10	0	0	0	0	0
Aerobic mesophilic spores	cfu/g	NMT 10	0	0	0	0	0
Salmonella spp.	cfu/100 g	negative	negative	negative	negative	negative	negative
Staphylococcus aureus	cfu/10 g	negative	negative	negative	negative	negative	negative
Enterobacteriaceae[4]	cfu/30 g	negative	negative	negative	negative	negative	negative
Cronobacter sakazakii[4]	cfu/10 g	negative	negative	negative	negative	negative	negative

1. not less than 2. not more than 3. colony-forming unit 4. Tentative specifications for Enterobacteriaceae and *C. sakazakii* were established recently. The 5 samples tested were non-consecutive samples different from those tested for the other specifications, which were no longer available.

3. Intended Technical Effect

The intended effect of the addition of scFOS to infant formula and follow-on formula is to enhance the organoleptic properties and palatability of formula, and to provide a non-digestible oligosaccharide which might induce in infants fed such formula softer stool consistency, reduce the risk of constipation, serve as a source of colonic fermentation, lower colonic pH, and modify colonic bacterial colonization.

3.1 Human Milk Oligosaccharides

The composition of the intestinal microbiome of the infant is greatly influenced by the foods consumed. The predominance in the microbiota of the breastfed infant of beneficial genera such as *Bifidobacterium* and *Lactobacillus* spp. is believed to result from fermentation of human milk oligosaccharides (HMO; Sherman et al. 2009). Human breast milk contains more than 130 different oligosaccharides (Kunz et al. 2000; Vandenplas 2002; Ballard and Morrow 2013), accounting, after lactose and fat, for the largest solid constituent (Newburg 1997). The total concentration of oligosaccharides in human milk may exceed 1200 mg/100 ml, and levels in colostrum may reach concentrations as high as 2300 mg/100 ml (Newburg 1997; Kunz et al. 2000). Vandenplas (2002) reported that the highest concentration of HMO, 2000 mg/100 ml, is reached on the fourth day of life. Differences between term and preterm milk have not been observed (Kunz et al. 2000).

Based on milk samples from 50 human donors, Chaturvedi et al. (1997) identified twelve major types of oligosaccharides ranging from tri- to octasaccharides, accounting for more than 70% of the total. HMO exist in many different molecular forms—linear or branched, composed of simple sugars or containing sugar derivatives, acidic or neutral (Vandenplas 2002).

HMO are resistant to enzymatic digestion in the upper GI tract (Engfer 2000). Reaching the colon intact, they may serve as substrates for colonic fermentation, and it has been shown that HMO induce an increase in the number of bifidobacteria in the colonic microbiome, along with a reduction in the number of potentially pathogenic bacteria such as *Streptococcus pneumoniae*, *Campylobacter jejuni*, and enteropathogenic *Escherichia coli* (Newburg 1997; Kunz et al. 2000; Vandenplas 2002; Chierici et al. 2003; Barile and Rastall 2013). Hester et al. (2013) showed in *in vitro* studies as well as in *in vivo* studies in piglets infected with rotavirus that treatment with HMO significantly reduced the ability of rotavirus to replicate as measured by mRNA expression of non-structural protein-4.

Different HMO have been shown to have species-specific bifidogenic properties and sometimes even strain-level specificity (Ward et al. 2007; Xiao et al. 2010), implying a co-evolution of HMO and the genetic capability of select intestinal bacteria to utilize them (German et al. 2008).

In a proteomic analysis of *Bifidobacterium longum* ssp. *infantis*, Kim et al. (2013) identified the specific proteins deployed for the catabolism of HMO. They found that, in general, utilization of these substrates occurs by means of specific transport mechanisms and glycosyl hydrolases that break them into monosaccharides.

The immediate and longer-term benefits of HMO to the health of breast- and formula-fed infants remain a subject of active investigation. For example, Stepans et al. (2006) measured the

levels of one primary oligosaccharide, lacto-N-fucopentaose II, in breast milk of 49 mothers at 2 weeks postpartum and found that these levels were associated with significantly fewer enteric and respiratory problems at 6 and 12 weeks. Wang (2009) reviewed data showing the importance of sialic acid in brain development and cognition, including expression of two learning-related genes, with long-term effects on learning and memory. In more recent research, He et al. (2014) found that HMO modulate the signaling pathways of the immature mucosa, which tends to hyperreact to newly colonizing unfamiliar bacteria, to attenuate mucosal response to surface inflammatory stimuli while enhancing signals that support maturation of the mucosal immune system. After noting the presence of HMO in the urine of breast-fed infants, Lin et al. (2014) found that HMO protect bladder epithelial cells from cytotoxic and proinflammatory effects of uropathogenic bacteria, resulting in reduced incidence of urinary tract infections.

3.2. Bovine Milk

In bovine milk, only trace amounts of oligosaccharides are detectable, with sialyllactose being the major component and a general trait of high sialylation and low fucosylation versus a general trait of low sialylation and high fucosylation in humans (Kunz et al., 2000; Sundekilde et al. 2012; Nwosu et al. 2012). Thus, most infant formulas based on bovine milk are essentially devoid of oligosaccharides unless they are added.

3.3. Infant Formula

Breastfeeding is regarded as the normative standard for infant feeding (Vandenplas 2002; AAP 2012). As a result, multiple health organizations, including the World Health Organization (WHO 2002), the American Academy of Pediatrics (AAP 2012), and the U. S. Department of Health and Human Services (USDHHS 2000), endorse breastfeeding as the optimal form of nutrition for infants during the first year of life. In recognition of the advantages of human milk over infant formula, infant formula manufacturers have made changes in formulas to more closely simulate either human milk composition or breastfeeding performance (IOM 2003). The IOM's Committee on the Evaluation of the Addition of Ingredients New to Infant Formula noted that the attempt to match human milk composition is a "quixotic quest since human milk is a complex body fluid that is variable not only between individuals, but within an individual over time" (IOM 2003, p 3–4). IOM also suggested that the alternative to matching human milk composition is to match breastfeeding performance.

Because of the tremendous variety, variability in levels, and complexity of the structure of HMO, it is not feasible to add a similar oligosaccharide composition to infant formula (SCF 2003). Thus, it is proposed to add other oligosaccharides that, while not precisely duplicating the structure of those found in human milk, may mimic at least some of their beneficial functions such as the prebiotic effect on gut microbiota.

Infant formulas containing 800 mg oligosaccharides/100 ml, comprising a combination of 90% galactooligosaccharides (GOS; 720 mg/100 ml) and 10% long-chain inulin (80 mg/100 ml) have been marketed in Europe since March 2000 (Savino et al. 2003). Such formulas are marketed under the names "Omneo" (manufactured by Nutricia) and "Conformil" (manufactured by Milupa), both owned by Numico, a member of Groupe Danone. In the U.S., the addition of GOS to infant formula has been determined to be GRAS by several manufacturers. FDA has responded with "no questions" letters in response to four GRAS notices: GRN No. 233 from

Mead Johnson (FDA 2009a), GRN No. 236 from Friesland Foods Domo (FDA 2008), GRN No. 286 from GTC Nutrition (FDA 2009b), and GRN No. 334 from Yakult Pharmaceutical Industry Co. (FDA 2010). The Mead Johnson GRAS determination provided for the addition of 200 mg/100 ml polydextrose to infant formula in combination with 200 mg/100 ml GOS (FDA 2009a). Friesland Foods Domo determined that the addition of up to 500 mg GOS/100 ml formula is GRAS while GTC Nutrition and Yakult found addition of 720 mg GOS/100 ml to be GRAS.

Additionally, in 2011, Pfizer Nutrition and BENEO-Orafti determined that the addition of oligofructose to milk-based infant formula powder at a maximum level of 300 mg/100 ml of formula as consumed is GRAS and notified FDA in GRN No. 392. FDA responded with "no questions" on May 7, 2012 (FDA 2012).

As discussed in detail in Section 5.2 on pharmacokinetics, scFOS, like HMO, is resistant to enzymatic hydrolysis in the upper GI tract and thus reaches the colon largely intact, where it is susceptible to fermentation by the colonic microbiota. Like HMO, scFOS is bifidogenic: infants given formula supplemented with scFOS tend to develop a colonic microbiota that more closely resembles that found in breastfed infants than does the microbiota of infants fed the same formula but without added scFOS. Additionally, as will be discussed in detail, infants consuming formula containing scFOS produce more frequent and softer stools than do those receiving control formula, more closely resembling the stooling behavior of breastfed infants. The tendency of cow's-milk formula-fed infants to produce less frequent and harder stools than do breastfed infants has been observed in many studies; Weaver et al. (1988), for example, observed 120 healthy formula-fed and 120 healthy breastfed infants aged 2-20 weeks and reported that the breastfed infants produced significantly more frequent and significantly softer stools.

Sherman et al. (2009), reporting on a global summit meeting focusing on prebiotics and infant formula, illustrated the potential and proven effects of prebiotics in a figure reproduced below as Figure 3.

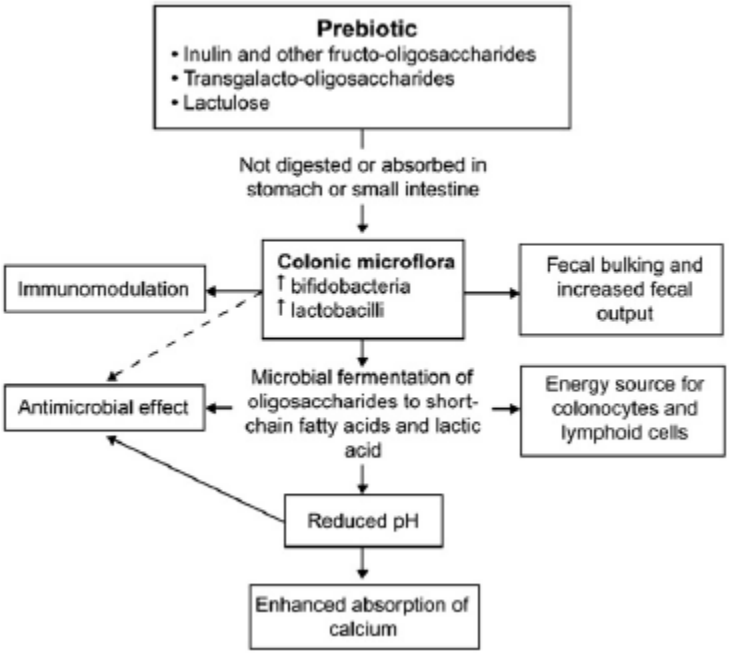

Figure 3. Effects of Prebiotics (from Sherman et al. 2009).

4. Intended Use and Consumer Exposure

4.1. Addition Level of scFOS to Infant Formula

The intended use of Ingredion's scFOS is addition to non-exempt infant formula as a prebiotic ingredient as defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP 2009): "Prebiotics are selectively fermented dietary ingredients that result in specific changes in the composition and/or activity of the GI microbiota, thus conferring benefit(s) upon host health." The maximum intended addition levels are 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed. For infant formula powders with a hydration rate of 12.7 g/100 ml, these levels are equivalent to 31.5 and 39.4 mg scFOS/g powder, respectively.

4.2. Estimated Daily Intake of scFOS

According to tables of daily energy intake by formula-fed infants provided by Fomon (1993), the subpopulation of infants with the highest intake per kg body weight is boys age 14–27 days. The 90th percentile energy intake by this group is 141.3 kcal/kg bw/day. Among girls, the highest energy intake is found in the same age group, 14–27 days, and is nearly as high as boys: 138.9 kcal/kg bw/day[1]. Most standard formulas contain 67 kcal/100 ml when ready to consume. Therefore, to obtain 141.3 kcal energy/kg bw, an infant boy must consume 209.0 ml formula/kg bw. To reach her 90th percentile of energy consumption, 138.9 kcal/kg bw/day, an infant girl must consume 205.5 ml formula/kg bw. The 90th percentile of formula intake for the two sexes combined is about 207 ml/kg bw/day.

The 90th percentile daily intake of scFOS, added at a maximum concentration of 500 mg/100 ml, is thus estimated to be 1.035 g/kg bw/day. This maximum is based on the addition level of scFOS in follow-on formula; the 90th percentile daily intake of scFOS from starter formula, with a maximum concentration of 400 mg/100 ml, is estimated to be 828 mg/kg bw/day.

As the infant grows, formula intake increases, but more slowly than weight gain, so that consumption assessed as ml formula per kg body weight is lower for infants older than 27 days. As a result, intake of scFOS per kg body weight decreases as the infant grows older and larger. Estimates of the mean and 90th percentile intakes of scFOS at various ages, based on Fomon's (1993) data on energy intakes, are shown in Figure 7.

Figure 4 shows that the estimated 90th percentile intake of scFOS peaks at about 1.035 g/kg bw/day during the first 6 weeks of life, then begins to decline, reaching about 840 mg/kg bw/day by weeks 8–12. Establishing the EDI of scFOS at 1.035 g/kg bw/day

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[1] These estimates are corroborated by data from the 2008 Feeding Infants and Toddlers Study (FITS; Butte et al. 2010), which reported the 90th percentile energy intake for infants aged birth to 5 months as 779 kcal. Although body weights of the FITS participants on the days diets were assessed were not available, infant growth charts issued by the Centers for Disease Control and Prevention indicate that the median body weights for the two sexes combined at birth and at 5 months are about 3.4 and 7.4 kg, respectively. A reasonable estimate of the median body weight of infants aged birth to 5 months is the average of these two body weights, or 5.4 kg. The 90th percentile energy intake of 779 kcal thus represents about 144 kcal/kg, very close to the estimates in Fomon (1993).

(achieved by consuming follow-on rather than starter formula) is thus a very conservative basis for estimating long-term exposure.

Since bovine milk does not contain fructans, there are no other sources of scFOS or other fructans in the diets of formula-fed infants and, therefore, the above estimated 90th percentile daily intake constitutes the total daily exposure of formula-fed infants to scFOS and to fructans.

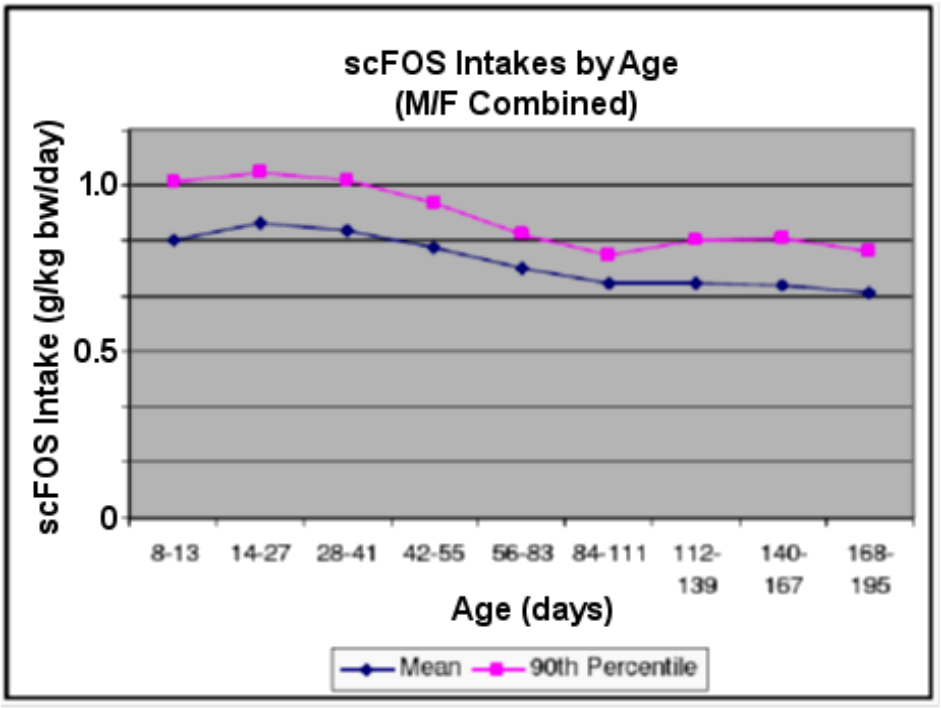

Figure 4. Estimated Intakes of scFOS by Age (based on data from Fomon 1993).

5. Safe Use, Pharmacokinetics, and Toxicity of Fructans

5.1. Safe Use and GRAS Status of Inulin, Oligofructose, and scFOS

5.1.1. Conventional Foods

Several fructans are already GRAS for addition to conventional foods in the U.S. The first GRAS determination for a fructan—inulin—was completed in 1992 (Coussement 1999). The review was sponsored by Orafti (now Beneo-Orafti) and concerned a product containing >95% inulin from chicory roots and sold under the name Raftiline® as well as an oligofructose sold as Raftilose®. This latter product is now trade named Orafti®P95, and its use in infant formula was the subject of a 2011 GRAS notice (GRN 392). The GRAS panel convened by Orafti based its determination that inulin and oligofructose are GRAS for addition to food, limited only by cGMP, on the history of consumption of naturally occurring inulin and oligofructose, corroborated by scientific evidence. The panel concluded:

> "We find there is no scientific evidence in the available data and literature on the food uses of these substances that demonstrates or suggests reasonable grounds to suspect a hazard to the public when used at levels that are current or that might reasonably be expected to be used in the future. Our position regarding the safety of inulin and oligofructose is based on the long human experience of consuming inulin containing foods as well as evaluation of available scientific evidence relating to inulin and its hydrolysis products. Since inulin and oligofructose have been natural components of many foods consumed safely by humans over millennia, there is no reason to suspect a significant risk to the public health when used in foods. Therefore, we conclude that these food substances are generally recognized as safe, both by long-established history of use in foods and by the opinion of experts qualified by scientific training and experience in food safety after a thorough review of the available scientific evidence" (Orafti GRAS Panel, quoted in Coussement 1999).

Other inulin products that have been determined to be GRAS for addition to food include Roxlor International's BakeFlora™ and its companion oligofructose product BeFlora®, which were determined to be GRAS in 2001 (Roxlor 2002), TIC Gums' TIC Pretested® Inulin LV-110 (TIC Gums 2005), and Cargill's Oliggo-Fiber™ Inulin (Cargill 2005), self-determined to be GRAS for use limited only by cGMP.

Roxlor's products both combine fructans extracted from chicory with mung bean extract; the average DP in BakeFlora™, containing both inulin and FOS, is reported to be 23, while in BeFlora® (containing FOS produced by enzymatic partial hydrolysis of inulin), the average DP is reported to be 2-8. Cargill offers a number of formulations of Oliggo-Fiber™, inulin extracts derived from chicory roots, with average reported DP of 8-10 or 20.

Makers of one scFOS product and one inulin product have notified FDA of their GRAS determinations regarding addition of their products to conventional foods. In 2000, GTC Nutrition Company determined that scFOS manufactured by the enzymatic treatment of sucrose is GRAS for use as a bulking agent; FDA responded to GRAS Notice No. GRN No. 44 that it had "no questions at this time" regarding this determination (FDA 2000). 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's intended use would be as high as 12.8 g/day. In 2007, GTC notified FDA that it had determined that the addition of scFOS to foods in general, including infant and toddler foods but excluding infant formula, at levels resulting in intakes up

to 20 g/day in the general population and up to 4.2 g/day in infants less than one year of age, is also GRAS; FDA had no questions regarding this conclusion (FDA 2007b).

Imperial Sensus notified FDA in GRAS Notice No. GRN No. 118 in 2002 that it had concluded that inulin from chicory root is GRAS for use as a bulking agent. The product reportedly includes a range of DP up to 60. The estimated 90th percentile intake of this inulin from its intended uses was estimated at 20 g/day for the general population. FDA again had no questions regarding this conclusion (FDA 2003).

5.1.2. Infant Formula

As noted earlier, Pfizer Nutrition and BENEO-Orafti determined that the addition of chicory-derived oligofructose to milk-based infant formula powder at a maximum level of 300 mg/100 ml of formula as consumed is GRAS and notified FDA in GRN No. 392. FDA responded with "no questions" on May 7, 2012 (FDA 2012).

On May 23, 2013, Food Standards Australia New Zealand (FSANZ 2013) issued a report approving an application from GTC Nutrition requesting permission for the optional addition of scFOS 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 cited the following conclusions:

- 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 scFOS has no identifiable hazard at concentrations likely to be encountered under Good Manufacturing Practice;
- Digestion of scFOS 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; and
- 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 scFOS up to 3.0 g/L for periods ranging from 1 week to approximately 3 months.

5.2. Pharmacokinetics

5.2.1. Animal Studies

Oku et al. (1984) studied the digestibility of scFOS in one *in vitro* experiment and two animal studies. In the *in vitro* study, scFOS was exposed to pancreatic and small-intestinal homogenates and to purified sucrase-isomaltase complex. In the first animal experiment, group-housed male Wistar rats weighing about 50 g (number not reported) were given *ad libitum* access to water and diets containing either 0 or 20% scFOS for 6 weeks. After sacrifice, the pancreas was removed and mucosa from the duodenum, jejunum, and ileum were scraped and tested for the activities of enzymes that hydrolyze 1-kestose and nystose. In the second experiment, 0.5 ml of isotopically labeled scFOS was injected into the subcardinal vein of male Wistar rats weighing

about 230 g (number not reported), which were then placed in metabolic cages; excreted CO_2 was measured and urine and feces were collected.

Results of the *in vitro* study demonstrated that little or no digestion of 1-kestose or nystose occurred; no activity of α-amylase (AMY) could be observed in pancreatic, liver, or kidney homogenate and only trace levels in duodenal, jejunal, and ileal mucosa—about 0.1% of the activity level produced by maltose—and hydrolysis of nystose was significantly less than that of 1-kestose. It was also found that combinations of scFOS with sucrose or maltose had no effect on the rate of digestion of those free sugars.

The 6-week feeding study was intended to test whether long-term ingestion of scFOS might induce greater activity of hydrolyzing enzymes, but this did not occur. Indeed, it was reported that the activities of sucrase and maltase tended to decrease during long-term feeding of scFOS.

The purpose of the intravenous study was to determine if any small amounts of 1-kestose or nystose that might be absorbed intact could be hydrolyzed by enzymes of some internal organ. Nearly all injected radioactivity was excreted in the urine; less than 1% appeared in expired CO_2 and only 1.24% appeared in feces. Thin-layer chromatography of the excreted radioactivity determined that it was in the form of unchanged scFOS, indicating that no hydrolysis of scFOS occurred.

In an *in vitro* study, Tsuji et al. (1986) studied absorption of glucose, sucrose, maltose, maltitol, maltotriose, maltotriitol, maltosylfructose, maltotriosylfructose, isomaltose, palatinose, trehalulose, kestose, and nystose (components of scFOS) by measuring the transmural potential difference evoked by Na^+-dependent active transport of glucose. Male Wistar rats weighing about 250 g were given free access to water and chow for 13-15 hours and then sacrificed; the small intestine was excised and isolated everted intestinal segments were prepared. In order to measure absorption, a small amount of the test article was dissolved in 1 ml water and injected into mucosal medium.

All of the sugars except kestose and nystose, components of scFOS, displayed evoked action potentials indicating absorption, and all of the sugars showed evidence of hydrolysis except the monosaccharide glucose and the scFOS constituents kestose and nystose.

In a study of the digestibility of scFOS (Tokunaga et al. 1989), conventional male Wistar rats weighing about 250 g, similar rats weight about 240 g treated with penicillin and chloramphenicol for a week, and germfree male rats weight about 120 g were fasted overnight and then gavaged with 3 µCi of [U-^{14}C]scFOS (2.1 mg). The rats were placed in metabolic cages to allow trapping of expired $^{14}CO_2$ and collection of urine and feces. About 55% of the radioactivity administered to conventional rats was expired as $^{14}CO_2$ within 24 hours while 3.2% and 3.0% was excreted in urine and feces, respectively. Rats that had received antibiotic treatment had significantly less radioactivity as $^{14}CO_2$ and about twice as much in feces. Germfree rats expired little radioactivity as $^{14}CO_2$ during the first 8 hours, and the total within 24 hours was about 40% while radioactivity in the feces was significantly higher than in the other 2 groups. The authors concluded: " These observations suggest that [scFOS] is metabolized by intestinal microorganisms in the gastrointestinal tract," and suggested that the antibiotic-treated

and germfree rats had begun recolonization with colonic microbiota during their residence in the metabolic cage.

Bjorck and Nilsson (1991) studied gastric-acid hydrolysis of inulin derived from dahlia tubers, with DP up to 30, in 10 male Wistar rats (average weight = 101.8 g) receiving diets containing 5% inulin for 5 days. Analysis of the inulin revealed a fructose:glucose ratio of 23:1 and no detectible sucrose or free fructose or glucose. One group of n = 5 rats was given Omeprazol to inhibit gastric-acid secretion while 5 other rats served as controls; both groups received the antibiotic drug Nebacitin to suppress the hindgut microbiota. Feed was restricted to 10 g/day, but water was freely available. Feed consumption was measured and feces were collected daily.

There were no significant differences between groups in feed intake or in the dry weight of fecal matter. Inulin recovered in feces from the control group with normal levels of secretion of gastric acid was significantly hydrolyzed, whereas only minor hydrolysis occurred in rats with suppressed gastric-acid secretion, indicating the key role of gastric acid in the hydrolysis of inulin to lower molecular-weight fractions. The authors suggested that this gastric hydrolysis, observed under the conditions of the rat study, may liberate small amounts of free fructose, which would explain the apparent digestibility of a small fraction of ingested inulin. This effect would appear to be dependent on the pH of the gastric contents.

In a review article, Oku and Nakamura (2002) reported a study in which ^{14}C-scFOS was orally administered to both conventional and germ-free male Wistar rats weighing about 230 g housed in metabolic cages. In the conventional rats, about 60% of the administered radioactivity was exhaled as CO_2 within 24 hours. The total release of radioactivity was similar to that observed with ^{14}C-sucrose, but was delayed about 3 hours. However, administration of ^{14}C-scFOS to germ-free rats resulted in only trace exhalation of radioactivity, demonstrating that metabolism is provided only through fermentation by intestinal microbes.

In summary, the results of *in vitro* and animal studies indicate that scFOS, like other fructans, is not hydrolyzed by the intrinsic enzymes of the animals, but is specifically fermented by the gut microbiota. Absent microbial fermentation, scFOS is not absorbed. Further, ingestion of scFOS or inulin has no effect on secretion of GI enzymes.

5.2.2. Human Studies

5.2.2.1. Healthy Adults

In a randomized, double-blind, placebo-controlled study, Stone-Dorshow and Levitt (1987) tested the effect of scFOS on breath hydrogen excretion. Fifteen healthy volunteers aged 21-65 ingested 10 g scFOS and provided breath samples at hourly intervals for 6 hours. For the next 12 days, 10 participants ingested 15 g scFOS/day in 3 doses while 5 controls ingested 15 g sucrose; all volunteers then provided fasting blood samples and again ingested 10 g scFOS and gave breath samples for 6 hours. A breath test after ingestion of 10 g lactulose was performed 10 days later. During the feeding period all subjects maintained a daily diary to record any GI effects.

The peak excretion of hydrogen following 10 g scFOS was at 3 hours[1]; neither this figure nor the total area under the curve was affected by 12 days consumption of scFOS or sucrose. The time/area-under-the-curve profiles of the breath tests of scFOS and lactulose were similar, suggesting that no metabolism was occurring. The 10 subjects who consumed 15 g scFOS/day reported significantly greater flatulence than did the 5 taking 15 g sucrose.

Rumessen et al. (1990) investigated the intestinal handling of inulin with a high content of FOS from Jerusalem artichokes and its effects on blood glucose, insulin, and C-peptide. The inulin tested was composed of unbranched chains of fructofuranose units, mostly with terminal glucose residues, with $DP_{average} = 7$ and 39% of the molecules having DP > 8; i.e., a minimum of 60% FOS. Eight healthy adults (6 males and 2 females aged 23-33 years) took lactulose challenges and produced significant breath hydrogen. They then received single-dose challenges of 5, 10, and 20 g artichoke inulin; 20 g fructose; 50 g wheat starch; and 50 g wheat starch + 10 g artichoke inulin. Breath hydrogen tests were performed before, during, and every 15-30 minutes after dosing for 12 hours. Blood samples were drawn for analysis of glucose, insulin, and C-peptide before dosing and every 15-30 minutes after dosing for 3 hours.

Regardless of dose, artichoke inulin was apparently completely unabsorbed, and traces of fructan (less than 1% of the administered dose) were detected in the urine of only one participant after a 20-g dose[2]; extensive fermentation was indicated by significantly increased dose-dependent breath hydrogen, beginning 3 hours after administration with a peak at 5 hours. Orocecal transit times ranged from 145 minutes after a 20-g dose up to 270 minutes after a 5-g dose; all of these times were significantly longer than those of lactulose and fructose. Artichoke inulin, even at 20 g, had little effect on blood glucose and insulin, significantly lower than after fructose ingestion. There was no apparent interference of inulin with starch absorption. Only mild flatulence was reported except one volunteer who complained of moderate flatulence after 10 g artichoke inulin; there was no overall significant difference in reported side effects between artichoke inulin, fructose, or wheat starch. No diarrhea or abdominal pain was reported.

A comparison of the kinetics of this study using 10 g artichoke inulin with that of 10 g scFOS by Stone-Dorshow and Levitt (1987) suggests that fermentation begins to occur about 3 hours after ingestion with both fructans, but peak breath hydrogen production occurs slightly later with the longer-chain fructan. This would be consistent with the hypothesis that the kinetics of fermentation depend on the degree of polymerization.

Molis et al. (1996) studied the digestion and excretion of scFOS in a non-blinded study with 6 healthy adults, 3 of each sex, aged 20-27 years. Subjects consumed the control diet with 3 10-g daily supplements of sucrose for 6 days, followed by a washout period of 1 week or longer, and the control diet with supplementary scFOS in 3 daily doses for 11 days. The scFOS dose began at 5 g/day and was increased over the first 3 days to the full daily dose of 20.1 g/day, which was ingested on days 4 through 11. On the last few days, the subjects provided stools and urine and on day 10 were intubated to collect distal ileum fluid samples while a perfusion lumen

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[1] This does not represent bifidobacterial fermentation, which does not result in production of hydrogen.
[2] The authors offered no explanation for this anomalous finding, but one likely possibility is contamination from feces rather than absorption and urinary excretion.

was used to estimate flow through the ileum. Throughout the dietary periods, the subjects were asked to record any GI symptoms.

A small amount of the ingested scFOS may have been hydrolyzed in the stomach or fermented by small-intestinal microbiota; only 0.12% of ingested scFOS was recovered in urine. While 89% of the ingested scFOS reached the distal ileum, none was excreted in stools, indicating that scFOS reaching the colon was completely fermented by colonic microbiota. Ingested scFOS appeared in the distal ileum within 30-60 minutes after ingestion and continued to be recovered for 8 hours. No GI symptoms were reported during either the placebo or scFOS regimens.

Alles et al. (1996) investigated the metabolic fate of two different levels of oligofructose as compared to a glucose control. Twenty-four non-smoking non-overweight healthy males aged 19-28 years (mean = 22.1 years) participated in a balanced multiple crossover trial using an orthogonal Latin-square design for three periods, with supplement periods of 7 days and two 7-day wash-out periods. The daily supplements were: 15 g oligofructose + 0 g glucose; 5 g oligofructose + 2.7 g glucose; and 0 g oligofructose + 4.1 g glucose. The supplements were dissolved in water, producing solutions of equal osmolarity, and were consumed in 2 daily doses at breakfast and dinner. Body weights were measured on the first day of each supplement period and participants maintained a diary to record supplement consumption, defecation, and any GI complaints. Breath samples and fecal samples were collected at the end of each feeding period.

All participants completed the study and, other than a significant increase in reported flatulence during ingestion of 15 g oligofructose/day, there were no differences in reported GI effects, nor in defecation frequency, stool wet or dry weights, stool pH, or stool form. Ingestion of 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 the total concentration of short-chain fatty acids (SCFA) or the relative proportions of the individual SCFA. The authors concluded that "fructooligosaccharides added to the diet of young Western subjects are fully metabolized in the large intestine."

Rumessen and Gudmand-Hoyer (1998) investigated the intestinal transport and fermentation of chicory-derived long-chain inulin and oligofructose in a single-blind crossover study of healthy adults. Five healthy men and 5 women aged 18-25 years received a lactulose challenge followed by 9 further single-dose tests in random order, separated by 48 hours or more: 20 g lactulose; 10, 20, and 30 g oligofructose; 20 g long-chain inulin; 20 and 50 g fructose; 20 g sorbitol; and 20 g sorbitol + 20 g glucose. Breath samples were taken every 30 minutes for 12 hours after each test and analyzed for H_2 and CH_4. Blood was sampled via catheter every 30 minutes for 3 hours for measurement of glucose and acetate. Urine was collected after administration of short-chain fructans for assessment of the presence of fructans.

One volunteer failed to complete the tests due to a non-related condition. All participants showed a significant rise in hydrogen after lactulose challenge. Average orocecal transit times for both shorter- and longer-chain fructans, lactulose, sorbitol, and fructose ranged from 30 to 105 minutes, with considerable overlap across all tested carbohydrates, but with long-chain inulin having a significantly longer transit or fermentation time than oligofructose. Breath hydrogen and venous acetate production increased in proportion to increasing fructan dose and

were similar to responses to lactulose. All volunteers showed malabsorption of 50 g fructose, resulting in significantly more symptoms than 20 g fructose. Ingestion of sorbitol with equimolar amounts of glucose did not reduce malabsorption or abdominal distress. Reported abdominal symptoms (most often mild gas) increased with increasing intakes of indigestible carbohydrates (lactulose, oligofructose, long-chain inulin, high doses of fructose, and sorbitol). Abdominal upset appeared to be inversely related to degree of polymerization: the incidence with 20 g lactulose was comparable to that with 20 g oligofructose and higher than with 20 g long-chain inulin.

In accordance with a Latin-square design, Castiglia-Delavaud et al. (1998) assigned 9 healthy young men with an average age of 21.5 years to receive 1 of 3 diets for a total of 28 days: control diet, control diet + 50 g sugar-beet fiber/day, or control diet + 50 g inulin/day. Each 28-day test period included 2 days on the control diet, 14 days with increasing content of inulin or sugar-beet fiber, and 12 days with 50 g/day of inulin or sugar-beet fiber. The tested inulin product included 62% inulin derived from chicory; other components were not reported but may have included oligofructose, other non-starch polysaccharides, and digestible mono- and disaccharides. During the last 8 days of each experimental period, feces and urine were collected for analysis of energy, nitrogen, total lipids and SCFA, starch, cellulose and hemicellulose, glucose, uronic acids, and bacterial counts. Energy expenditure was measured by whole-body indirect calorimetry.

Ingestion of sugar-beet fiber or the inulin-containing product 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 among diets were not significantly different. The fermentability of sugar-beet fiber averaged about 89% while that of the inulin-containing product approached 100%. The calculated energy contents of the two non-starch polysaccharides were 2.8 kcal/g for sugar-beet fiber and 1.2 kcal/g from the inulin-containing product.

A Latin square, randomized, double-blind, diet-controlled study was designed to investigate the effect of daily intake of 15 g nondigestible poly- or oligosaccharides on large-bowel function, blood lipid concentrations, and glucose absorption (van Dokkum et al. 1999). Twelve healthy men (mean age = 23 years) received 4 successive intervention periods of 3 weeks each: chicory inulin, oligofructose, GOS, and control, apparently with no washout periods. The daily 15-g dose of non-digestible oligosaccharide was consumed in 3 doses of 5 g each. During the final week of each treatment, analyses were conducted of stool weight, intestinal transit, fecal pH, SCFA, bile acids, fecal enzymes, blood lipids, glucose absorption, and reported GI symptoms.

All participants completed the study with no significant differences among treatments in reported GI tolerance issues. The treatments had no effect on colonic function, transit time, or fecal weight 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 hydrogen 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. No differences in blood lipids reached significance.

In summary, these studies in healthy humans are consistent with the findings of animal studies in showing that nearly all ingested inulin, oligofructose, and scFOS reach the colon where they are fermented by colonic bacteria. The kinetics of orocecal transit time and bacterial fermentation are inversely proportional to the degree of polymerization of the fructan. Ingestion of inulin or oligofructose may result in modification of fecal markers such as defecation frequency or stool characteristics, as well as GI symptoms typical of those regularly observed with ingestion of high doses of dietary fiber.

5.2.2.2. Compromised Adults

Bach Knudsen and Hessov (1995) investigated the recovery of ingested inulin from Jerusalem artichokes from the small intestine of 7 ileostomy subjects. The patients (1 male and 6 females aged 22-73 years, median age = 38 years) had been proctocolectomized for ulcerative colitis or familial polyposis coli, but were otherwise in good health with no GI resections or antibiotic usage. The patients were hospitalized and given a low-fiber diet for 3 days. On day 1, they were also given 10 g inulin and on day 2 they received 30 g inulin, both dissolved in 200 ml water. The osmolarities of the load were 68 and 204 mOsm/L for the 10-g and 30-g doses, respectively. The inulin product included 71% inulin and 21% glucose, fructose, and sucrose. The average DP of the inulin product was not reported.

Ileostomy effluent was collected every 2 hours from 8:00 am to 8:00 pm, at 11:00 pm, and the following morning at 8:00 am. The dry-matter content was assessed, including low-molecular-weight sugars, inulin, organic acids, protein, starch, non-starch polysaccharides, and ash.

The total weight of the ileal dry matter increased significantly in a dose-dependent manner with increasing inulin load, due almost entirely to increased carbohydrate excretion, which in turn resulted primarily from excretion of inulin. The recovery of inulin was approximately 87% at both ingestion levels. Inulin excretion increased rapidly after ingestion, peaking at 3 hours with a mean transit time of 4.9 hours at an intake of 10 g inulin product and 3.4 hours at 30 g. Analysis of the fructose:glucose ratio (4:1) of the inulin as ingested and as recovered in ileal effluent (4.5-4.7) revealed that the low-DP inulin fractions were more sensitive to hydrolysis than the higher DP fractions, suggesting that scFOS would be still more sensitive. Ileal concentrations of SCFA (acetic, propionic, and butyric acids) were significantly reduced by inulin ingestion, while the concentration of lactic acid was significantly increased. The authors concluded that this study demonstrated that, while slight hydrolysis of inulin was seen (due to acids, enzymes, or microbial degradation), “inulin is practically indigestible in the small intestine of man” and that “the transit of inulin through the small intestine was rapid.” They also noted that ingestion of the tested levels of inulin resulted in minor increases in non-absorbed sugars in the effluent.

In a double-blind crossover study of 10 ulcerative colitis patients (5 of each sex, aged 30-71 with mean age 54 years) with conventional ileostomy, Ellegard et al. (1997) added 17 g/day chicory inulin, 17 g/day oligofructose, or 7 g/day sucrose, in 3 daily doses, to a controlled diet for 3 days each. Ileostomy effluents were collected and analyzed on the 2nd and 3rd days of each feeding period.

None of the patients noticed any difference among the 3 diets, indicating that daily ingestion of 17 g/day inulin or oligofructose in 3 daily doses was well tolerated. Nearly all—over 88%--of the ingested inulin and oligofructose were recovered in the ileostomy effluents. These data are consistent with the recovery from Bach Knudsen and Hessov (1995); the disappearance of small amounts of inulin and oligofructose can be explained by slight stomach hydrolysis and fermentation occurring in the lower small intestine. Dry solid and energy excretion both increased significantly on inulin and on oligofructose compared to control diet. Cholesterol absorption, excretion of cholesterol, bile acids, nitrogen, fat, calcium, magnesium, zinc, and iron were not significantly affected by inulin and oligofructose. The authors concluded that any physiological effects of inulin and oligofructose are probably mediated through other mechanisms than altered excretion from the small intestine.

In summary, the data from ileostomy studies consistently show nearly complete nondigestibility of fructans from chicory and artichoke in either the stomach or small intestine.

5.2.3. Conclusions Regarding Pharmacokinetics of Fructans

In addition to the results of *in vitro* testing showing the resistance of fructans to hydrolysis by human enzymes and the absence of absorption of non-hydrolyzed fructosyl chains, four published studies in rats and five in humans—two of the latter involving ileostomy subjects—have provided convincing evidence that digestion of fructans—inulin, oligofructose, or scFOS—in the small intestine is close to nil. Although Bjorck and Nilsson (1991) found that a small amount of inulin was apparently hydrolyzed by gastric acid in rats, in this study the rats were restricted to only 10 g feed/day, producing abnormally low gastric pH levels. It is not at all clear that this phenomenon would be observed when fructans are consumed *ad libitum* or with food—especially with dairy-based food—which provides significant buffering.

Most significant, the two studies in ileostomy patients (Bach Knudsen and Hessov 1995 and Ellegard et al. 1997) both recovered about 90% of the ingested fructans in effluents and concluded that little or no digestion or absorption of fructans occurs in either the stomach or the small intestine. Reviewing this work, Andersson et al. (1999) concluded first that “the ileostomy model is considered to be a reliable model to reflect small bowel absorption,” and second that “inulin or oligofructose passes through the small bowel without degradation.”

The conclusion of Andersson et al. (1999) was supported by Cummings et al. (2001) and Cummings and Macfarlane (2002) after a review of the *in vitro*, animal, and human data. It may be concluded that infants consuming scFOS in infant formula at the intended addition level do not digest a significant fraction of the scFOS and, therefore, do not absorb free glucose or fructose hydrolyzed from the scFOS. Further, the scFOS reaches the large bowel largely intact, where it provides a substrate for bacterial fermentation.

Two studies in healthy adults (Molis et al. 1996 and Alles et al. 1996) found no traces of ingested fructans in the feces, and Castiglia-Delavaud et al. (1998), based on energy release, concluded that fermentation of inulin in the colon approached 100%. These three studies together provide firm evidence of nearly complete fermentation of ingested fructans in healthy adults. The end products of this fermentation are mostly lactate, SCFA (acetate, propionate, and butyrate), H_2, and CO_2.

5.3. Toxicity

Studies of oral toxicity discussed in the following sections are summarized in Table 3 at the end of the section.

5.3.1. Acute Oral Toxicity of scFOS

Takeda and Niizato (1982, summarized in Carabin and Flamm 1999) studied the acute oral toxicity of scFOS. The test article was administered to 4-week-old male and female JcL-IcR mice. Six mice of each sex were randomly assigned to groups that were administered by gavage single doses of 0, 3, 6, or 9 g scFOS/kg bw dissolved in 0.5 ml water. The animals were observed for 1 week for signs of toxicity. No deaths occurred, there were no differences in body weight gain between the test and control animals, and no abnormalities were seen in either sex in the general state of health. The authors concluded that scFOS did not cause adverse reactions in mice when given as a single dose of up to 9 g/kg bw; the LD_{50} for oral administration of scFOS to rats in this study was therefore > 9000 mg/kg bw.

Takeda and Niizato (1982, summarized in Carabin and Flamm 1999) also evaluated the acute toxicity of scFOS in Sprague-Dawley rats. Groups of 6-week-old male and 10-week-old female rats were randomly assigned to 1 of 4 groups (n = 6 rats/group) that received by gavage 0, 3, 6, or 9 g scFOS/kg bw dissolved in 2 ml water in a single dose. The animals were observed for 1 week for any signs of toxicity. There were no deaths and no abnormalities or changes in body weight were seen in animals of either sex. It was concluded that scFOS is not toxic to male or female rats when administered as a single dose of up to 9 g/kg bw and the LD_{50} for oral administration of scFOS to rats in this study was > 9000 mg/kg bw.

These studies indicate that scFOS has low potential for oral toxicity, with an LD_{50} for oral administration >9000 mg/kg bw, far in excess of the 2000 mg/kg bw which today is regarded as the limit dose in such tests.

5.3.2. Subacute Oral Toxicity of scFOS

Takeda and Niizato (1982; presented in Carabin and Flamm 1999) administered scFOS to male Wistar rats for 6 weeks. Groups of 18 rats, 6 to 7 weeks old, were administered single daily doses of 1.5, 3, or 4.5 g/kg bw/day of scFOS (test) or sucrose and glucose (controls). Blood samples were taken from 6 animals in each group during weeks 2, 4, and 6. Animals were weighed at baseline and termination; after sacrifice, the animals were necropsied.

There were no deaths or abnormalities during the study and no consistent treatment-related differences were observed in blood chemistries. Animals receiving 3 or 4.5 g/kg bw/day of scFOS showed a slight increase in body weight compared to controls. A slight swelling of the appendix was observed in the rats receiving scFOS, while this was not seen in the other groups. Histopathology of the following organs revealed no abnormalities: liver, pancreas, adrenal glands, kidneys, brain, cerebellum, heart, lungs, spleen, pituitary gland, and testes. It was concluded that no treatment-related toxicity occurred in any of the scFOS-treated groups; the no observed adverse effect level (NOAEL) for orally administered scFOS in this study was the highest dose tested, 4500 mg/kg bw/day.

In another 6-week subacute study (Takeda and Niizato 1982; presented in Carabin and Flamm 1999), 6- to 7-week-old male Wistar rats were again assigned to groups of 18 animals.

The test substance was scFOS and the controls were sucrose, glucose, and sorbitol. The animals were given *ad libitum* access to feed in which 5% sucrose and 5% starch were replaced with 5 or 10% of the test substances. At the end of weeks 2, 4, and 6, 6 animals from each group were sacrificed. Blood samples were taken and the liver, pancreas, and adrenal glands were removed for histopathologic examination. Kidneys, brain, cerebellum, heart, lungs, spleen, pituitary gland, and testes were also removed from the animals killed at the end of the 6th week.

Carabin and Flamm (1999) reported that no deaths occurred and no treatment-related abnormalities were observed. The sorbitol and scFOS groups had lower body weights in the 1st to 5th weeks, but 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 watery stools on day 2 and those in the scFOS group on day 10. The only blood chemistry finding regarded as treatment-related 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. The latter changes were most marked in the animals receiving sorbitol and sucrose. Isolated cases of calcium deposits were seen in all groups.

Overall, oral administration of scFOS resulted in decreased body weight, lowered cholesterol, and swelling of the appendix. Isolated cases were observed of adverse changes in the kidneys and liver and calcium deposits in the cortex, but these were similar in prevalence and severity to those seen in the control groups. Effects seen in the proximal renal tubules were less severe among scFOS-treated animals than those receiving sucrose. The reduction in body weight gain was regarded as a result of the lower caloric content of scFOS as compared to sucrose and glucose. The researchers concluded that there was no evidence of greater toxicity of scFOS than the sorbitol or sugars used as controls even at concentrations as high as 10% of the diet and the NOAEL in this study was the highest dietary concentration, 10%, equivalent to approximately 7500 mg/kg bw/day.

Tokunaga et al. (1986) studied the safety of repeated-dose intake of scFOS. Groups of n = 6 male Wistar rats weighing 40-50 g were given *ad libitum* access to a control diet containing 67% cornstarch, or one of 3 diets in which cornstarch was replaced by 10% scFOS, 20% scFOS, or 20% glucomannan as a fiber reference group. Although the report is not completely clear, it appears that each group of 6 rats was housed in a single stainless-steel, wire-bottom cage. The rats were kept on their assigned diets for 4-6 weeks with weekly recording of body weight. During the last week, the animals were individually housed in metabolic cages and feed intake was measured, feces were collected, and GI transit time was measured using carmine red as a marker. The rats were starved for the final 18 hours before sacrifice, after which blood was collected and the liver, kidney, small intestine, cecum, and colon were removed, cleaned, and weighed. Blood was analyzed for TC and TAG concentrations and feces were analyzed for neutral and acidic sterols and SCFA.

Feed intake was similar in all 4 feeding groups. There was no difference in weight gain between control rats and those receiving diets containing 10% scFOS, but the diets with 20% scFOS or glucomannan resulted in significantly reduced weight gain. Diet had no effect on the absolute weights of the liver or kidneys, but—as has often been found with nondigestible carbohydrates—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 fiber ingestion were also seen in increased fecal weight and shortened GI transit time; glucomannan was more potent than scFOS in producing these effects.

With regard to serum lipids, ingestion of scFOS or glucomannan resulted in significant dose-dependent decreases in TAG concentration but no effect on cholesterol. Excretion of both neutral and acidic sterols was significantly enhanced by ingestion of fiber, as was that of SCFA, especially acetic and propionic acids.

Ingestion of both scFOS and glucomannan resulted in transient watery stools during the first few days of feeding, but no evidence of toxicity was seen from ingestion of scFOS at dietary concentrations at 10% or 20% (equivalent to approximately 7500 and 15000 mg/kg bw/day), assuming that the reduction in body weight gain seen with 20% scFOS was due to its not being fully utilized as an energy source or to nutritional imbalances resulting from fiber constituting 20% of the feed. The authors did not establish a NOAEL in this study.

5.3.3. Subchronic Oral Toxicity of Other Fructans

Genta et al. (2005) fed dried yacon root flour containing FOS[1] to 3-month-old male and female Wistar rats for 4 months. After 7 days' acclimatization, the rats were randomly assigned to 3 groups of 20 animals (10 male and 10 female) and individually housed with free access to both powdered rodent diet and water. Control rats received the rodent feed alone; test rats also received tablets containing either 340 or 6800 mg FOS/kg bw/day. Feed consumption, body weight, and clinical observations (mortality, general condition, behavior, hair, skin and mucous aspect, abdomen and external genitalia, secretions, autonomic activity, and nervous central activity) were recorded weekly. Yacon doses were adjusted weekly as needed. Blood samples were taken at several time points. A glucose tolerance test was performed on day 90 by depriving half of the animals in each group of feed for 24 hours and then force-feeding them 2 g glucose/kg bw. Blood samples were taken 0, 15, 30, 60, 90, and 120 minutes after the glucose administration.

Hematology outcomes measured included erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, total leukocyte count, and differential leukocyte count. Clinical chemistries included glucose, blood urea nitrogen (BUN), creatinine, TC, TAG, total proteins, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT). Urinalysis outcomes were volume, specific gravity, pH, glucose, protein, ketones, bilirubin, urobilinogen, blood pigments, and presence of sediment. The liver, GI tract, and kidneys were examined in situ, then excised, weighed, and fixed for histological examination.

There was no mortality during the study and no signs of toxicity were apparent during the weekly clinical observations. No signs of adverse GI effects, such as diarrhea or constipation,

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[1] Not further characterized by Genta et al. (2005) but described by Lobo et al. (2007) as containing 55.3% FOS with DP < 10, along with 13.5% fructose, 13.4% sucrose, and 8.9% glucose.

were noted. There were no effects on body weight or body weight gain in either male or female rats, nor were any significant differences observed in feed consumption. The 3 groups did not differ significantly in their responses to the glucose tolerance test in either latency or maximum glucose concentration. No significant 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. The only significant difference was noted in the postprandial state, in which the rats receiving yacon flour exhibited decreased serum TAG, but no differences in cholesterol or glucose. Urinalysis at 2 and 4 months revealed no significant differences among the groups.

The necropsy revealed no treatment-related abnormalities except that both absolute and relative weights of the entire GI tract were increased in both males and females in the high-dose group due to enlargement of the cecum. Histopathological examination showed no effects other than increased cecal hypertrophy and increased size of epithelial cells, but no sign of cell tumefaction, in the high-dose yacon flour group. These effects, commonly observed with large doses of poorly digestible carbohydrates, were regarded as trophic effects of the extremely high dose level (equivalent to about 400 g yacon flour/day for a human) rather than evidence of toxicity. The NOAEL for yacon flour oral toxicity in this study was the highest dose tested, 6800 mg/kg bw/day, equivalent to 3760 mg oligofructose/kg bw/day.

A 13-week study of the oral toxicity of oligofructose was carried out by Boyle et al. (2008) in accordance with the Redbook 2000 guideline IV.C.4.a, Subchronic Toxicity Studies with Rodents (FDA 2007a). Male and female 7-week-old Sprague-Dawley CD® rats (male weights 191-287 g; female weights 155-197 g) were randomly assigned to receive standard rodent chow with 0, 0.55, 1.65, 4.96, or 9.91% oligofructose (20 animals/sex/dose) replacing cornstarch. Feed and water were available *ad libitum* to the individually caged rats. Animals were observed twice daily for mortality and once daily for appearance, motor and behavioral activity, feed intake, and bodyweight. They received detailed physical examinations weekly and ophthalmoscopic examinations prior to study initiation and during weeks 5 and 11. Fecal samples were also collected from 10 males/dose before the study began and during week 12. Blood samples were drawn from 10 rats/sex/dose prior to the study and during weeks 1, 6, and 13. Hematology measures included red blood cell count, reticulocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width, differential, platelet count, white blood cell count, and lymphocyte, monocyte, eosinophil and basophil counts. Clinical chemistry measures were AST, ALT, ALP, glucose, BUN, creatinine, BUN/creatinine ratio, total protein, albumin, total globulin, albumin/globulin ratio, total bilirubin, direct bilirubin, indirect bilirubin, TC, HDL, LDL, LDL/HDL ratio, TAG, sodium, potassium, chloride, calcium, and inorganic phosphorous.

After sacrifice, complete necropsies were performed, including examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities, adrenal glands, aorta, bones and joints (femoral–tibial joint), bone marrow, brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, jejunum, kidneys, liver, lungs, cervical and mesenteric lymph nodes, mammary gland, ovaries, pancreas, parathyroid glands, peripheral nerve (sciatic), pituitary gland, prostate gland, salivary gland, seminal vesicles, skeletal muscle (thigh), skin, cervical and lumber spinal cord, spleen, squamous and glandular stomach, testes,

thymus, thyroid glands, tongue, trachea, urinary bladder, uterus, and vagina. All tissue lesions were examined. The adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, testes, thyroid/parathyroid glands and cecum were weighed. Blood was taken for all hematological and biochemical measures described above as well as coagulation measures, including prothrombin time, activated partial thromboplastin time, and fibrinogen. Fecal samples were analyzed for total bacteria and bifidobacteria.

One high-dose male rat died during the study, apparently due to multifocal prostatitis; this was not regarded as test-article related. Feed intake was significantly lower in rats fed higher levels of oligofructose, especially during the first half of the study. As a result, body weights tended to be lower, reaching statistical significance only for male rats receiving the highest dose. Since the test article was added at a constant fraction of the diet, the intake per kg body weight decreased throughout the study, from a high dose of 5730 mg/kg bw/day during the first week to a high dose of 3630 mg/kg bw/day during the final week for males, and 6130 and 5310 mg/kg bw/day for females. There were no significant clinical observations and no significant test-article-related differences in hematology, biochemistry, or coagulation parameters.

A significant dose-related increase was noted in both absolute and relative cecal weights of both sexes, but there was no associated histopathology. No other significant organ-weight or histopathological differences were noted. Total fecal bacteria populations were slightly but statistically significantly higher in rats fed the two higher doses of oligofructose; bifidobacteria population changes were twice as great and also statistically significant. Based on these data, the authors concluded that the NOAEL of oligofructose in this study was 9.91% dietary concentration, the highest level tested. Although the authors did not calculate the NOAEL in mg/kg bw, the average daily doses of high-dose-group males and females were 4680 and 5720 mg/kg bw/day, respectively, and 4680 mg oligofructose/kg bw/day may be regarded as the NOAEL.

5.3.4. Chronic Oral Toxicity of scFOS

Clevenger et al. (1988) performed a combined 104-week chronic toxicity and carcinogenicity study with 100 4-week-old male and female Fischer 344 rats using scFOS. The animals were acclimatized for 7 days, then randomized into 4 groups of individually caged rats that received scFOS in their diet at concentrations of 0, 8000, 20,000, and 50,000 ppm, equivalent to 0, 341, 854, and 2170 mg/kg bw/day, respectively, for male rats and 0, 419, 1045, and 2664 mg/kg bw/day, respectively, for female rats. Rats were observed twice daily and feed consumption was measured weekly. Body weights were taken weekly for the first 26 weeks and every other week for the remainder of the study. At the conclusion of the study, feed conversion efficiency and scFOS intake were calculated and blood samples were taken. Hematology outcomes were hematocrit, hemoglobin, red-blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, total leukocyte count, and differential leukocyte counts. Clinical chemistry outcomes included sodium, potassium, chloride, calcium, inorganic phosphorus, glucose, BUN, uric acid, creatinine, TC, albumin, total bilirubin, AST, ALT, ALP, and lactate hydrogenase. Brain, adrenal gland, heart, lung, spleen, liver, kidney, testis, and ovary weights were recorded and tissue samples were obtained for histopathological examination.

There was some mortality in all groups of both males and females. (Survival rates in the control and low-, mid-, and high-dose groups were 88%, 72%, 68%, and 84%, respectively, for males and 82%, 74%, 74%, and 88%, respectively, for females.) The higher mortality in the male mid-dose group was not considered treatment-related since no dose–response relationship was evident. Ingestion of scFOS did not affect feed intake, body weight gain, feed conversion efficiency, or absolute organ weights for either males or females. No effect was seen on any hematology outcomes. Blood chemistry results showed slight but significant elevations of sodium and chloride in male rats. Male rats in the mid-dose scFOS group also exhibited slightly elevated levels of blood glucose and creatinine; however, creatinine levels in males in the high-dose group decreased. All other outcomes were not significantly different between test groups and controls. In females, all blood chemistry outcomes were similar to those of 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.

Clevenger et al. (1988) observed that neoplastic lesions (e.g., pheochromocytomas, thyroid C-cell adenomas, leukemias, and pituitary adenomas) often occur spontaneously in F-344 rats. Further, the non-neoplastic lesions observed were common to aging rats of this strain as demonstrated by their historical control incidence. In the scFOS-treated animals, the incidence of rare tumors (those with incidences below 1%) in male or female rats was not increased. The incidence of spontaneous tumors in the scFOS-treated animals was comparable to that of controls, with the exception of pituitary adenomas. In the 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, while 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 in the present study 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. Further statistical analysis was carried out employing generally accepted tests of trend. 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. Based on this analysis it was concluded that higher incidence of pituitary adenomas in males was not treatment related.

The results of this study indicate that scFOS is not carcinogenic and does not produce chronic toxicity in rats. 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.

5.3.5. Developmental and Reproductive Toxicity of scFOS

Henquin (1988; presented in Carabin and Flamm 1999) demonstrated the lack of developmental toxicity of scFOS fed to rats at a 20% dietary concentration. Twelve female Wistar rats with a copulation plug were fed a diet containing 20% scFOS from day 1 to day 21 of gestation while 17 female Wistar rats with a copulation plug were fed a control diet. In the first 6 hours after birth, the litters were numbered, sexed, and weighed. Thirty-six hours after delivery, the newborns were equally distributed (9 neonates/mother) among the lactating mothers, which continued on their gestational diets.

The scFOS had no effect on the number of pregnancies but produced a reduction in body weight gain of the pregnant rats. This may be due to a lower caloric value for scFOS, decreased

intake of food for this group, or diarrhea observed in the first week and softer stools in the second and third weeks. Despite the reduction in body weight gain of the pregnant rats, the fetus and newborn weights were not affected. However, during the nursing period, a growth delay was observed for the male pups in the test group. This may be indicative of the restricted nutritional status of the lactating mothers. The study concluded that a diet containing 20% scFOS has no significant effects on the course of pregnancy in rats and on the development of their fetuses and newborns.

Sleet and Brightwell (1990; presented in Carabin and Flamm 1999) also evaluated possible maternal and developmental toxicity of scFOS using Sprague Dawley rats (strain Crl CD (SD) BR). Four groups of 24 to 27 pregnant females were administered scFOS from day 0 to 6 post coitum at 4.75% dietary concentration. On post-coital day 6, the dietary concentration of scFOS in the 4 groups was changed to 0, 5%, 10%, or 20%. On day 15, all of the animals were placed on an scFOS-free diet. A fifth group received an scFOS-free diet throughout the entire study. On day 20, the rats were sacrificed and their litters examined. About half of the fetuses were dissected and examined fresh prior to skeletal staining. No treatment-related effects were observed during days 0 to 6 or during days 6 to 15. There was no mortality and no diarrhea was observed in any of the test animals. scFOS administered at 4.75% dietary concentration during days 0 to 6 did not affect body weight and body weight change in any of the groups, but, beginning on day 8, body weight was reduced dose-dependently in all 3 scFOS groups relative to controls. The 5% group exhibited a lower weight gain than the controls while the 10 and 20% groups actually lost weight. From day 11 to the end of the study, the body weight and body weight change were similar in all groups except the 20% scFOS group, which remained below controls.

At necropsy, the findings in dams were unremarkable. The number of pups per litter, the sex ratio, and viability of both the embryo and the fetus were not affected by the dietary administration of scFOS. 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 the slightly lowered body weights of the dams, seen only in the 20% scFOS group. It was concluded that scFOS at dietary concentrations up to 20% does not cause adverse effects or adversely affect the pregnancy outcome or *in utero* development of the rat.

5.3.6. Genotoxicity/Mutagenicity of scFOS and Other Fructans

Short-chain FOS was tested for genotoxicity in three assays (Clevenger et al. 1988):

1. Microbial reverse mutation assays (Ames assay) in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 and *Escherichia coli* WP2 *uvr*A, with 0, 50, 150, 500, 1500, or 5000 µg/plate, with and without S9 metabolic activation. The study was OECD-compliant and was replicated with three test plates per strain per treatment condition. No indication of mutagenicity was observed.

2. Mammalian cell mutation assay with mouse lymphoma L5178Y cells. Preliminary toxicity was tested with scFOS concentrations of 50, 100, 250, 1000, 2500, or 5000 µg/ml. Mutagenicity was tested with concentrations of 2000, 3000, 4000, or 5000 µg/ml in the presence

and absence of an Arochlor-induced rat liver microsomal metabolic activation system. No indication of mutagenicity was observed.

3. An OECD compliant assay for the induction of unscheduled DNA synthesis (UDS) in human epithelioid cells (HeLa S3) derived from a human cervical carcinoma. A wide range of test doses was used for each assay (25, 50, 100, 200, 400, 800, 1600, 3200, 6400, 12,800, 25,600, 51,200 µg/ml), with and without metabolic activation. The results indicated no genotoxic potential from the use of scFOS.

The results of the mutagenicity studies demonstrated that, over a wide range of dose levels, the scFOS did not cause gene mutations in bacteria or mammalian cells and did not induce UDS in mammalian cells either in the absence or in the presence of a metabolic activation system.

Boyle et al. (2008) tested the mutagenic potential of oligofructose in a reverse-mutation bacterial assay (Ames test) and the potential clastogenicity in a chromosome aberration test. In the Ames test, dose levels of 100, 333, 1000, 3333, and 5000 µg oligofructose/plate were plated in triplicate with *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 in both the presence and absence of rat liver S9 activation. No positive responses were observed at any level with any strain either with or without S9 activation, and it was concluded that oligofructose is not mutagenic under the conditions tested.

In the chromosome aberration assay, cultures of Chinese hamster ovary cells to concentrations of 313, 625, 1250, 2500, and 5000 µg oligofructose/ml for 4 hours and 20 hours in the absence of S9 metabolic activation and for 4 hours in its presence. No statistically significant increases in structural or numerical chromosome aberrations were observed in either the activated or non-activated groups at any tested dose, indicating an absence of toxicity and clastogenicity.

5.3.7. Toxicity: Conclusions

Several fructans have been subjected to toxicological study, including scFOS, oligofructose contained in yacon root flour, and inulin-derived oligofructose. With the exception of one acute oral toxicity study in mice, all research used the rat as the animal model. In a total of 10 studies of oral toxicity—2 acute, 3 subacute, 2 subchronic, 1 chronic, and 2 developmental—no consistent, statistically significant, dose-dependent effects were reported. NOAELs have invariably been the highest concentration or dose levels. In several repeated-dose studies with high dietary concentrations of fructans—10% or more—animals have shown dose-dependent decrements in weight gain due to poor caloric utilization of the fructans constituting a significant portion of the animals' diets and not indicative of toxicity. Large doses of fructans may result in dose-dependent cecal enlargement, a trophic effect and not evidence of toxicity.

The NOAEL in a subchronic study of oligofructose was 9.91% dietary concentration (4680 mg/kg bw/day) in a study by Boyle et al. (2008), while a 2-year feeding study of scFOS in rats reported by Clevenger et al. (1988) found no evidence of carcinogenicity and a NOAEL of 5% dietary concentration, equivalent to 2170 mg/kg bw/day. No evidence of mutagenicity or clastogenicity was reported in *in vitro* and *in vivo* studies of the genetic toxicity of scFOS and oligofructose.

In summary, the complete body of toxicological research on scFOS and oligofructose demonstrates that the oral toxicity of these substances is extremely low.

Table 3. Toxicity Studies					
Reference	**Study Design & Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Acute Oral Toxicity					
Takeda and Niizato (1982) [acute mouse study]	Acute oral toxicity study of scFOS	48 4-week-old male and female JcL-IcR mice (6 mice/sex/dose)	scFOS	Single gavage doses of 0, 3, 6, or 9 g scFOS/ kg bw	No deaths occurred, there were no differences in body weight gain between the test and control animals, and no abnormalities were seen in either sex in the general state of health. The LD_{50} for oral administration of scFOS to rats in this study was > 9000 mg/kg bw.
Takeda and Niizato (1982) [acute rat study]	Acute oral toxicity study of scFOS	48 6-week-old male and 10-week-old female Sprague Dawley rats (6 rats/sex/ dose)	scFOS	Single gavage doses of 0, 3, 6, or 9 g scFOS/ kg bw	There were no deaths and no abnormalities or changes in body weight were seen in the general state of health of animals of either sex. The LD_{50} for oral administration of scFOS to rats in this study was > 9000 mg/kg bw.

Table 3. Toxicity Studies					
Reference	**Study Design & Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Subacute Oral Toxicity					
Tokunaga et al. (1986)	Study of the safety and metabolic handling of scFOS or gluco-mannan	24 male Wistar rats weighing 40-50 g (6 rats/ dose)	scFOS	0, 10, or 20% dietary concentration for 4-6 weeks	Feed intake was similar in all 4 feeding groups. There was no difference in weight gain between control rats and those receiving diets containing 10% scFOS, but the diets with 20% scFOS or glucomannan resulted in significantly reduced weight gain. Diet had no effect on the absolute weights of the liver or kidneys, but 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 also 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. 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 transient diarrhea during the first few days of feeding, but no evidence of toxicity was seen from ingestion of scFOS at dietary concentrations at 10% or 20% (equivalent to approximately 7500 and 15000 mg/kg bw/day), if the reduction in body weight gain seen with 20% scFOS was due to its not being fully utilized as an energy source. The authors did not indicate a NOAEL.
Takeda and Niizato (1982) [1st sub-acute rat study]	Subacute oral toxicity study of scFOS	72 6-7-week-old male Wistar rats (18 rats/dose)	scFOS	0, 1.5, 3, or 4.5 g/kg bw/ day for 6 weeks	There were no deaths or abnormalities during the study 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 body weight compared to controls. A slight swelling of the appendix was observed in the rats receiving scFOS, while this was 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 in this study was the highest dose tested, 4500 mg/kg bw/day.

Table 3. Toxicity Studies					
Reference	**Study Design & Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Takeda and Niizato (1982) [2nd sub-acute rat study]	Subacute oral toxicity study of scFOS and sorbitol	108 6-7-week-old male Wistar rats (18 rats/treat-ment/dose)	scFOS	5 or 10% dietary concentration for 6 weeks	No deaths occurred and no treatment-related abnormalities were observed. The sorbitol and scFOS groups had lower body weights in the 1st to 5th weeks, but 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. The only treatment-related blood chemistry finding was 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. The latter changes were most marked in the animals receiving sorbitol and sucrose. Isolated cases of Ca deposits were seen in all groups. Overall, oral administration of scFOS resulted in decreased body weight, lowered cholesterol, and swelling of the appendix. Isolated cases were observed of adverse changes in the kidneys and liver and Ca deposits in the cortex, but these were similar in prevalence and severity to those seen in the control groups. Effects seen in the proximal renal tubules were less severe among scFOS-treated animals than those receiving sucrose. The reduction in body weight gain was regarded as a result of the lower caloric content of scFOS as compared to sucrose and glucose. The researchers concluded that there was no evidence of greater toxicity of scFOS than the sugars used as controls even at concentrations as high as 10% of the diet and the NOAEL in this study was the highest dietary concentration, 10%, approximately equivalent to 7500 mg/kg bw/day.

Table 3. Toxicity Studies					
Reference	**Study Design & Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Subchronic Oral Toxicity					
Boyle et al. (2008)	Oral toxicity (feeding) study of oligofructose	Male and female 7-week-old Sprague-Dawley CD® rats (male weights 191-287 g; female weights 155-197 g); 20 rats/sex/dose	oligofructose	0, 0.55, 1.65, 4.96, or 9.91% dietary concentration (high doses = 4680 and 5720 mg/kg bw/day for males & females, respectively) for 13 weeks	Feed intake was significantly lower in rats fed higher levels of oligofructose, especially during the first half of the study. As a result, body weights tended to be lower, reaching statistical significance only for male rats receiving the highest dose. There were no significant clinical observations and no significant test-article-related differences in hematology, biochemistry, or coagulation outcomes. A significant dose-related increase was noted in both absolute and relative cecal weights of both sexes, but there was no associated histopathology. No other significant organ-weight or histopathological differences were noted. Total fecal bacteria populations were slightly but statistically significantly higher in rats fed the two higher doses of oligofructose; bifidobacteria population changes were twice as great and statistically significant. The authors concluded that the NOAEL of oligofructose in this study was 9.91% dietary concentration, the highest level tested, equivalent to 4680 and 5720 mg/kg bw/day in males and females respectively.

Table 3. Toxicity Studies					
Reference	**Study Design & Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Genta et al. (2005)	Subchronic oral toxicity (feeding) of flour from yacon root containing oligofructose	60 3-month-old male and female Wistar rats (10 rats/sex/ dose)	yacon root flour containing 55.3% oligofructose	0, 340, or 6800 mg flour/kg bw/ day providing 0, 188, or 3760 mg oligofructose/ kg bw/day for 4 months	There was no mortality during the study and no signs of toxicity were apparent during the weekly clinical observations. No signs of GI effects, such as diarrhea or constipation, were noted. There were no effects on body weight gain in either male or female rats, nor in feed consumption. The 3 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. The only 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. The necropsy revealed no treatment-related abnormalities except that both absolute and relative weights of the entire GI tract were increased in both sexes in the high-dose groups due to enlargement of the cecum. Histopathological examination showed no effects other than increased cecal hypertrophy and increased size of epithelial cells, but 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 in this study was the highest dose tested, 6800 mg/kg bw/day, equivalent to 3760 mg oligofructose/kg bw/day.

Table 3. Toxicity Studies					
Reference	**Study Design & Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Chronic Oral Toxicity					
Clevenger et al. (1988)	Combined 104-week chronic toxicity and carcino-genicity study of scFOS	100 4-week-old male and female Fischer 344 rats (12-13 rats/ sex/dose)	scFOS	0, 0.8, 2.0%, and 5.0% dietary concentration (equivalent to 0, 341, 854, and 2170 mg/kg bw/day for male rats and 0, 419, 1045, and 2664 mg/kg bw/day for female rats for 2 years	There was some mortality in all groups of both males and females, not considered treatment-related. scFOS did not affect feed intake, body weight gain, feed conversion efficiency, absolute organ weights, or any hematology outcomes. Blood chemistry results showed 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 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 the 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, while 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 in the present study 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. Further statistical analysis was carried out employing generally accepted tests of trend. 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. Based on this analysis it was concluded that higher incidence of pituitary adenomas in males was not treatment related. The results indicate that scFOS is not carcinogenic.

Table 3. Toxicity Studies					
Reference	**Study Design & Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Developmental Toxicity					
Henquin (1988)	Develop-mental toxicity study of oligo-fructose	19 female Wistar rats with copula-tion plugs (12 test, 17 control)	oligofructose	20% dietary concentration from day 1 to day 21 of gestation	Oligofructose had no effect on the number of pregnancies but produced a reduction in body weight gain of the pregnant rats, perhaps due to a lower caloric value for oligofructose, decreased intake of food for this group, or diarrhea observed in the first week and softer stools in the second and third weeks. Despite the reduction in body weight gain of the pregnant rats, the fetus and newborn weights were not affected. 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 study 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)	Study of maternal and develop-mental toxicity of oligofructose	~100 pregnant female Crl CD (SD) BR Sprague Dawley rats (~24 rats/dose)	oligofructose	4.75% dietary concentration from post coitum day 0 to 6, then 0, 5, 10, or 20% dietary concentration to day 15	No treatment-related effects were observed during days 0 to 6 or during days 6 to 15. There was no mortality and no diarrhea was observed in any of the test animals. Oligofructose administered at 4.75% dietary concentration during days 0 to 6 did not affect body weight and body weight change in any of the groups, but, beginning on day 8, body weight was reduced dose-dependently in all 3 oligofructose groups relative to controls. The 5% group exhibited 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 controls. At necropsy, the findings in dams were unremarkable. 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, seen only in the 20% oligofructose-group. It was 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.

6. STUDIES OF INFANT TOLERANCE, GROWTH, AND SAFETY

Studies discussed in this section are summarized in Table 4 at the end of the section.

6.1. Studies of scFOS

In an unpublished randomized, double-blind, placebo-controlled study submitted to Food Standards Australia New Zealand (FSANZ) in support of a proposal to allow the addition of scFOS to infant formula, 63 healthy term infants aged 4-10 weeks were given control formula for 2 weeks, followed by a whey-enriched formula containing 0, 150, or 310 mg scFOS/100 ml formula for 2 weeks (Abbott 1992). The groups as enrolled included 21, 22, and 20 infants, respectively, with a mean age of 43±4 days. Formula intake, growth, stool characteristics, and tolerance were assessed on days 1, 15, and 29. At the end of the feeding period, stool samples were analyzed for selected microbial species (*Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Clostridium*, *Peptostreptococcus*, *Escherichia*, enteric gram-negative rods (GNR) other than *Escherichia*, *Pseudomonas*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, and *Candida* spp.). Urine collected on days 15 and 29 was analyzed for scFOS and ketones, and blood drawn on day 29 was analyzed for the liver enzymes alanine aminotransferase (ALT) and AST, and the scFOS components kestose and nystose.

One infant from the control group, 5 from the low-scFOS group, and 4 from the high-scFOS group failed to complete the study; withdrawal of the single control-group infant, 2 of the low-scFOS infants, and 3 of the high-FOS infants was due to intolerance, while the remainder was attributed to protocol failures. Intolerance withdrawals were based on vomiting or spit-up, diarrhea or watery stools, fussiness, increased stool frequency, or weight loss; there were no differences in reported adverse events among feeding groups.

No significant differences among groups were reported in formula intake, growth, stooling patterns, tolerance, or in any of the outcomes measured in blood or urine. No kestose or nystose was detected in the blood of any infant, but these scFOS trimers and tetramers were recovered from the urine of most of the infants who had received scFOS-containing formula for 2 weeks (GF_2 in 55% and GF_3 in 64%)[1]. The only statistically significant difference in the microbiota was a reduction in *Clostridium* spp. in infants receiving scFOS as compared to the control group. The authors concluded that "Infant formulas containing added FOS at the levels provided … are well tolerated and support normal growth in term infants."

In an unpublished randomized, double-blind, placebo-controlled, multicenter study (Abbott 1993) submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula, 102 healthy term infants aged 1-8 days were randomized to receive formula containing 0 (n = 52) or 300 (n = 50) mg scFOS/100 ml formula for approximately16 weeks, to 112 days of age. Additionally, 25 healthy breast-fed infants aged 0-9 days constituted a human-milk reference group. Length, weight, and head circumference were measured at study entry and during study visits on days 28, 56, 84, and 112, when stool samples were collected for analysis

[1] The authors offered no explanation for the appearance of scFOS residues in urine but not in blood. However, the levels found in urine exceeded the detection limits by only small amounts and, although the analytical methods and limits of detection in the blood analyses were not described, it may be that these limits were higher for the blood analyses than for those in urine and scFOS residue levels simply failed to reach detection limits.

of selected microbial species (*Bifidobacterium*, *Lactobacillus*, *Bacteroides*, and *Clostridium* spp., and *C. difficile*). Parents kept records of formula intake, defecation frequency and stool characteristics, and indicators of tolerance. Stool samples were analyzed for selected microbial species (*Bifidobacterium*, *Lactobacillus*, *Bacteroides*, and *Clostridium* spp., and *C. difficile*). Urine was collected on days 8, 28, 56, 84, and 112 and analyzed for scFOS and ketones, and blood drawn on days 28 and 112 was analyzed for the liver enzymes ALT and AST, the scFOS components kestose and nystose, and TC.

Seventy infants receiving formula completed the study, 34 (65%) of those receiving formula without scFOS and 36 (72%) of those consuming scFOS-containing formula, as well as 23 (92%) human milk-fed infants. Protocol errors were responsible for the loss of 12, 6, and 2 infants from the non-scFOS formula group, the scFOS formula group, and the human milk group, respectively. Six infants were withdrawn from the non-scFOS formula group and 8 from the scFOS group due to adverse events: symptoms of milk intolerance (2 and 4 infants, respectively), diarrhea or watery stools (2 and 1 infants), constipation (2 and 1 infants), and colic or gassiness (1 scFOS-group infant each). Differences among groups were not statistically significant.

There were no differences among groups in measures of weight, length, or head circumference at any time during the study, nor did the formula groups differ in feeding frequency or intake, feedings with spit-up or vomit, stool frequency, or stool consistency, although the human milk-fed infants had significantly softer and more frequent stools than the 2 formula groups. Levels of TC were significantly higher in the human milk group than in either formula group, but levels of AST and ALT were similar in all groups. No blood samples from any infant had detectible scFOS trimers or tetramers, but they were consistently found in urine from infants receiving formula containing scFOS. No urine sample contained detectible ketones.

No differences were seen between the groups in populations of *Bifidobacterium*, *Bacteroides*, or *Clostridium* spp., or *C. difficile*, but counts of *Lactobacillus* spp. were significantly higher among infants receiving the scFOS-supplemented formula than those consuming the formula without scFOS. The authors concluded that "infant formulas containing added FOS at … up to 3 g/L are well tolerated and support normal growth in term infants. The addition of the fermentable fiber at these levels, however, has only small effects on fecal microflora."

Guesry et al. (2000) reported on a randomized double-blind study comparing the effects of 3 concentrations of scFOS in infant formula. Fifty-three 7-20-day-old infants were randomized to receive 5 bottles of formula per day for 2 weeks; each bottle provided either 200 mg lactose or 200, 400, or 600 mg scFOS providing daily intakes of 1.0 g lactose or 1.0, 2.0, or 3.0 g scFOS. The infants were examined and weighed weekly and mothers recorded daily formula consumption, stooling patterns, diaper rash, spitting up, vomiting, or other events. Stool samples were taken as baseline, at the end of feeding, and 2 weeks later for measurement of pH and enumeration of bifidobacteria.

At completion, the number of infants in each group was: 1.0 g/day lactose (13), 1.0 g/day scFOS (11), 2.0 g/day scFOS (11), 3.0 g/day scFOS (12); drop-out rates did not differ by group. There were no differences in fecal pH, bifidobacteria counts, or adverse effects; however, stooling frequency increased dose-dependently with scFOS intake.

The tolerance of healthy term infants to 2 experimental soy-based infant formulas with supplemental scFOS and mixed carotenoids was evaluated in a randomized, double-blind, placebo-controlled, multi-center study (Lasekan et al. 2010). The control formula was Similac® Isomil® Advance® soy formula with 20% of the carbohydrate as sucrose. Experimental soy formula A also included sucrose as 20% of the carbohydrate and also contained mixed carotenoids (lutein, lycopene, and beta-carotene) and 250 mg scFOS/100 ml while experimental formula B had all carbohydrate as corn syrup and also contained mixed carotenoids and 250 mg scFOS/100 ml.

A total of 186 healthy term infants aged 0-8 days were enrolled and received one of the 3 formulas (n = 62 infants/formula) until 35 days of age. Parents kept daily records of formula intake, incidence of spit-up and vomiting at feedings, occurrence of fussiness or gas, and infant's stool characteristics. There were follow-up visits at 14 and 35 days of age, at which growth (weight, length, and head circumference) was measured for comparison to baseline and urine collected by parents was analyzed for specific gravity. A physical examination including hydration status was performed at both follow-up visits and parents were interviewed to identify adverse events, serious adverse events, and the use of medications, supplements, home remedies or other sources of nutrition.

Of the 186 infants meeting eligibility criteria and enrolled, 142 were available at the 14-day follow-up visit and 120 at the 35-day visit. There were no significant differences among formula groups in completion rates, which were 81% for the control formula and 86% and 87% for formulas A and B, respectively. There were no significant differences among groups in formula intake, growth, stool frequency or consistency, feeding-associated spit-up or vomit, urine specific gravity, hydration status, adverse events, or serious adverse events. Two serious adverse events were reported in each formula group, but all were considered not study-related. The authors concluded that, "This study demonstrated that the addition of FOS at 2.5 g/L and mixed carotenoids to soy protein-based formulas, with or without sucrose, was safe and well tolerated in healthy term newborn infants."

In a randomized, double-blind, placebo-controlled, multi-center study, Xia et al. (2012) enrolled 97 healthy term infants aged ≤6 days (mean = 2.3±0.3 days) in a 4-week trial assessing the effects of 4 types of feeding on the intestinal microbiota. Infants in the control group (n = 24) received cow's-milk formula while those in the test groups received the same formula supplemented with either 240 (n = 25) or 340 mg (n = 26) scFOS/100 ml; a human-milk reference group (n = 22) received unsupplemented human milk. Parents maintained diaries of dietary intake, stooling patterns, medication use, and intolerance symptoms. Infants were weighed and medically evaluated at baseline and at the end of weeks 2 and 4; at the latter 2 visits, parents completed questionnaires regarding feeding, stooling patterns, adverse events, and overall satisfaction with the formula. At the end of week 4, a fresh stool sample was collected from each infant, community DNA was isolated, and bacterial groups were quantified based on real-time PCR assays using primers for total bacteria, *Bifidobacterium* spp., *Clostridium difficile, Escherichia coli,* and *Lactobacillus, Bacteroides*, and *Prevotella* spp.

A total of 65 infants completed the study and provided valid fecal samples; the dropouts from each group were as follows:

- Control group: 10 drop-outs, 1 due to parental report of intolerance

- 240 mg scFOS group: 11 drop-outs, 3 due to parental report of intolerance, 2 withdrawn by investigators due to an adverse event (not described, but not test-article related)
- 340 mg scFOS group: 6 drop-outs, 1 due to parental report of intolerance
- Human-milk reference group: 5 drop-outs

No differences were reported among groups in stool frequency or consistency, nor in the frequency of feedings with spit-ups or vomit. The groups did not differ in total bacterial loads. The highest abundance of bifidobacteria was observed in the high-scFOS group, but the variances were large and differences among groups were not statistically significant. Lactobacilli were less prevalent than bifidobacteria and also did not significantly differ among groups; similarly, bacteroides, *E. coli*, and *C. difficile* levels were not significantly different across groups although they tended to be lower in the infants fed human milk than formula-fed infants. The authors concluded that infant formula is similar to human milk in its ability to support bifidobacteria and lactobacilli, but suggested that "future improvement of infant formula should be directed to reduce the abundance of potentially harmful bacteria including *E. coli* and *C. difficile*."

Ripoll et al. (2014) enrolled 75 healthy 4-month-old infants in a 6-month prospective, randomized, double-blind, placebo-controlled, multicenter study (registered at www.clinicaltrials.org) of the effect of supplementation of infant formula with 500 mg scFOS/100 ml formula on growth, digestive tolerance, fecal bifidobacteria count, and fecal specific poliovirus secretory IgA level following vaccination. Infants visited the clinic 4 times—at baseline and at 1, 2, and 6 month follow-ups—and telephone contacts were made at 3, 4, and 5 months. At each interview, parents reported digestive intolerance (regurgitation, vomiting, soft stools, constipation, and crying), any adverse effects, and any infectious events, symptoms, and antibiotic treatment. Stool samples were provided at each clinic visit.

In the initial assignment, 38 infants received the scFOS formula while 37 infants were given control formula containing maltodextrins. While 81% of the infants suffered at least one adverse event, there were no significant differences between groups; the most prevalent adverse events were nasopharyngitis (28%) and bronchitis (12%); few were regarded as feeding-related and these did not differ between groups. Similarly, no differences were observed between groups in the incidence or severity of intolerance symptoms. No differences were seen in growth (weight and height) between the scFOS and control groups. Secretory IgA levels were extremely variable and no significant differences were observed between the 2 feeding groups. A significantly greater number of fecal bifidobacteria was noted in the scFOS group as compared to controls after one month of feeding, but the difference was no longer significant after 2 months.

The authors concluded that, "The overall digestive tolerance of the scFOS supplemented follow-on milk formula is very good and confirms that scFOS can be used safely at 5 g/L in infants older than 4 months."

In a prospective, randomized, double-blind, placebo controlled, multicenter trial, Paineau et al. (2014) assigned 61 healthy term infants aged 0-7 days (mean age = 4.1±0.8 days) to receive formula supplemented with 400 mg/100 ml of either scFOS or maltodextrins to the age of 4 months. Stool samples were collected prior to clinic visits at baseline and at the ages of 2, 3, and 4 months for analysis of bifidobacteria and antipoliovirus IgA; weight and length were also

measured at each clinic visit. Parents maintained diaries on formula consumption, digestive tolerance (assessed by incidence of abdominal pain, diarrhea, and vomiting), and adverse effects.

Formula consumption did not differ between the groups, nor did growth. The most frequent adverse event was abdominal pain, followed by liquid stools, but there was no difference in incidence or severity between the feeding groups. Only one serious adverse event occurred—an episode of bronchitis unrelated to feeding. Fecal bifidobacteria counts were significantly higher among infants receiving scFOS than those receiving maltodextrins, but no significant difference was seen in poliovirus-specific IgA. The authors concluded that, "This study demonstrates that a milk-based infant formula supplemented with scFOS at 4 g/L will increase the faecal content of Bifidobacteria in healthy term infants in comparison to a pacebo formula without inducing any problem of digestive tolerance."

6.2. Studies of Other Fructans

Firmansyah et al. (2000) reported on a randomized, double-blind, placebo-controlled study in which 50 8-month-old Indonesian infants consumed infant cereal with milk supplemented with 0 or 4% oligofructose-enriched inulin under the trade name Prebio1; further information regarding the test substance was not provided. The infants consumed the assigned cereal for 10 weeks, receiving measles vaccine at 4 weeks. Blood was collected for IgG measles antibody measurement before and 6 weeks after vaccination.

Only 1 infant (from the supplement group) failed to complete the study. Post-vaccination levels of IgG antibody were significantly higher among infants receiving the prebiotic and fewer side reactions were observed. There were no effects on health or growth. The authors interpreted these findings as indicating an improved immune response to the vaccination.

In a pair of randomized, double-blind, placebo-controlled trials, Duggan et al. (2003) studied the effect on the prevalence of infant diarrhea of adding oligofructose with or without zinc to infant cereal. The studies were performed in a "shantytown community" near Lima, Peru. In the first trial, which compared unsupplemented cereal with cereal containing 550 mg oligofructose/15 g cereal (3.67%), 282 healthy infants aged 6-12 months (mean age = 8.8 months) were enrolled; this study was powered to detect a 25% reduction in diarrhea. Anthropometric measures were taken at baseline and at 2, 4, and 6 months, the end of the controlled feeding period. Blood samples were taken at baseline and at 5 and 6 months. Fieldworkers visited each family 5 times/week during the first 2 months and twice a week thereafter to measure cereal consumption and question parents about stooling, side effects, and health of the infant. Stool samples were collected if diarrhea was reported.

Each feeding group had 141 infants at baseline; 12 infants from the prebiotic group and 19 from the control group failed to complete the study, none for reasons related to treatment. Daily consumption of cereal did not differ between the groups. There were no significant differences between the groups in the incidence or severity of diarrhea, the frequency with which diarrheal pathogens were isolated, constipation, blood chemistry, respiratory infections, side effects such as vomiting, or growth.

In the second feeding trial (Duggan et al. 2003), 349 enrolled infants aged 6-12 months (mean age = 8.6 months) were assigned to receive control cereal with 1 mg supplementary zinc (n = 175) or cereal with both oligofructose and zinc (n = 174). The study protocol was identical

to that of the first study. As in the first study, the reasons for study withdrawal (10 from the prebiotic group and 21 from the control group) did not differ. Also as in the first study, no differences were seen in any of the measured endpoints.

Moore et al. (2003) studied the GI effects of FOS-supplemented infant cereal in a randomized, double-blind, placebo-controlled, 28-day trial. Fifty-six healthy term infants aged 4-11 months (mean age = 7.7 months) with demonstrated tolerance for rice cereal and milk-based formula were assigned to receive infant cereal with 750 mg of either FOS or maltodextrin/ serving (n = 27 and 29, respectively). The source and chemical description of the FOS were not reported. Parents maintained a daily diary recording cereal intake, stooling pattern and characteristics, flatulence, vomiting, spitting up, crying, and abdominal cramping. Anthropometric measures were taken as baseline and study completion

One infant receiving the prebiotic cereal and 4 receiving the placebo dropped out. The mean daily consumption of FOS was 740 mg/day and was as high as 3000 mg/day. There were no tolerance issues with the prebiotic cereal, and infants receiving FOS had more significantly frequent stools with more regular and softer consistency than those receiving placebo. No serious adverse events were reported, but 17 infants in the experimental group had 24 reported non-serious events as compared to 16 infants with 21 non-serious events in the placebo group. No adverse event was regarded as being related to the intake of FOS. There were no differences between the 2 groups in growth.

Using a randomized, double-blind, parallel-group design, Yap et al. (2005) studied the effects of 3 different levels of supplementation with chicory inulin on mineral absorption and production of SCFA in healthy term infants. Thirty-six healthy infants aged 5-12 months (mean age = 7.7 months) were randomly assigned to receive a control formula or 3 different levels of chicory inulin for 14 days. The dose levels provided 0.75 g, 1.0 g, or 1.25 g inulin/day. Fecal samples were collected prior to dosing, at the end of the 14-day feeding period, and 2 weeks after cessation of feeding and analyzed for SCFA and the minerals calcium, iron, zinc, magnesium, and copper.

An apparent dose-response effect was noted for increasing levels of both lactic acid and SCFA with increasing inulin dose, but the differences did not reach statistical significance. However, a statistically significant dose-dependent decrease in pH was also observed. As with SCFA, calcium absorption tended to increase with increasing inulin doses, but differences were not statistically significant. Effects on absorption of the other minerals, while consistently in the direction of improved absorption with increased inulin intake, were generally less than those for calcium. No adverse events were reported.

Euler at al. (2005) compared the effects of different doses of prebiotic in a randomized, double-blind, crossover study with a non-randomized human milk-fed reference group. The test prebiotic was oligofructose. Eighty-seven healthy term infants aged 2-6 weeks were enrolled. The formula-fed infants were randomized to receive unsupplemented cow's milk-based term infant formula and, during the 1-week crossover feeding period, formula supplemented with 1.5 or 3.0 g oligofructose/L. The study duration was 5 weeks, and each formula-fed infant received control formula for 4 of those weeks and oligofructose-supplemented formula for 1 week at either week 2 or week 4. Parents maintained diaries of formula intake, and stool frequency and characteristics; infants received weekly physical examinations including anthropometric

measures and questioning about any possible adverse effects. Fecal samples were collected weekly for analysis of lactobacilli, bifidobacteria, enterococci, bacteroides, clostridia, and *Clostridium difficile* cytotoxin. If an infant exhibited diarrhea, stool samples were taken and tested for occult blood, leukocytes, rotavirus, enteric adenovirus, *Giardia lamblia*, *Cryptosporidium* spp., and *C. difficile* toxin.

Seventy-two infants (14 human milk-fed, 28 receiving 1.5 g oligofructose/L, and 30 receiving 3.0 g oligofructose/L) completed the study. There were no significant differences among the formula groups in growth as indicated by weight, length, and head circumference. There were no differences in counts of *Lactobacillus* or *Bifidobacterium* spp. among the groups at baseline. Following oligofructose supplementation, *Lactobacillus* spp. colony counts remained similar; however, *Bifidobacterium* spp. counts were significantly higher in the 1.5 g/L oligofructose groups than in the 3.0 g/L oligofructose or human-milk groups. By a week after oligofructose withdrawal, however, the counts had equalized. The formula-fed infants had significantly higher counts of enterococci and bacteroides at baseline than did the human milk-fed infants; by the end of oligofructose supplementation, there were no differences among the 3 groups in bacteroides counts but enterococci remained significantly lower in the human milk-fed infants. However, the baseline bacteroides difference had returned by 7 days after cessation of oligofructose feeding. Clostridia counts were similar at baseline, but 1.5 g/L oligofructose supplementation resulted in a transient significant increase while 3.0 g oligofructose/L produced a transient significant decrease in clostridia.

No human milk-fed infants had detectible *C. difficile* toxin at any time; this toxin was detected in fecal samples from 19% of the formula-fed infants at baseline while it was detected in only 10% of the samples at the end of oligofructose feeding. This reduction, however, was not statistically significant. Most infants (59% of human milk-fed, 83% of those receiving 1.5 g oligofructose/L, and 97% of those receiving 3.0 g oligofructose/L) experienced adverse events—these figures include adverse events experienced during the 4 weeks the formula-fed infants were on control formula as well as the 1 week they were consuming oligofructose-supplemented formula. During the week of oligofructose-supplementation, the formula-fed infants experienced significant increases in the incidence of flatulence and spit-ups; there was a tendency for adverse events to be more frequent in the higher oligofructose-dose group. Stool frequency was greater and consistency was softer for human milk-fed infants compared to formula-fed infants. Stool frequency decreased and consistency changed minimally during 1.5-g/L feeding, while stool frequency and consistency increased during the 3.0-g/L feeding as compared to baseline. The stool consistency of the 3.0-g oligofructose/L group was significantly softer than in the 1.5-g/L group, more closely resembling that of the human milk-fed infants.

With regard to the effect of oligofructose supplementation on stooling patterns and stool consistency, the authors observed:

> "No parents reported overt diarrhea during [oligofructose] supplementation. Parents, however, did report that stools became more frequent and significantly softer, but not loose, during 3.0 g/L [oligofructose] supplementation. This is an important finding because hard stools are a common problem in [formula-fed] infants and softer or loose stools are more typical of the breast-fed infant" (Euler et al. 2005).

Euler et al. (2005) concluded that, "Infant formula supplemented with 1.5 or 3.0 g/L fructooligosaccharides was safe but had minimal effect on fecal flora and *C. difficile* toxin," although they conceded that a feeding period longer than 1 week might result in significant beneficial effects.

In a randomized, prospective, double-blind, placebo-controlled study of the management of infant colic, Campoy et al. (2005) enrolled 106 colicky infants aged 15 days to 4 months (mean age = 42 days). Twenty infants (non-randomized) were breastfed while the remaining 86 infants were randomized to receive either conventional formula (n = 31) or the study formula (n = 55), which consisted of partially hydrolyzed whey protein, low lactose, high β-palmitate, carob seed flour, long-chain polyunsaturated fatty acids (LC-PUFA), and FOS. Further details of the composition of the formula—including the characteristics and concentration of FOS—were not provided. Infants were physically examined at baseline and after 10 and 30 days.

There were no significant differences in growth or any problems with feeding intolerance in any of the 3 groups. The infants receiving the test formula had a significant reduction in colic symptoms (prolonged crying, constipation, regurgitation, and hypertonia) as compared to those receiving breast milk or standard formula.

Bettler et al. (2006) studied the safety of administration of oligofructose and *B. animalis* ssp. *lactis* strain Bl-07 to healthy toddlers aged 12-34 months (mean = 22.1 months). After a 7-day washout period, 318 children were randomly assigned to receive control formula (n = 105), probiotic formula with 2.6×10^8 cfu bifidobacteria/100 ml (n = 108), or synbiotic formula with bifidobacteria and 150 mg oligofructose/100 ml (n = 105) for 28 days, with parental instructions to feed the children 200 to 400 ml formula/day, resulting in a daily intake of oligofructose of 300-600 mg. Stool samples were collected on days 0, 7, 28, and 43, and interviews were scheduled on days 7, 14, 21, and 28 to learn about any tolerance issues or adverse effects, which parents were encouraged to report by means of probing questions.

A total of 170 toddlers (54%) completed the study. Both numbers of and reasons for withdrawal were similar in all feeding groups, and none of the reasons were related to the study formulas. A total of 186 toddlers reported at least one adverse event, but there were no differences between groups in the frequency, severity, or nature of the adverse events. The amount of formula consumed was similar in all groups and the gains in body weight did not differ between the groups. The experimental strain of bifidobacteria, *B. animalis* ssp. *lactis* Bl-07, was not detected in any fecal samples prior to the study, and in 1% of control toddlers and 38% of toddlers in the probiotic and synbiotic groups on day 7. By day 28, the proportion of toddlers with detectible fecal Bl-07 had risen to 41% of those fed the probiotic formula and 50% of those receiving the synbiotic formula. By day 43—2 weeks after feeding cessation—strain Bl-07 was recovered in the feces of only one toddler. However, the groups did not differ significantly in fecal bifidobacteria, streptococci, or bacteroides counts. Relative to control, the probiotic and synbiotic groups had significantly increased counts of lactobacilli at day 7 and a small but statistically significant reduction in clostridia at day 28.

The authors concluded that the control, probiotic, and synbiotic formulas were equally tolerable, equally safe, and equally supportive of normal growth over the 28-day feeding period.

Brunser et al. (2006b) compared the effects on infants' fecal microbiota of a standard milk-based infant formula, the same formula with 200 mg/100 ml of oligofructose, the same formula with10^8 cfu *L. johnsonii* NCC533 (La1)/g powder, or breast feeding. Following a prospective, randomized, double-blind, parallel-group design, 116 healthy term infants were randomized to receive one of the 3 formulas for 13 weeks, followed by a 2-week washout with standard formula, while 26 infants were breastfed throughout the period. Parents maintained a record of formula intake and any adverse effects and returned to the clinic every 15 days for health status evaluation and anthropometric measurements. Fecal samples were collected at baseline and at 7 and 15 weeks for assessment of bacterial populations, including enteropathogens (*Salmonella, Shigella,* and *Campylobacter* spp., and *E. coli*), *L. johnsonii* La1, bifidobacteria, lactobacilli, clostridia, enterococci, enterobacteria, and bacteroides.

Seventy-six formula-fed infants (66% of those enrolled) completed the entire study; primary reasons for withdrawal were failure to follow the protocol, antibiotic use, or illness. Withdrawal rates did not differ across the 3 formula groups and none of the withdrawals was associated with adverse reaction to the formula. All formulas were well tolerated and average formula intake was similar for all 3 groups, resulting in an average intake of oligofructose of 252 mg/kg bw/day and an average intake of *L. johnsonii* La1 of 1.8×10^8 cfu. The number of adverse events per infant did not differ between the 3 formula groups or between the formula-fed and breastfed infants, nor were there any differences in growth measured by gain in weight and length.

At baseline, bifidobacteria counts were significantly higher in the breastfed group than among formula-fed infants, but there were no differences after 7 weeks of feeding or 2 weeks after cessation of pre- or probiotic feeding (Brunser et al. 2006b). Lactobacilli were also significantly more numerous in breastfed than formula-fed infants at baseline, and enterobacteria were less numerous. While the infants receiving probiotic formula showed a transient increase in lactobacilli, enumerations of these bacteria did not generally change significantly in the formula-fed groups. There were no differences between groups or over time in counts of *C. perfringens*, *Bacteroides* spp., or *Enterococcus* spp. No *Salmonella* or *Shigella* spp. were detected, but *E. coli* and *C. jejuni* were found in a small number of samples, with no significant differences among feeding groups.

Brunser et al. (2006b) concluded that the study confirms a predominance of bifido-bacteria in breastfed infants, and that the concentration of oligofructose used in this study (200 mg/100 ml formula) was too small to have a significant effect on the host microbiota.

Using a prospective, randomized, double-blind, multi-center trial, Bettler and Euler (2006) assessed growth and tolerance in healthy full-term infants fed formula supplemented with oligofructose for 12 weeks. The study was conducted at 17 outpatient physician offices and 297 infants were enrolled, including whites, blacks, Hispanic, Asian, and Native American populations. Infants aged 14 days or less who were being fed formula were randomized to receive standard milk-based formula with LC-PUFA (n = 98) or the same formula supplemented with 150 or 300 mg oligofructose/100 ml (n = 98 and 101, respectively). Weight, length, and head circumference were recorded at baseline and every 4 weeks. Adverse events and reports of formula acceptance and tolerance were recorded at these same visits as well as during telephone calls between each visit. Blood was drawn at baseline and termination for analysis of albumin,

blood urea nitrogen, calcium, magnesium, phosphorus, creatinine, TAG, low-density lipoprotein, and TC.

A total of 85 infants discontinued, 32 from the control group, 26 from the 150-mg oligofructose group, and 27 from the 300-mg oligofructose group. Only 12 of these withdrawals were due to adverse events, 5 each from the control and low-dose oligofructose groups and 2 from the high-dose oligofructose group. Overall, there were no significant differences in the number of withdrawals, reason for withdrawal, or length of study participation prior to withdrawal. Mean weight, length, and head circumference and calculated mean *z* scores were not significantly different among the 3 groups based on 2000 reference ranges issued by the Centers for Disease Control and Prevention. There were no differences in the mean values for routine blood chemistries at week 12 and the values were within normal reference ranges. Overall, at least one adverse event was reported for 55% of the infants, but the lowest incidence of formula-related adverse events was in the group receiving the higher dose of oligofructose, 300 mg/100 ml. None of the formula-related adverse events was considered to be serious. There were no differences among groups in formula acceptance and tolerance. The authors concluded that "the experimental cow's milk-based formula supplemented with either 1.5 or 3.0 g oligofructose/L is safe and supports normal infant growth." The formulas were well tolerated, with the 3.0-g/L group having fewer observed adverse events than the control group. The difference among formula groups was significant for all reported events of constipation, with fewer events in the 3.0-g/L group, but this comparison was not significant for constipation events that were considered by the investigators to be formula-related. Of particular importance is the observation that "There were no significant differences in incidence of diarrhea, loose stools, dehydration or allergic reaction among the three groups."

Waligora-Dupriet et al. (2007) reported a randomized, double-blind, placebo-controlled study in which 35 healthy term infants aged 7-19 months were assigned to receive 2 g/day of either oligofructose or maltodextrin for 21 days, followed by 15 days observation. Parents kept daily diaries to record appetite, stooling, symptoms of intolerance, use of medication, and adverse events. Fecal samples were collected at baseline, in the middle and at the end of feeding, and at the end of the observation period and analyzed for staphylococci, enterococci, enterobacteria, lactobacilli, bacteroides, bifidobacteria, and clostridia, as well as the enteropathogens *Campylobacter* and *Salmonella* spp., and *Clostridium difficile*.

Because common infections such as pharyngitis, otitis, and bronchitis and consequent use of antibiotics were frequent, only 10 infants in each group completed the study; all exclusions were due to antibiotic use. Bifidobacteria populations increased while staphylococci and clostridia declined in the oligofructose-supplemented group; the difference from the placebo group was statistically significant for clostridia but not for bifidobacteria or staphylococci. No other bacterial counts differed significantly between the prebiotic and placebo groups, and all differences had disappeared by the end of the observation period. Tolerance manifestations of flatulence, diarrhea, and vomiting were observed significantly less frequently in the oligofructose group than in the placebo group.

In a prospective, randomized, double-blind, crossover study, Kim et al. (2007) studied the effect of supplementation of infant formula with chicory inulin on the gut microbiome, the frequency of defecation, and the pH and consistency of feces. Fourteen healthy term infants

averaging 12.4 weeks of age were randomly assigned to receive control formula for 3 weeks followed by inulin-supplemented formula or the 2 treatments in reverse order. Inulin from chicory roots was added to the experimental formula at a concentration of 1500 mg/100 g powder (~200 mg/100 ml hydrated formula). Hospital nurses recorded defecation frequency and amount and consistency of stools daily. Anthropometric measures were taken and fecal samples were collected at the end of each feeding period.

The mean intake of inulin was 1500 mg/day. No infants were withdrawn from the study, no formula-related adverse effects were observed, and there were no differences in growth between control and inulin feeding periods. Stool characteristics during inulin feeding apparently changed in the expected directions (toward increased frequency, softer stools, and lower pH) but none of the changes reached statistical significance. There were no differences between control and inulin feeding in total anaerobic bacteria or bacteroides, but both bifidobacteria and lactobacilli increased significantly during inulin intake periods. The authors concluded that the addition of native inulin to infant formula elicits a prebiotic response.

Kapiki et al. (2007) enrolled 56 healthy bottle-fed preterm infants in a randomized, double-blind, placebo-controlled study of the bifidogenic effect of infant formula supplemented with chicory-derived FOS. All enrolled infants were less than 14 days old (mean age = 7.0±4/5 days), had gestational ages less than 36 weeks (mean = 33.7±1.6 weeks), and had been admitted to a neonatal unit, but were otherwise healthy. Twenty-four infants were randomly assigned to receive preterm formula with 400 mg maltodextrin (placebo)/100 ml formula while 41 infants were fed similar formula with 400 mg FOS/100 ml formula. The duration of feeding was 14 days. At baseline, the infants were measured anthropometrically and a stool sample was taken for bacterial analysis. During the study a diary was maintained of formula intake, stool frequency and characteristics, and any side effects. Additional stool samples were taken after 7 and 14 days.

Nine infants failed to complete the study, 5 from the FOS group and 4 from the placebo group, for reasons not related to the study. Over the full 14 days, infants in the placebo group gained significantly more weight and had significantly greater arm circumference, while those in the FOS group gained non-significantly greater length. Both formulas were well tolerated. Intake of the FOS-supplemented formula produced a significantly higher frequency of defecation and softer stools as well as significantly greater concentrations of fecal bifidobacteria and bacteroides and significantly lower numbers of *E. coli* and enterococci. The authors stated that "All infants tolerated well the two formulae," although the evidence supporting this claim was not described. The authors concluded, "We have documented that the addition of a small quantity of FOS in the normal diet of preterm infants was well tolerated and resulted in a rapid increase in the numbers of bifidobacteria and the proportion of infants colonized by bifidobacteria."

Bettler and Kullen (2007) reported on a randomized, double-blind, multi-center parallel 8-week trial with healthy term infants aged 5-14 days (mean age = 7.6±2.6 days) in which formula-fed infants were randomized to receive α-lactalbumin-enriched milk-based formula either with 300 mg/100 ml oligofructose (n = 47) or unsupplemented (n = 48). Human milk-fed infants (n= 50) were not randomized into a treatment group and continued to be breastfed throughout the study. Stool samples were collected at baseline and at week 8 and analyzed for fecal concentrations of bifidobacteria, clostridia, lactobacilli and bacteroides. The primary

endpoint was comparison of fecal bifidobacteria concentrations among the feeding groups while a secondary endpoint was fecal concentrations of lactobacilli, bacteroides, and clostridia.

Eighty-four infants (58%) completed the study. The number of infants who discontinued and the reasons for discontinuation were similar among treatment groups. Fecal bifidobacteria concentrations for both formula groups were statistically equivalent to the breastfed group at 8 weeks with all 3 groups showing a significant increase in bifidobacteria from baseline. There was no significant difference among the three treatment groups in fecal counts of bacteroides, clostridia, or lactobacilli. In conclusion, the formula-fed groups had similar fecal bacteria concentrations to the breastfed reference group after 8 weeks of feeding.

In a prospective, randomized, double-blind, placebo-controlled study, Panigrahi et al. (2008) investigated long-term colonization of *L. plantarum* when administered to breastfed neonates along with FOS. Thirty-one healthy term infants aged 1-3 days were enrolled and randomized to receive daily oral administration via syringe of a combination of 150 mg FOS and 10^9 cfu *L. plantarum* (n = 19) or a dextrose saline placebo (n = 12) for 7 days. No information was provided regarding the source or chemical characteristics of the FOS. The infants were weighed on days 0, 7, and 28 and monthly to 6 months, and stool samples were collected on days 0, 3, 7, 14, 21, 28, and monthly to age 6 months. They were monitored for bowel habits and any adverse effects.

L. plantarum was cultured from 84% of the infants in the synbiotic group on day 3 and from 95% on day 28; 100%, 88%, 56%, and 32% remained colonized at months 2, 4, 5, and 6, respectively. *L. plantarum* was not detected in the feces of any infants in the placebo group. The infants receiving the synbiotic exhibited a significantly greater number and variety of bacterial species than did the control infants. No serious adverse events were reported in either group, and there were no differences in non-serious events.

Yap et al. (2008) enrolled 36 healthy formula-fed term infants aged 5-12 months (mean age = 7.7 months) in a prospective, randomized, double-blind, placebo-controlled trial of the effects of inulin supplementation on fecal characteristics and microbial composition. Infants all received the control diet consisting of follow-on infant formula, baby food, and biscuits along with Frutafit® chicory-derived inulin to be added to the formula at levels needed to provide daily intakes of 0 (n = 8), 750 mg (n = 10), 1000 mg (n = 9), or 1250 mg (n = 9) for 14 days. Parents maintained a diary of feeding, stooling, and any symptoms of intolerance. Stool samples were collected at baseline, at the end of feeding, and 2 weeks after cessation of feeding.

Inulin supplementation decreased fecal pH dose-dependently, but effects on defecation frequency or fecal consistency were not significant. Total fecal anaerobe populations were significantly decreased in the high-inulin-dose group as compared with the other groups, but this difference disappeared after feeding ceased. All levels of inulin supplementation significantly decreased numbers of clostridia and the 2 higher doses resulted in significant reductions in Gram+ cocci and total coliforms. No effect was seen in lactobacilli, but bifidobacteria increased dose-dependently with inulin supplementation, reaching statistical significance compared to controls only at the high dose. There were no reports of adverse events.

In a prospective, randomized, double-blind, placebo-controlled study of combinations of probiotics and prebiotic ingredients, Underwood et al. (2009) assessed effects on weight gain and

fecal SCFA and microbiota of preterm infants. Ninety healthy infants with gestational age at birth <35 weeks were randomized during their first week of life to receive one of 3 twice-daily supplements for 28 days. One supplement contained $5x10^8$ cfu *L. rhamnosus* strain GG + inulin (n = 30), the second contained $5x10^8$ cfu each of *L. acidophilus, B. longum, B. bifidum,* and *B. infantis* + inulin (n = 31), and the third (placebo) contained only the formula Pregestamil (n = 29). Neither the chemical characteristics nor the quality of inulin were reported. The infants' weight, length, and head circumference were measured and stool samples were obtained weekly.

The only infants withdrawn from the study were ones transferred out of the ICU, none for study-related reasons. There were no differences in growth. All formulas were well tolerated; symptoms such as emesis, gastric distention, or excessive gastric residuals were minor. There were no significant differences between groups in fecal SCFA. The only difference in fecal bacteria was a significant increase in numbers of bifidobacteria among premature infants receiving the multi-strain probiotic+prebiotic formula.

Yao et al. (2010) studied the effects of infant-formula composition on stool characteristics and composition. In a randomized, double-blind, parallel-group study, 300 healthy term infants aged 7-14 days were assigned to one of 4 α-lactalbumin-enriched formulas for 8 weeks: standard term infant formula, formula with 40% of the palmitate in the sn-2 position, formula with high sn-2 and 3.0 g oligofructose/L, or formula with high sn-2 and 5.0 g oligofructose/L. An additional 75 infants served as a human milk-fed reference group. Tolerance was assessed via a parental questionnaire and physician-reported study events. The primary outcome measure was stool soap and mineral content at week 8; secondary outcome measures included stool characteristics and GI tolerance.

The infants receiving the high sn-2 formula, with or without oligofructose, had significantly less stool palmitate soaps, softer stools, and higher bifidobacteria counts than infants receiving the control formula, resembling the human-milk reference group; there was no difference in stool frequency. Addition of oligofructose to the high sn-2 formula was associated with further decreases in the number of formed stools and in stool calcium as compared to the control group.

Only 6 of the 375 enrolled infants (1.6%) failed to complete the study. Parental responses to the questionnaire indicated no increase in the incidence of watery stools, gassiness, vomiting, fussiness, or other symptoms of intolerance with the addition of up to 5.0 g oligofructose/L to the formula. The authors concluded that "addition of oligofructose further improved stool consistency without physician or parent reported concerns."

Lugonja et al. (2010) conducted a non-randomized, non-blinded, non-placebo-controlled study comparing the bifidogenic effects of breast milk and prebiotic-supplemented infant formula. Twenty-one healthy infants aged 5 to 16 weeks (mean = 8.6 weeks) were enrolled; 10 infants (7 boys and 3 girls) were breastfed while 11 infants (6 boys and 5 girls) received formula containing 400 mg/100 ml of a blend of inulin and oligofructose derived from chicory; the fructans were not further described. The trial had a duration of 28 days, during which daily measures were taken of weight, length, number of feeds, any indications of intolerance (GI symptoms, flatus, regurgitation, loss of appetite), frequency of stooling, and stool consistency (soft, normal, or hard). At baseline and on days 14 and 28, stool samples were collected and

analyzed for pH, organic acids, and numbers of bifidobacteria, lactobacilli, total aerobes, total anaerobes, and fungi/yeasts.

All infants in both groups grew at normal rates and there was no difference between groups. There were no significant differences between groups in measures of intolerance, stool frequency, or stool consistency, although there was a tendency for stooling to be more frequent in the formula group. The number of daily feeds was significantly higher in the breastfed group. Counts of bifidobacteria increased significantly over the 28 days in both groups, but the rate of increase and the final numbers were significantly greater among infants receiving prebiotic-supplemented formula than those who were breastfed. Lactobacilli increased in both groups while aerobes, anaerobes, and fungi and yeasts decreased, but there were no significant differences between the formula and breastfed groups. Total organic acids increased and pH decreased over time in both groups, but the changes were significantly greater among infants receiving prebiotic-supplemented formula.

Most stools from infants in both groups (73% of the formula group and 60% of the breastfed group) were regarded as of normal consistency; the remainder—27% of the formula-fed and 40% of the breastfed infants—were judged to be soft. The mean water content of the stools of infants receiving formula containing inulin+oligofructose was 77.9% at 28 days, non-significantly lower than the mean water content of breastfed infants' stools (81.2%). Thus, there was no indication of greater water loss among infants receiving prebiotic formula than those receiving breast milk.

Veereman-Wauters et al. (2011) investigated the effect of supplementation of infant-formula with a 1:1 blend of oligofructose and long-chain inulin on infants' intestinal microbiota. Eighty-one healthy neonates less than 5 days of age were enrolled in a 28-day prospective, randomized, double-blind, placebo-controlled, multi-center study on tolerance and bifidogenic effect of supplemented formula. Infants were randomly assigned to one of 4 feeding groups: powdered unsupplemented standard formula (n = 21) or the same formula supplemented with 400 mg oligofructose-enriched inulin/100 ml as prepared (n = 21), 800 mg oligofructose-enriched inulin/100 ml (n = 20), or 800 mg 90:10 GOS+long-chain inulin/100 ml (n = 19). Additionally, a non-randomized group of 29 breastfed infants was included as a reference group.

Parents maintained a diary of feeding, stooling frequency and consistency, any intolerance symptoms such as crying, regurgitation, or vomiting, and any side effects. Anthropometric measures were taken at baseline and at the end of 2 and 4 weeks while stool samples were collected for microbiological evaluation on days 3, 14, and 28.

A total of 34 infants did not complete the study—6 each from the control and reference groups, 7 each from the 2 oligofructose-enriched inulin groups, and 8 from the GOS+long-chain inulin group. Neither drop-out rates nor reasons for drop-out differed significantly between groups. All of the infants exhibited significant growth over the 4 weeks of the study, with no significant differences between groups in weight, length, or head circumference. The 4 formula-fed groups also showed no differences in food intake (which was not measured in the breastfed reference group). The breastfed reference infants exhibited a significantly higher frequency of defecation than did the 4 formula-fed groups, which did not differ significantly from each other. With regard to stool consistency, the breastfed infants' stools were significantly softer than the formula-fed infants', and the 3 groups receiving prebiotic-supplemented formula had

significantly softer stools than did those receiving the control formula; the 3 prebiotic-supplemented groups did not differ from each other. While there was a tendency for the frequency of crying to increase over time, there were no significant differences in crying frequency in any week among the 4 feeding groups. The other 2 markers of intolerance, regurgitation and vomiting, showed no significant trends over time and no significant differences among feeding groups. No serious adverse events were observed, and there were no significant differences between groups in non-serious events.

The 3 prebiotic groups, but not the control group, showed significant increases in total fecal bacteria counts over time, but there were no significant differences between groups. There were nonsignificant trends for increases in bacteroides, clostridia, and lactobacilli over time in all groups, with no differences among the groups. While bifidobacteria increased significantly over time in all groups, the increase was slower in the control group not receiving prebiotics. By day 14, the fecal bifidobacteria counts for the infants receiving 800 mg/100 ml of either oligofructose-enriched inulin or GOS+long-chain inulin were significantly higher than the controls or those receiving 400 mg oligofructose-enriched inulin/100 ml.

In summary, the 3 prebiotic formulas studied by Veereman-Wauters et al. (2011) and the control did not differ in tolerance measures, amount of formula consumed, or resulting growth. Consumption of the prebiotic formulas produced softer stools, and consumption of formulas containing 800 mg prebiotic (either oligofructose-enriched inulin or GOS+long-chain inulin)/ 100 ml resulted in significant increases in the prevalence of bifidobacteria.

6.3. Reviews of Infant Feeding of Prebiotic Ingredients

Two comprehensive systematic reviews of randomized controlled trials of infants were undertaken by a group in Western Australia and published in 2009—one review (Srinivasjois et al. 2009) focused on preterm neonates while the other (Rao et al. 2009) focused on full-term neonates. In both reviews, trials were included only if they included adequate randomization, were fully controlled, used unsupplemented vs. prebiotic-supplemented milk-based formula, did not employ combinations of pre- and probiotics, commenced within the first 28 days of life, and had a duration of at least 2 weeks.

In the assessment of studies of preterm neonates (Srinivasjois et al. 2009), only trials of neonates with gestation ≤ 37 weeks at birth and ≤ 40 weeks at randomization were included. From an initial sample of 55 potentially relevant published articles, only 4 randomized controlled trials met the inclusion criteria: Boehm et al. (2002), Mihatsch et al. (2006), Indrio et al. (2007), and Kapiki et al. (2007), all of which were discussed in the previous sections. Srinivasjois et al. (2009) concluded that "prebiotic oligosaccharide supplementation (GOS and/or FOS [sic]) is well tolerated by preterm infants and results in higher stool colony counts of bifidobacteria, reduced growth of pathogenic bacteria, accelerated GI transit time, softer and acidic stools similar to those of breastfed infants without adversely affecting the weight gain."

The Rao et al. (2009) review of studies of full-term neonates began with the same 55 articles as provided the starting point for the preterm-infant review, of which 13 articles

reporting on 11 clinical trials were deemed eligible for inclusion[1]. Three of these studies did not involve fructans (2 were of GOS only and 1 was of GOS+lactulose+polydextrose); the remaining 8 studies were discussed in the previous sections. Only 1 study (Bettler and Euler 2006) included supplementation with oligofructose alone; the remaining studies were of GOS+long-term inulin blends, 1 with the further addition of acidic oligosaccharides from pectin. Nevertheless, Rao et al. (2009) reported consistent findings, particularly with respect to bifidogenesis. They found that, in term neonates, prebiotic supplementation of formula consistently resulted in higher stool colony counts of bifidobacteria, irrespective of the heterogeneity among studies with regard to the specific prebiotic tested, dosage, or duration of supplementation. In addition, stools in the supplemented groups had higher lactobacilli counts, lower pathogenic bacteria counts, and more acidic pH and were softer and more frequent, similar to those of breastfed neonates. No study indicated any adverse effect on growth or any other adverse effect. With the sole exception of the GOS+lactulose+polydextrose formula, which has no relevance for the evaluation of fructans, prebiotic supplementation was well tolerated.

The authors concluded that "our results show that prebiotic supplementation of formula milk in full-term neonates is well tolerated and results in various short-term beneficial effects, including increased stool colony counts of bifidobacteria and lactobacilli, decreased counts of pathogenic enteric bacteria, more acidic stools, and softer and frequent stools, without adversely affecting weight gain."

Additionally, a "global prebiotic summit meeting" was held in the summer of 2008 to review the potential roles and clinical utility of prebiotics in newborns, infants, and children (Sherman et al. 2009). After an extensive review of the published literature, including clinical studies in both preterm and term infants, Sherman et al.'s (2009) review of the meeting concluded that, despite "theoretical concerns" discussed by the group, a search of the biomedical literature does not provide consistent evidence of adverse effects. The group noted that, "The relevance of reported outcome measures, however, still is not clear, at least for decreasing disease and promoting health. In addition, a number of questions remain about the mechanisms underlying the potential benefits of prebiotics." Nevertheless, "Most studies indicate that, when taken in sufficient amounts, prebiotics soften stools, increase stool frequency (without episodes of diarrhea), and increase the ratio of bifidobacteria to total fecal bacteria. In infants receiving prebiotic-supplemented formula, water balance remains normal and, in most studies, the infants continue to grow appropriately."

6.4. Infant Tolerance, Growth, and Safety: Conclusions

The totality of the extensive body of research discussed in Sections 6.1 and 6.2, which includes reports of open-label studies and other research not included in Srinivasjois et al. (2009) or Rao et al. (2009), clearly supports the conclusions reached by these reviewers that prebiotic supplementation of formula intended for consumption by preterm and term infants is safe and

[1] Alliet et al. (2007), Bakker-Zieriksee (2005a, 2005b, 2006), Ben et al. (2004), Bettler and Euler (2006), Costalos et al. (2008), Decsi et al. (2005), Fanaro et al. (2005), Moro et al. (2002, 2003, 2006), and Ziegler et al. (2007).

has physiological effects that bring the performance of formula-fed infants closer to that of breastfed infants.

Twenty-seven studies enrolling 3,163 infants have appeared in the literature in which infants were given formula or infant food supplemented with fructans[1]. Seven of these studies, including 637 infants, assessed the effects of ingestion of scFOS; the test articles in remaining studies were oligofructose, inulin, and blends of intact and hydrolyzed inulin. Study durations varied from 1 week to 6 months with a median duration of 1 month. Addition levels ranged from 150 to 800 mg/100 ml formula, with a median addition level of 300 mg prebiotic/100 ml formula.

The results of theses studies have consistently demonstrated that prebiotic addition to infant formula intended for consumption by either preterm or term infants is safe, with no indications of serious adverse effects or reduction in growth. In no study were any serious adverse events associated with ingestion of the added prebiotic, and the less serious symptoms such as flatulence or fussiness were generally reported with approximately equal frequency in both prebiotic and placebo groups. Fifteen studies measured growth and none found any difference between prebiotic and placebo groups.

The beneficial effects of prebiotic supplementation were less consistently demonstrated than was the safety of the supplementation, possibly because of the great variability in both the test article and the addition level across studies. Fecal bifidobacteria were enumerated in 15 studies and lactobacilli in 5; significant increases in both genera were seen in the majority of studies, but not in all. Little effect was generally observed with regard to other colonic bacteria. Similarly, defecation frequency increased significantly in 5 of the 10 studies that recorded this endpoint, but not in the others, and stools were significantly softer in 5 of the 7 studies that included assessment of stool consistency, bringing the performance of formula-fed infants into closer resemblance to that of human milk-fed infants. Fecal pH, however, was significantly lowered in all 5 studies that measured acidity.

In summary, 27 studies have been conducted in which fructans have been administered to infants for periods as long as six months (in addition to the many studies of GOS-fructan blends). While there is some variability in the effects seen on stooling, bifidobacteria, and other colonic bacteria, there has been complete consistency in the absence of prebiotic-associated adverse effects and in the ability of the prebiotic-containing formula to support normal growth of both preterm and term infants. It may be concluded from this body of literature that the addition of scFOS and other fructans to infant formula has been shown to be without likelihood of harm.

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[1] This is in addition to a large number of studies of infant formulas supplemented with a blend of GOS and long-chain inulin, a body of literature that is not reviewed in this document.

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

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Abbott (1993) [unpublished; submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula	Randomized, double-blind, placebo-controlled, multicenter study of the safety and bifidogenic effect of scFOS in infant formula	102 healthy term infants aged 1-8 days (and 25 healthy breast-fed infants aged 0-9 days as a human-milk reference group)	scFOS	0 or 3 g/L formula for about 16 weeks (to 112 days of age)	Six infants were withdrawn from the non-scFOS formula group and 8 from the scFOS group due to adverse events: symptoms of milk intolerance (2 and 4 infants, respectively), diarrhea or watery stools (2 and 1 infants), constipation (2 and 1 infants), and colic or gassiness (1 scFOS-group infant each). Differences between groups were not statistically significant. There were no differences between groups in measures of weight, length, head circumference, feeding frequency or intake, feedings with spit-up or vomit, stool frequency, or stool consistency, although the human-milk-fed infants had significantly softer and more frequent stools than the 2 formula groups. Levels of AST and ALT were similar in all groups. No blood samples from any infant had detectible scFOS trimers or tetramers. No urine sample contained detectible ketones. No differences were seen between the groups in populations of *Bifidobacteria, Bacteroides,* or *Clostridia* spp., or *C. difficile*, but counts of *Lactobacillus* spp. were significantly higher among infants receiving the scFOS-supplemented formula. The authors concluded that "infant formulas containing added FOS at … up to 3 g/L are well tolerated and support normal growth in term infants."
Guesry et al. (2000)	Prospective, randomized double-blind study comparing the effects of 3 concentration levels of scFOS in infant formula	53 infants aged 7-20 days	scFOS	200, 400, or 600 mg/day for 2 weeks	Drop-out rates did not differ by group. Stooling frequency increased dose-dependently with scFOS intake. There were no differences in fecal pH, bifidobacteria counts, or adverse effects.

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Lasekan et al. (2010)	Randomized, double-blind, placebo-controlled, multi-center study of tolerance to soy-based infant formulas with scFOS and mixed carotenoids	186 healthy term infants aged 0-8 days	scFOS	0 or 2.5 g/L formula until 35 days of age	There were no significant differences between formula groups in completion rates, formula intake, growth, stool frequency or consistency, feeding-associated spit-up or vomit, urine specific gravity, hydration status, adverse events, or serious adverse events. Two serious adverse events were reported in each formula group, but all were considered not study related. The authors concluded that, "This study demonstrated that the addition of FOS at 2.5 g/L and mixed carotenoids to soy protein-based formulas, with or without sucrose, was safe and well tolerated in healthy term newborn infants."
Paineau et al. (2014)	Prospective, randomized, double-blind, placebo controlled, multicenter trial of effect of scFOS on bifidogenesis and antipoliovirus IgA	61 healthy term infants aged 0-7 days (mean age = 4.1±0.8 days)	scFOS	4 g/L to age 4 months	Formula consumption and growth did not differ between the group receiving scFOS and a control group that received maltodextrin. There was no difference in incidence or severity of adverse effects between groups. Fecal bifidobacteria counts were significantly higher among infants receiving scFOS than those receiving maltodextrins, but no significant difference was seen in poliovirus-specific IgA. The authors concluded that, "This study demonstrates that a milk-based infant formula supplemented with scFOS at 4 g/L will increase the faecal content of Bifidobacteria in healthy term infants in comparison to a placebo formula without inducing any problem of digestive tolerance."

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Ripoll et al. (2014); unpublished	Prospective, randomized, double-blind, placebo-controlled, multicenter study of the effect of scFOS on growth, digestive tolerance, fecal bifidobacteria count, and specific poliovirus secretory IgA	75 healthy 4-month-old infants	scFOS	5 g/L for 6 months	81% of the infants suffered adverse events, but there were no significant differences between groups receiving scFOS or maltodextrin placebo; few were regarded as feeding-related and these did not differ between groups. No differences were observed between groups in the incidence or severity of intolerance symptoms, growth (weight and height), or secretory IgA levels. A significantly greater number of fecal bifidobacteria was noted in the scFOS group as compared to controls after one month of feeding, but the difference was no longer significant after 2 months. The authors concluded that, "The overall digestive tolerance of the scFOS supplemented follow-on milk formula is very good and confirms that scFOS can be used safely at 5 g/L in infants older than 4 months."
Xia et al. (2012)	Randomized, double-blind, placebo-controlled, multi-center study of the effects of feeding on the intestinal microbiota	97 healthy term infants aged ≤6 days (mean = 2.3±0.3 days)	scFOS	0, 2.4, or 3.4 g/L formula for 4 weeks	Dropouts from each group were: Control group—10 drop-outs, 1 due to parental report of intolerance; 2.4-g scFOS group—11 drop-outs, 3 due to parental report of intolerance, 2 withdrawn by investigators due to non-test-article related adverse events; 3.4 g scFOS group—6 drop-outs, 1 due to parental report of intolerance. No differences were reported among groups in stool frequency or consistency, frequency of feedings with spit-ups or vomit, or total bacterial loads. The highest abundance of bifidobacteria was in the high-scFOS group, but differences among groups were not significant. Lactobacilli, bacteroides, *E. coli*, and *C. difficile* levels were not significantly different across groups. The authors concluded that infant formula is similar to human milk in its ability to support bifidobacteria and lactobacilli, but suggested that "future improvement of infant formula should be directed to reduce the abundance of potentially harmful bacteria including *E. coli* and *C. difficile*."

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Other Fructans					
Bettler and Euler (2006)	Prospective, randomized, double-blind, parallel-group, multi-center trial to assess the effect of oligofructose on growth and tolerance in healthy full-term infants	297 healthy term infants aged 14 days or less who were being fed formula	Oligofructose from chicory	0, 1.5, or 3 g/L formula for 12 weeks	12 withdrawals were due to adverse events, 5 each from the control and low-dose oligofructose groups and 2 from the high-dose oligofructose group. There were no significant differences in the number of withdrawals, reason for withdrawal, or length of study participation prior to withdrawal. Mean growth in all groups was within CDC reference ranges and did not differ from each other. Adverse events were reported for 55% of the infants; the lowest incidence was in the group receiving the higher dose of oligofructose. None of the formula-related adverse events was considered to be serious. There were no differences among the groups in formula acceptance and tolerance. The authors concluded that "the experimental cow's milk-based formula supplemented with either 1.5 or 3.0 g FOS [sic]/L is safe and supports normal infant growth."
Bettler et al. (2006)	Prospective, randomized, double-blind, parallel-group trial to assess the safety of addition of oligofructose and *B. animalis* ssp. *lactis* strain BI-07 in formula for healthy toddlers	318 healthy toddlers aged 12-34 months (mean = 22.1 months)	Oligofructose from chicory and *B. animalis* ssp. *lactis* strain BI-07	0 or 1.5 g/L formula for 28 days	Both numbers of and reasons for withdrawal were similar in all feeding groups, none related to the study formulas. The amount of formula consumed was similar in all groups and gains in body weight did not differ between groups. By day 28, the proportion of toddlers with detectible fecal BI-07 had risen to 41% of those fed the probiotic formula and 50% of those receiving the synbiotic formula. By 2 weeks after feeding cessation, strain BI-07 was recovered in the feces of only 1 toddler. However, the groups did not differ significantly in fecal bifidobacteria, streptococci, or bacteroides counts. Relative to control, the probiotic and synbiotic groups had significantly increased counts of lactobacilli at day 7 and a small but statistically significant reduction in clostridia at day 28. 186 toddlers reported at least one adverse event, but there were no differences between groups in the frequency, severity, or nature of the adverse events.

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Bettler and Kullen (2007)	Prospective, randomized, double-blind, placebo-controlled, multi-center trial of oligofructose effects on bifidogenesis, the incidence of adverse events, normal growth, fecal concentra-tions of other bacteria, level of fecal calprotectin, stool frequency and consistency, and anti-rotaviral activity of feces.	145 healthy term infants aged 1-13 days	Oligofructose from chicory	0 or 3 g/L formula for 8 weeks	Differences in the incidence of and reasons for withdrawal and numbers of reported adverse events and serious adverse events were not significant between the groups. Treatment-related adverse events were significantly less prevalent in the breastfed reference group than in the formula-fed groups, but the 2 formula-fed groups did not differ from each other. There were no differences in intake of formula between those receiving oligofructose-supplemented formula and the control group. No difference was observed in bifidobacterial numbers between the 2 formula groups or between the formula groups and breastfed reference group at any time during the study, although all 3 groups showed increases in bifidobacteria from baseline to study completion. The groups did not differ in numbers of bacteroides, but clostridia were consistently more numerous in the feces of the formula-fed infants than the breastfed reference group until week 8, when there was no difference; the 2 formula groups did not differ from each other. Lactobacilli were higher in the oligofructose-fed group than in the control-formula group, which in turn had significantly higher counts than did the breastfed infants, at all time points, including baseline. There were no differences between the groups in changes in numbers of clostridia or lactobacilli over time. Similarly, no differences were seen between groups in fecal calprotectin concentration of anti-rotaviral activity. The breastfed infants had significantly higher frequency of defecation and significantly softer stools than did the formula-fed infants, who did not differ significantly from each other. There were no differences between groups in body weight at any visit, nor in hydration status.

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Brunser et al. (2006b)	Prospective, randomized, double-blind, parallel-group study of the effects of oligofructose or *L. johnsonii* NCC533 (La1) on infants' fecal microbiota	116 healthy term infants	Oligofructose from chicory	0 or 2 g/L formula for 13 weeks	Withdrawal rates did not differ across the 3 formula groups (oligofructose, *L. johnsonii*, and control groups) and none of the withdrawals was associated with adverse reaction to the formula. At baseline, bifidobacteria counts were significantly higher in the breastfed group than among formula-fed infants, but there were no differences after 7 weeks of feeding or 2 weeks after cessation of pre- or probiotic feeding. Lactobacilli were also significantly more numerous in breastfed than formula-fed infants at baseline, and enterobacteria were less numerous. While the infants receiving probiotic formula showed a transient increase in lactobacilli, enumerations of these bacteria did not generally change significantly in the formula-fed groups. There were no differences between groups or over time in counts of *C. perfringens, Bacteroides* spp., or *Enterococcus* spp. No *Salmonella* or *Shigella* spp. were isolated, but *E. coli* and *Campylobacter jejuni* were found in a small number of samples, with no significant differences among feeding groups. The authors oncluded that the study confirms a predominance of bifidobacteria in breastfed infants, and that the concentration of oligofructose used in this study had little effect on the host microbiota. All formulas were well tolerated and average formula intake was similar for all 3 groups. The number of adverse events per infant did not differ among the 3 formula groups or between the formula-fed and breastfed infants, nor were there any differences in growth measured by gain in weight and length.

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Campoy et al. (2005)	Prospective, randomized, prospective, double-blind, placebo-controlled study of the management of infant colic	106 colicky infants aged 15 days to 4 months (mean age = 42 days)	FOS (source & characteristics not reported)	Intake not reported; duration was 30 days	The infants receiving the test formula had a significant reduction in colic symptoms (prolonged crying, constipation, regurgitation, and hypertonia) as compared to those receiving breast milk or standard formula. There were no significant differences in growth or any problems with feeding intolerance in any of the 3 groups.
Duggan et al. (2003) [Trial 1]	Prospective, randomized, double-blind, placebo-controlled study of the effect of OS + Zn on the prevalence of infant diarrhea	282 healthy infants aged 6-12 months (mean age = 8.8 months)	Oligofructose from chicory	0 or 550 mg/15 g cereal for 6 months	Daily consumption of cereal did not differ between the groups. There were no significant differences between the groups in the incidence or severity of diarrhea, the frequency with which diarrheal pathogens were isolated, constipation, blood chemistry, respiratory infections, side effects such as vomiting, or growth.
Duggan et al. (2003) [Trial 2]	Prospective, randomized, double-blind, placebo-controlled study of the effect of OS + Zn on the prevalence of infant diarrhea	349 healthy infants aged 6-12 months (mean age = 8.6 months)	Oligofructose from chicory	0 or 550 mg/15 g cereal for 6 months	The reasons for study withdrawal (10 from the prebiotic group and 21 from the control group) did not differ. No differences were seen in the incidence or severity of diarrhea, the frequency with which diarrheal pathogens were isolated, constipation, blood chemistry, respiratory infections, side effects such as vomiting, or growth.

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Euler at al. (2005)	Prospective, randomized, double-blind, crossover study of the safety and effects of different doses of oligofructose; included a non-randomized breastfed reference group	87 healthy term infants aged 2-6 weeks	Oligofructose from chicory	0,1.5, or 3 g/L formula for 1 week	There were no significant differences among the groups in growth as indicated by weight, length, and head circumference. Bifidobacteria counts were higher in the oligofructose groups immediately after supplementation than among the breastfed infants, although there was no apparent dose relationship. By a week after oligofructose cessation, the counts had equalized. Oligofructose supplementation had no effect on lactobacilli. The formula-fed infants had significantly higher counts of enterococci and bacteroides at baseline than did the breastfed infants, but by the end of oligofructose supplementation, there were no differences in bacteroides counts among the 3 groups. However, the baseline difference had returned by 7 days after cessation of oligofructose feeding. Clostridia counts were similar at baseline, but oligofructose supplementation resulted in a transient significant increase in clostridia in the formula-fed groups. *C. difficile* toxin was found in no breastfed infants, in 19% of formula-fed infants at baseline, and in 11% at the end of oligofructose feeding. During the week of oligofructose supplementation, the formula-fed infants experienced significant increases in loose stools and the incidence of flatulence and spit-ups.
Firmansyah et al. (2000)	Prospective, randomized, double-blind, placebo-controlled study of the effect of oligofructose and long-chain inulin on the immune response	50 8-month-old infants	Blend of long-chain inulin and oligofructose from chicory	0 or 600 mg/15 g infant cereal for 10 weeks	Post-measles-vaccination levels of IgG antibody were significantly higher among infants receiving the prebiotic and fewer side reactions were observed. The authors interpreted these findings as indicating an improved immune response to the vaccination. There were no effects on health or growth.

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Kapiki et al. (2007)	Prospective, randomized, double-blind, placebo-controlled study of the bifidogenic effect of FOS in infant formula	56 bottle-fed preterm infants less than 14 days old (mean age = 7.0 days) with gestational ages < 36 weeks (mean = 33.7 weeks), and admitted to a neonatal unit but other-wise healthy	FOS described as having been produced by partial enzymatic hydrolysis of chicory inulin	0 or 4 g/L formula for 14 days	Infants in the placebo group gained significantly more weight and had significantly greater arm circumference, while those in the FOS group gained non-significantly greater length. Intake of the FOS-supplemented formula produced a significantly higher frequency of defecation and softer stools as well as significantly greater concentrations of fecal bifidobacteria and bacteroides and significantly lower numbers of *E. coli* and enterococci. Both formulas were well tolerated.
Kim et al. (2007)	Prospective, randomized, double-blind, crossover study of the effect of inulin on the infant's gut microbiome, frequency of defecation, and the pH and consistency of feces	14 healthy term infants averaging 12.4 weeks of age	Inulin from chicory	0 or 1.5 g/100 g powder for 3 weeks	Stool characteristics during inulin feeding changed non-significantly toward increased frequency, softer stools, and lower pH. There were no differences between control and inulin feeding in total anaerobic bacteria or *Bacteroides* spp., but both bifidobacteria and lactobacilli increased significantly during inulin intake periods. No infants were withdrawn from the study, no formula-related adverse effects were observed, and there were no differences in growth between control and inulin feeding periods.

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Lugonja et al. (2010)	Non-randomized, non-blinded, non-placebo-controlled study comparing the bifidogenic effects of breast milk and prebiotic-supplemented infant formula	21 healthy infants aged 5 to 16 weeks (mean = 8.6 weeks)	Blend of inulin and oligofructose from chicory	0 or 4 g/L formula for 28 days	The number of daily feeds was significantly higher in the breastfed group. Counts of bifidobacteria increased significantly over the 28 days in both groups. Lactobacilli increased in both groups while aerobes, anaerobes, and fungi and yeasts decreased, but there were no significant differences between the formula and breastfed groups. Total organic acids increased and pH decreased over time in both groups. Most stools from infants in both groups were of normal consistency. The mean water content of the stools of infants receiving formula containing inulin+oligofructose was 77.9%, non-significantly lower than the mean water content of breastfed infants' stools (81.2%). All infants grew at normal rates and there was no difference between formula-fed and breastfed infants. There were no significant differences between groups in measures of intolerance, stool frequency, or stool consistency.
Moore et al. (2003)	Prospective, randomized, double-blind, placebo-controlled study of the GI effects of FOS-supplemented infant cereal	56 healthy term infants aged 4-11 months (mean age = 7.7 months) with demonstrated tolerance for rice cereal and milk-based formula	FOS (source and characterization not reported)	0 or 750 mg/ serving of cereal for 28-days	Infants receiving FOS had significantly more frequent stools with more regular and softer consistency than those receiving placebo. No serious adverse events were reported; 17 infants in the experimental group had 24 reported non-serious events as compared to 16 infants with 21 non-serious events in the placebo group. No adverse event was regarded as being related to the intake of FOS. There were no differences between the 2 groups in growth and no tolerance issues with the prebiotic cereal

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Panigrahi et al. (2008)	Prospective, randomized, double-blind, placebo-controlled study of long-term colonization of *L. plantarum* when administered to breastfed neonates along with FOS	31 healthy term infants aged 1-3 days	FOS (source and characterization not reported) and 10^9 cfu *L. plantarum*	150 mg/day for 7 days	*L. plantarum* was cultured from 84% of the infants in the synbiotic group on day 3 and from 95% on day 28; 100%, 88%, 56%, and 32% remained colonized at months 2, 4, 5, and 6, respectively. *L. plantarum* was not detected in the feces of any infants in the placebo group. The infants receiving the synbiotic exhibited a significantly greater number and variety of bacterial species than did the control infants. No serious adverse events were reported in either group, and there were no differences in non-serious events.
Underwood et al. (2009)	Prospective, randomized, double-blind, placebo-controlled study of the effects of combinations of probiotics and prebiotics on weight gain, fecal SCFA, and microbiota of preterm infants	90 healthy infants <7 days old with gestational age at birth <35 weeks	Inulin (source and characterization not reported); with $5x10^8$ cfu each of *L. acidophilus, B. longum, B. bifidum,* and *B. infantis or* $5x10^8$ cfu *L. rhamnosus* strain GG	Intake was not reported; duration = 28 days	The only infants lost to the study were ones transferred out of the ICU, none for study-related reasons. There were no significant differences between groups in fecal SCFA. The only difference in fecal bacteria was a significant increase in numbers of bifidobacteria among premature infants receiving the multi-strain probiotic+prebiotic formula. There were no differences in growth. All formulas were well tolerated; symptoms such as emesis, gastric distention, or excessive gastric residuals were minor.

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

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Waligora-Dupriet et al. (2007)	Prospective, randomized, double-blind, placebo-controlled study of tolerance and bifidogenic effects of oligofructose	35 healthy term infants aged 7-19 months	Oligofructose from chicory	2 g/day for 21 days	Because common infections such as pharyngitis, otitis, and bronchitis and consequent use of antibiotics were frequent, only 10 infants in each group completed the study; all exclusions were due to antibiotic use. Bifidobacteria populations increased while staphylococci and clostridia declined in the oligofructose-supplemented group; the difference from the placebo group was statistically significant for clostridia but not for bifidobacteria or staphylococci. No other bacterial counts differed significantly between the prebiotic and placebo groups, and all differences had disappeared by the end of the 15-day observation period. Flatulence, diarrhea, and vomiting were observed significantly less frequently in the oligofructose group than in the placebo group.

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Yao et al. (2010)	Prospective, randomized, double-blind, parallel-group study of the effects of infant-formula composition (high sn-2 palmitate w/ or w/o oligofructose) on stool characteristics and composition	300 healthy formula-fed term infants aged 7-14 days; n = 75 human-milk-fed reference group	Oligofructose from chicory	0, 3, or 5 g/L formula for 8 weeks	Withdrawals: 2 each from the human-milk reference group and the high sn-2 group, 1 each from the control group and the 3.0-g oligofructose group, and 0 from the 5.0-g oligofructose group. The infants receiving the high sn-2 formula, whether with or without oligofructose, had significantly less stool palmitate soaps and higher bifidobacteria counts than control infants, resembling the human-milk reference group; there was no difference in stool frequency. The high sn-2 group also had significantly softer stools than did the control infants, and addition of oligofructose resulted in a further dose-dependent increase in stool softness; the 5.0 g oligofructose group was not significantly different from the human-milk reference infants. Similarly, the addition of oligofructose significantly decreased stool calcium in a dose-dependent manner. Physician-reported GI events were few and not different among the 4 formula groups and the human-milk reference group; parental reports indicated no increase in the incidence of watery stools, gassiness, or other symptoms of intolerance with the addition of oligofructose. Addition of up to 5.0 g oligofructose/L to formula had no effect on growth (weight, length, head circumference).

Table 4. Studies of Fructans in Infants.					
Reference	**Study Design & Objective**	**Subjects**	**Source and Characteristics of Test Article**	**Dose & Duration**	**Results**
Yap et al. (2005)	Prospective, randomized, double-blind, parallel-group study of the dose effects of inulin on mineral absorption and production of SCFA in healthy term infants	36 healthy infants aged 5-12 months (mean age = 7.7 months)	Inulin from chicory	0, 750, 1000, or 1250 mg for 14 days	A non-significant dose-response effect was noted for increasing levels of both lactic acid and SCFA with increasing inulin dose and a significant dose-dependent decrease in pH was also observed. Ca absorption increased non-significantly with increasing inulin doses. Effects on absorption of the other minerals, while consistently in the direction of improved absorption with increased inulin intake, were generally less than those for Ca. No adverse events were reported.
Yap et al. (2008)	Prospective, randomized, double-blind, placebo-controlled trial of the effects of inulin on infants' fecal characteristics and microbial composition	36 healthy formula-fed term infants aged 5-12 months (mean age = 7.7 months)	Inulin from chicory	0, 750, 1000, or 1250 mg for 14 days	Inulin supplementation decreased fecal pH dose-dependently, but effects on defecation frequency or fecal consistency were not significant. Total fecal anaerobe populations were significantly decreased in the high-inulin dose group as compared with the other groups, but this difference disappeared after feeding ceased. All levels of inulin supplementation significantly decreased numbers of clostridia and the 2 higher doses resulted in significant reductions in Gram+ cocci and total coliforms. No effect was seen in lactobacilli, but bifidobacteria increased dose-dependently with inulin supplementation, reaching statistical significance compared to controls only at the high dose. No adverse events were reported.

7. Studies of Fructans in Adults and Children

7.1. Healthy Adults

Studies discussed in this section are summarized in Table 5 at the end of the section.

7.1.1. Studies with scFOS

Hidaka et al. (1986) compared blood glucose and insulin in healthy individuals given 50 g glucose and, one week later, 25 g scFOS; glucose was measured every 2 minutes for 3 hours after administration of the test article. No information was given regarding the number, sex, or age of subjects. Confirming the findings of in vitro tests comparing hydrolysis of scFOS, maltose, and sucrose by human salivary enzymes and by rat pancreatic and small-intestinal homogenates, the in vivo test in humans found no effect of scFOS ingestion on either blood glucose or insulin, indicating the nondigestibility of the substance.

Hidaka et al. (1986) administered 8 g scFOS/day in the diet to healthy adults (no further information was reported) for 2 months. Fecal bifidobacteria counts increased significantly, as did fecal SCFA and urinary phenol and p-cresol.

Tokunaga et al. (1993) enrolled 27 apparently healthy adults (21 males aged 36.8±9.0 years and 6 females aged 25.2±3.3 years) in an open-label study of the effect of scFOS on the intestinal microbiota. Volunteers were randomly allocated to 3 groups receiving 1, 3, or 5 g scFOS/day in single administrations following breakfast for 2 weeks, followed by a 2-week follow-up period. All participants completed daily questionnaires regarding defecation, and stool samples were collected at baseline, at the end of the intervention, and at the end of the follow-up period for analysis of microbiota.

All 3 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, but the increased counts of bifidobacteria disappeared over the 2-week follow-up. All 3 groups also had statistically significantly increased frequency of defecation and softening of the stool, with no apparent dose effect.

In an uncontrolled open-label study, Williams et al. (1994) enrolled 10 apparently healthy adults, 5 of each sex, aged 20-40 years, to consume 4 g scFOS/day for 14 days. The scFOS was given in 2 daily doses—a 1-g chewable tablet in the morning and a drink containing 3 g scFOS in the evening. Fecal samples were collected at baseline and at the end of the intervention, placed immediately in anaerobic chambers, and analyzed for total aerobes and anaerobes, bifidobacteria, and lactobacilli.

Nine of the 10 individuals showed increased bacterial counts (statistical significance was not reported). Analyses of changes across all 10 participants showed that the largest change was the increase in total anaerobes, but this change was not statistically significant due to large variance. Somewhat smaller increases in total aerobes, bifidobacteria, and lactobacilli were statistically significant, while a nonsignificant decrease was seen in Enterobacteriaceae. The authors 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)."

In a randomized, double-blind, placebo-controlled crossover study (Luo et al. 1996), 12 apparently healthy males aged 19-32 years (mean age = 24±1 year) received 20 g/day of either scFOS or sucrose for two 4-week periods separated by a 2-week washout. The test articles were prepared in cookies which the participants were instructed to consume in 3 or 4 daily snacks. All volunteers were seen weekly by a physician and fasting blood was drawn for analysis of plasma glucose, insulin, triacylglycerol (TAG), total and high-density lipoprotein (HDL) cholesterol, apolipoproteins A-1 and B, and lipoprotein(a). Blood taken at the end of each dietary period was also analyzed for insulin binding to erythrocytes, insulin-mediated glucose metabolism, and hepatic glucose production.

Compliance was excellent and none of the participants withdrew from the study, nor were there any complains of adverse symptoms during the dietary periods. The interventions had no significant effect on fasting glucose, insulin, TAG, total or HDL cholesterol, apolipoprotein A-1 or B, or lipoprotein(a) concentrations. Ingestion of scFOS did not affect insulin binding to erythrocytes or insulin-mediated glucose metabolism, but hepatic glucose production was lowered significantly. The authors concluded that "chronic ingestion of 20 g scFOS by healthy subjects has no detectable adverse metabolic effect but may reduce basal hepatic glucose production."

Buddington et al. (1996) conducted an open-label study in which 12 apparently healthy adults (6 of each sex aged 20-34 years) consumed 4 g scFOS/day for 25 days, ingesting 1 g in the morning as a chewable tablet and 3 g in the evening in a flavored drink mix. Participants maintained diaries and provided interviews regarding any GI or other problems. Fecal samples were collected at baseline and on days 5, 10, 15, 20, 25, 30, and 35 (the last 2 collections 5 and 10 days after feeding of scFOS was terminated), evaluated for hardness, and placed immediately in anaerobic chambers. Samples were taken from the center of each stool and analyzed for microbiota and activities of nitroreductase, β-glucuronidase, and glycocholic acid hydroxylase.

No participant reported digestive or health problems. 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 authors concluded that "4 g [scFOS] alters the fecal flora in a manner perceived as beneficial by decreasing activities of some reductive enzymes."

Bouhnik et al. (1996) gave 20 healthy adults (10 of each sex) aged 22-39 years 12.5 g/day of either sucrose placebo or scFOS in 3 oral doses for 12 days. Symptoms of intolerance (flatulence, borborygmi [noise], bloating, abdominal pain) and bowel function (stool number and consistency) were recorded daily. Stool samples were collected at 4-day intervals before, during, and for 12 days after scFOS administration and analyzed for bacterial counts, pH, lipids, and activities of azoreductase, nitroreductase, β-fructosidase, β-glucuronidase, and bile acids.

scFOS intake had no effect on fecal pH or total anaerobe counts, but bifidobacteria numbers increased significantly during scFOS feeding, returning to baseline levels within 12 days after cessation, consistent with a selective stimulation as observed by Gibson et al. (1995). β-Fructosidase activity also increased significantly during scFOS ingestion, returning to baseline within 12 days, but other enzyme activity levels and bile acids were not affected. No differences

were observed in the weight of stools or in lipid content. The only symptom of intolerance that was significantly higher in the scFOS group than the placebo group was bloating. The authors concluded that ingestion of 12.5 scFOS/day was well tolerated and produced significant bifidogenesis but no other significant effects.

In an investigation of the effect of the addition of scFOS to enteral formulas, Garleb et al. (1996) enrolled 27 apparently healthy male college students in a randomized, double-blind, controlled study. Nine participants designated as controls received a low-residue polymeric enteral formula, while 9 participants received the same formula with the addition of 5 g scFOS/liter and 9 participants received formula with 10 g scFOS/L. The students consumed their assigned enteral formula ad libitum at 3 set mealtimes/day for 14 days; no other food or drink were permitted other than water, diet soda, and sugar-free gum. The amount of formula consumed was recorded daily, participants completed a daily questionnaire regarding adverse GI symptoms (nausea, cramping, diarrhea, distension, flatus, vomiting, and regurgitation), participants were weighed and fecal samples were collected at baseline and study termination, and fasting blood samples were taken at the end of the intervention for analysis of glucose, BUN, creatinine, bilirubin, TC, TAG, protein, albumin, globuline, ALP, lactate dehydrogenase, ALT, AST, calcium, sodium, potassium, chloride, iron, phosphorus, and GGT.

Participants consumed a mean of 3 liters of formula per day (which did not differ significantly among groups), and so the low and high scFOS groups had daily intakes of about 15 and 30 g scFOS, respectively. There were no significant changes in body weights, nor differences or deviations from the normal range of blood chemistry values. Fecal acetate, isobutyrate, and isovalerate concentrations were significantly higher among students ingesting scFOS than the controls, but there were no differences in propionate or butyrate concentrations, fecal pH, or fecal percent dry matter. Consumption of scFOS significantly increased fecal bifidobacteria levels over levels of the controls. The authors reported that tolerance of the scFOS-containing formula was good, although one student in the high-scFOS group withdrew after one day due to intolerance; he was replaced and no further serious intolerance issues were observed. Complaints of nausea, cramping, distension, vomiting, diarrhea, and regurgitation were similar across all intervention groups and were present on fewer than 5% of participant-days. Flatus was reported significantly more frequently by those consuming 30 g scFOS/day than the controls, but most complaints occurred during the first 4 days. The authors 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."

In order to assess the safety of tablets containing scFOS and calcium prior to undertaking a trial of the effect of scFOS on calcium absorption and excretion, Ohta et al. (1999) administered tablets providing 9 g scFOS and 900 mg calcium to 10 healthy men aged 37±10 years. The men recorded subjective descriptions of side effects such as stomachache or discomfort, flatulence, borborygmi, flatus, diarrhea, or constipation. 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."

In a study of the effect of scFOS on calcium absorption and excretion, Ohta et al. (1999) enrolled 10 healthy men aged 37±10 years in a randomized, double-blind, placebo-controlled

crossover study. The men were given 200 ml distilled water at hourly intervals from 7:00 a.m. to 4:00 p.m. At 9:00 a.m., the men consumed tablets providing 0.3 g calcium and 3.0 g scFOS or placebo (sucrose) along with breakfast; they ate lunch at 1:00 p.m. Urinary samples were collected every 2 hours from 9:00 a.m. to 5:00 p.m. After a 1-week washout, the regimen was repeated with the other treatment.

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 authors concluded that "Calcium absorption was stimulated and the calcium supplementary effects of the calcium tablets were improved."

Bouhnik et al. (1999) investigated the dose-response of bifidogenesis to scFOS intake in a study with 40 apparently healthy volunteers (18 males and 22 females aged 18 to 47 years with a mean age of 29.6 years), who were randomly assigned to 5 groups (n = 8/group) to receive 2 daily doses totaling 0, 2.5, 5, 10, or 20 g scFOS/day for 7 days. The scFOS was given in a powdered mixture that included sucrose as needed to total 20 g. Blinding was not reported. Participants completed a daily diary recording flatus, borborygmi, bloating, abdominal pain, and stool frequency and consistency. Stools were collected at baseline and termination and placed immediately in anaerobic containers for determination of pH and microbial enumeration.

No increase in total anaerobes was observed in any group. Bifidobacteria did not increase significantly in groups receiving 0 or 2.5 g scFOS/day, but increased significantly in all 3 groups receiving larger doses of scFOS; the correlation between dose and bifidobacterial counts was a statistically significant r = 0.53. Flatus was reported significantly more frequently by those receiving 20 g scFOS/day than the other groups, but no other GI symptom differed among groups. The authors 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."

In a randomized, double-blind, placebo-controlled crossover trial (Tahiri et al. 2001), 11 apparently healthy postmenopausal women age 59±6 years received either scFOS or sucrose placebo for 5 weeks while their absorption and excretion of labeled magnesium were monitored. Crossover feeding periods were separated by a 3-week washout. Volunteers consumed 5 g/day of scFOS or sucrose at lunch for the first 4 days, then 10 g/day (5 g each at lunch and dinner) for the remaining 31 days. On day 28, the women received a dose of 87.5 mg ^{25}Mg in 100 ml water. Fecal collection began immediately and was continued for 5-7 days while 24-hour urine was collected for 2 days. Blood was drawn at the beginning and end of each dietary period.

Compliance was good; the women consumed a mean of 9.8±0.2 g/day of both scFOS and sucrose. Ingestion of scFOS significantly increased magnesium absorption by 12.3%, which resulted in higher levels of both plasma and urine magnesium, and the authors concluded that "ingestion of moderate doses of scFOS did improve intestinal magnesium absorption and status in postmenopausal women."

Fukushima et al. (2002) studied the effect of scFOS on calcium absorption in 2 similar randomized, double-blind, placebo-controlled, crossover trials, using a canned liquid malt drink

in the first trial and a dehydrated powder malt drink in the second. The canned drink in the first trial was prepared with 228 mg calcium as $^{44}CaCO3$ and either 0 or 1.9 g scFOS, while the powder drink included 180 mg ^{44}Ca and 0 or 1.5 g scFOS. The first trial enrolled 8 apparently healthy females aged 20.9±0.6 years while the second enrolled 5 apparently healthy females aged 21.4±0.9 years. Urinary calcium was significantly increased at 4, 6, and 8 hours after ingestion with both scFOS-containing drinks.

Additionally, Fukushima et al. (2002) assessed the safety of scFOS intake in 2 studies with the 2 drinks consumed at 3x the consumption level of the main studies (5.7 and 4.5 g scFOS/day), with 9 apparently healthy adults (6 males and 3 females) consuming the canned drink and 10 adults (7 males and 3 females) consuming the powder-type drink for 7 days. Effects were assessed based on a clinical interview and blood chemistry and hematology assessed before and after the intervention (analytes included total protein, AST, ALT, ALP, GGT, AMY, uric acid, BUN, creatinine, calcium, phosphorus, iron, TC, HDL-cholesterol, neutral lipid, and glucose as well as red and white blood cell counts, hemoglobin, hematocrit, MCV, MCH, MCHC, and platelet count), and urinalysis (specific gravity, pH, urobilinogen, bilirubin, ketone bodies, protein, glucose, occult blood). No abnormal changes were observed in any of these measures, nor in the clinical interview, and stooling was normal with no indication of diarrhea.

The effect of a malt beverage providing 3.0 g scFOS on calcium absorption in young women was studied by Uenishi et al. (2002) in a randomized, double-blind crossover study using a ^{44}Ca stable isotope tracer in calcium carbonate. Eight female university students aged 20-22 years (mean = 20.5±0.8 years) consumed the malt beverage with or without the added scFOS, with a 1-week washout between the arms, and the $^{44}Ca/^{43}Ca$ ratio of their urine was measured after 2, 4, 6, 8, 10, and 12 hours.

Consumption of scFOS significantly raised total urinary calcium and $^{44}Ca/^{43}Ca$ ratio measured 4 hours and longer after ingestion. The authors concluded that "addition of scFOS enhances absorption of calcium."

In an open-label study (Uenishi et al. 2002), 26 apparently healthy adults aged 31.8±7.5 years and 15 children aged 6.0±2.6 years consumed a malt beverage providing 3.0 g scFOS/day for 13 weeks. At baseline and at the end of feeding, blood samples were analyzed for biochemical outcomes (total protein, AST, ALT, GPT, ALP, GGT, AMY, uric acid, BUN, creatinine, calcium, phosphorus, iron, TC, HDL cholesterol, neutral fat, and blood glucose) and hematologic measures (leucocyte count, erythrocyte count, hemoglobin, hematocrit value, MCV, MCH, MCHC, and platelet count), and urine was analyzed for specific gravity, pH, urobilinogen, bilirubin, ketone bodies, protein, sugar, and occult blood).

No significant change was observed in mean values for any examination item after ingestion of the test drink and >3.0 g scFOS for 13 weeks and no adverse events were reported. There was no diarrhea. The authors concluded, "Adverse changes in the health status of subjects were not observed in subjective symptom, 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."

Oku and Nakamura (2003) studied the differences in digestibility and absorption of scFOS, GOS, and IMO in a crossover study with 38 apparently healthy adults (9 men and 29

women) aged 23.7±6.6 years. Participants were given single doses of 10 or 20 g scFOS, GOS, or IMO with 4-7-day washout periods between. Breath H_2 was collected before ingestion of the test oligosaccharide, at 40, 60, 80, 100, and 120 minutes after ingestion, and at half-hour intervals to 7 hours after ingestion.

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). Generation of H_2 was dose-dependent: breath hydrogen was approximately twice as abundant after ingestion of 20 g scFOS or GOS than after 10 g. The authors 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 participants showed abdominal symptoms such as flatus, distention, and borborygmi after ingestion of 10 or 20 g scFOS and after 20 g, but not 10, GOS, while no GI symptoms were reported after ingestion of 10 or 20 g IMO.

In a randomized, double-blind, crossover study, Tahiri et al. (2003) investigated the long-term effect of 10 g scFOS/day on calcium absorption in postmenopausal women. Twelve healthy postmenopausal women aged 50-70 years (mean = 59.8 years) received scFOS (5-g sachets twice a day) or placebo for 5 weeks (beginning with 4 days at a tolerance-building dose of 5 g/day) with a 3-week washout period between interventions. On day 29, during the midday meal, the women ingested a dose of calcium carbonate labeled with ^{44}Ca. Feces were collected for 5–7 days after the isotope intake to measure unabsorbed calcium isotope, blood was drawn on days 0, 28, and 29 of each experimental period, and urine samples were collected on days 0 and 28 of each period. Calcium status indices, calciotropic hormones, and bone turnover were also assessed.

The mean calcium absorption and urinary excretion with scFOS intervention and placebo were not significantly different, nor were plasma parathyroid hormone or 1,25-dihydroxy-vitamin D concentrations. No adverse effects were reported, and—based on non-significant findings with a small subgroup—the authors suggested that scFOS intake may improve calcium absorption by women in late post-menopause.

Bouhnik et al. (2004) studied bifidogenesis in healthy adults receiving nondigestible carbohydrates. Two hundred healthy volunteers (81 males, 119 females) aged 18-54 years with a mean age of 30 years participated in a randomized, parallel-group, placebo-controlled, double-blind dose-response study. In the first phase, 64 participants were allocated to 8 groups of n = 8 to ingest 10 g/day placebo (sucrose + maltodextrin) or scFOS, soy oligosaccharides, GOS, long-chain chicory inulin (DP ≥ 10), type III resistant tapioca starch, isomaltooligosaccharides from sucrose- or maltose-derived dextran, or lactulose in 2 daily doses of 5 g each for 7 days. All participants kept diaries in which they were instructed to record all GI side effects such as flatus, bloating, abdominal pain, and the frequency and consistency of stools were noted. Stools were collected at the beginning and end of the experimental period for analysis of pH and counts of total anaerobic bacteria, bifidobacteria, bacteroides, lactobacilli, and enterobacteria.

No differences were seen in any of the groups in fecal pH or counts of total anaerobic bacteria, bacteroides, lactobacilli, or enterobacteria, nor in stool frequency. Diarrhea was not reported in any of the groups, but all groups receiving 10 g nondigestible carbohydrates/day had similar significant increases in such common fiber-related GI effects as flatus, bloating, and

abdominal pain. Significant increases in bifidobacteria counts were seen in the groups receiving scFOS, soy oligosaccharides, type III resistant tapioca starch, and GOS, and these 4 substances were selected for further assessment in phase 2; long-chain inulin, isomaltooligosaccharides, and lactulose were not found to be significantly bifidogenic within the 7-day duration of the study.

During the second phase (Bouhnik et al. 2004), 136 subjects were assigned to a control group (n = 8) receiving a digestible carbohydrate placebo or to 1 of 4 groups of n = 32 who received doses of 2.5, 5.0, 7.5, or 10 g/day (n = 8/dose) of scFOS, soy oligosaccharides, type III resistant starch, or GOS in 2 equal daily doses for 7 days. All participants kept diaries in which they were instructed to record all GI side effects such as flatus, bloating, abdominal pain, and the frequency and consistency of stools were noted. Stools were collected at the beginning and end of the experimental period for analysis of pH and counts of total anaerobic bacteria, bifidobacteria, bacteroides, lactobacilli, and enterobacteria.

As in phase 1, no significant differences were seen in stool frequency, fecal pH, or counts of total anaerobic bacteria, bacteroides, lactobacilli, or enterobacteria, and diarrhea (defined as at least one watery stool per day) was not reported in any of the groups. All interventions were significantly bifidogenic, but a dose-response effect was significant only for scFOS. Although all interventions produced common fiber-induced GI side effects, there were no differences across interventions and the data do not appear to have been analyzed for dose-response effects. One key observation was that the degree of bifidogenesis was strongly influenced by starting levels of bifidobacteria, with initial low levels being significantly associated with increased post-intervention levels.

Ducros et al. (2005) studied the effect of scFOS on mineral absorption in healthy postmenopausal women. In a randomized, double-blind, cross-over design, 11 women aged 53-70 years (mean = 59 years) consumed diets providing 5-10 g/day of either scFOS or sucrose placebo for 5 weeks with a 3-week washout period intervening. (The women received single doses of 5 g scFOS/day for the first 4 days, then two 5-g servings/day providing 10 g scFOS/day for the remainder of the intervention period.) On day 28 of each intervention, the women received stable isotopes of zinc, copper, and selenium. Blood samples were taken at baseline for analysis of plasma levels of Zn, Cu, and Se. Feces were collected immediately after isotope ingestion and collection continued for 5-7 days; feces were analyzed for concentration of the stable isotopes.

All women had adequate plasma concentrations of trace elements at baseline. Intake of scFOS significantly enhanced 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.

Bouhnik et al. (2006) studied the dose-response relationship between scFOS ingestion and bifidogenesis in randomized, double-blind, placebo-controlled trial. Forty apparently healthy adults aged 20±1.3 years (18 men and 22 women) were divided into 5 groups (n = 8/group) and ingested 2 daily doses totaling 0, 2.5, 5.0, 7.5, or 10.0 g scFOS/day for 7 days. Participants kept a record of intolerance symptoms (flatulence, borborygmi, bloating, abdominal pain) and severity, defecation frequency, and stool consistency; stools were collected at baseline and termination for analysis of pH and microbiota.

Bifidobacteria counts increased at all doses of scFOS with a statistically significant correlation of 0.307 between dose and response. No significant differences were found in fecal pH or in numbers of lactobacilli, bacteroides, or enterobacteria. The frequency of symptoms of intolerance did not differ significantly 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 participant. The authors 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."

Fourteen healthy 12-14-year-old girls (mean age = 13±1 years) with chronically low calcium intake participated in a randomized, double-blind, placebo-controlled crossover trial in which they consumed 10 g/day of either scFOS or maltodextrin placebo for 36 days (van den Heuvel et al. 2009). Absorption of dietary calcium (given as $CaCO_3$) was estimated by comparing the abundance in urine of ^{44}Ca given orally with ^{46}Ca administered intravenously; similarly, dietary magnesium absorption (from MgO) was estimated based on the ratio of oral ^{25}Mg and intravenous ^{26}Mg. The girls and their parents reported any symptoms of intolerance or adverse events.

No short-term (8 days) or long-term (36 days) increase in calcium absorption was observed in response to scFOS intake, but 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," but provided no further information regarding adverse events.

In a randomized, double-blind, placebo-controlled crossover study of the effects of scFOS ingestion on hunger and satiety, Hess et al. (2011) enrolled 20 apparently healthy adults aged 18-64 years (10 men aged 28±2 years and 10 women aged 28±4 years) to consume 0, 5, or 8 g scFOS at breakfast and again 2 hours before dinner, providing a total dose of 0, 10, or 16 g. Satiety was assessed by self-report at 0, 15, 30, 45, 60, 90, 120, 180, and 240 minutes after breakfast and *ad libitum* food intake at lunch and dinner were measured. Breath H_2 was measured immediately after the morning dose and again 4 hours later. Participants self-reported any signs of GI intolerance.

No intolerance was reported. There were no consistent effects on hunger or satiety. Breath H_2 measured at 0 and 4 hours after scFOS ingestion increased dose-dependently, indicating that scFOS undergoes fermentation within 4 hours.

Slevin et al. (2014) investigated the effect of a calcium supplement with or without scFOS on bone density and bone turnover in non-osteoporotic postmenopausal women. In a randomized, double-blind, placebo-controlled trial, 300 women were randomly assigned to receive 800 mg calcium/day, 800 mg calcium and 3.6 g scFOS/day, or 9 g maltodextrin placebo for 24 months. While the calcium supplement had a beneficial effect on markers of bone turnover, scFOS had no significant additional effect. No adverse effects were reported.

7.1.2. Studies with Other Fructans

In an open-label dose-response study, Rumessen et al. (1990) investigated the intestinal handling of FOS-rich inulin from Jerusalem artichokes and its effects on blood glucose, insulin, and C-peptide. The fructan tested was composed of unbranched chains of fructofuranose units,

mostly with terminal glucose residues, with a DP range lower than that found in inulin derived from chicory. Eight healthy adults (6 males and 2 females aged 23-33 years took lactulose challenges and produced significant breath hydrogen. They then received single-dose challenges, separated by at least 72 hours, of 5, 10, and 20 g inulin; 20 g fructose; 50 g wheat starch; 50 g wheat starch + 10 g inulin. Breath hydrogen tests were performed before, during, and every 15-30 minutes after dosing for 12 hours. Blood samples were drawn for analysis of glucose, insulin, and C-peptide before dosing and every 15-30 minutes after dosing for 3 hours.

Artichoke inulin appeared to be completely unabsorbed at any dose; traces of fructan (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 hydrogen. Orocecal transit times ranged from 145 minutes after a 20-g dose up to 270 minutes after a 5-g dose; all of these times were significantly longer than those of lactulose and fructose. Administration of 20 g artichoke inulin significantly lowered postprandial blood glucose and insulin peaks. There was no apparent interference of inulin with starch absorption. Only mild flatulence was reported apart from one volunteer who complained of moderate flatulence after 10 g inulin; there was no overall significant difference in reported side effects between artichoke inulin, fructose, or wheat starch. No diarrhea or abdominal pain was reported.

In a study of the effects of oligofructose and inulin on colonic microbiota and colonic function, Gibson et al. (1995) gave healthy adults either oligofructose or chicory inulin with DP ≥ 2 for 15 days. Eight volunteers (7 males, 1 female aged 21 to 48 years; mean age = 33.6 years) consumed a controlled fructan-free diet containing 15 g sucrose for 15 days, then the same diet with the sucrose replaced by 15 g/oligofructose/day, and finally the sucrose-containing diet for 15 days. Stool samples were collected for an additional 5 days after the 45-day feeding period. Four of the volunteers participated in a follow-up study ingesting 15 g inulin/day for another 15 days. Bowel habits, transit time, stool composition, breath H_2 and CH_4, and the predominant genera of colonic bacteria were measured.

Both oligofructose and inulin significantly increased bifidobacteria concentrations in stools, while bacteroides, clostridia, and fusobacteria decreased when subjects were fed oligofructose, and gram-positive cocci decreased when subjects were fed inulin; however, total bacterial counts were not significantly different, suggesting selective stimulation of bifidobacteria. Fecal wet and dry matter, nitrogen, energy excretion, and breath H_2 increased significantly with both substrates, but SCFA product and breath CH_4 were not affected.

In a randomized double-blind crossover study of the effect of inulin on blood lipids and GI discomfort, Pedersen et al. (1997) instructed 72 healthy normolipidemic women aged 20-36 years to use 40 g/day of a low fat spread providing 0 or 14.4 g chicory inulin/day for 4 weeks and then to switch to the other spread. GI symptoms were assessed by questionnaire on days 0, 2, 4, 8, 16, and 24 of each period; fasting blood samples were drawn at baseline and during weeks 3 and 4 of each period. Three-day diet records were kept for the final days of each period.

No significant differences were observed in dietary intakes, plasma TC, HDL- or LDL-cholesterol, or TAG concentrations. During the period during which 14.4 g inulin/day was ingested, a significantly higher degree of GI discomfort from flatulence and other symptoms was reported, although these effects were regarded as mild.

In a study of the effect of chicory inulin (not clearly characterized) on mineral absorption, Coudray et al. (1997) assigned 9 healthy young men with an average age of 21.5 years to receive 1 of 3 diets for a total of 28 days: control diet (which provided 18 g dietary fiber/day), control diet + 40 g sugar-beet fiber/day, or control diet + 40 g inulin/day. Each 28-day test period included 2 days on the control diet, 14 days with increasing content of inulin or sugar-beet fiber, and 12 days with 40 g/day of inulin or sugar-beet fiber. Near the end of each experimental period, feces, urine, and blood were collected for analysis.

Ingestion of inulin significantly increased the absorption and balance of calcium. Sugar beet fiber ingestion resulted in a significant increase in dietary calcium intake from the beet-fiber preparation without affecting absorption. Absorption and balance of magnesium, iron, and zinc were not significantly altered by the ingestion of either experimental fiber.

Using a randomized, double-blind, placebo-controlled, crossover design, van den Heuvel et al. (1998) examined the effect of ingestion of 15 g/day of chicory-derived inulin, oligofructose, or GOS on absorption of iron and calcium in healthy men. Twelve healthy men aged 20-30 years consumed basal diet alone or supplemented with 15 g/day (188 mg/kg bw/day) of inulin, FOS, or GOS in 3 doses of 5 g each for 3 weeks. The duration of washout periods between interventions was not reported. Iron absorption was measured over the last 7 days of each intervention and calcium absorption was measured on the last day using double stable-isotopes.

All participants completed the study with no reported tolerance issues. The 3-week interventions resulted in no significant differences in either iron or calcium absorption, although mineral absorption was non-significantly higher with all 3 prebiotics. The authors concluded that 15 g/day inulin, FOS, or GOS did not have a negative effect on iron and calcium absorption in young healthy men.

Clausen et al. (1998) used a cross-over design to compare the induction of loose stools by ingestion of oligofructose or lactulose in healthy adults. Twelve healthy adults (4 males, 8 females) aged 27-56 years consumed either oligofructose or lactulose for 3 days at a dose of 20 g (taken in a single dose in the morning), then 3 days at a dose of 40 g, then 80 g, and finally 160 g; at least 1 week was provided between each dose level. A stool sample was taken on the 3rd day of each feeding period. After a 2-week washout period, the same regimen was followed with the other carbohydrate source.

Both oligofructose and lactulose produced significant dose-dependent increases in stool weights, with lactulose's effect significantly greater than that of oligofructose. Additionally, fecal pH decreased; sodium concentration increased while potassium decreased, but there was no change in fecal osmolarity. Little oligofructose appeared in feces until the intake reached 160 g/day, when about 20% of the ingested dose was excreted, about 40% as the free monosaccharide fructose. The authors concluded that a laxation effect of both saccharides was demonstrated, 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." They also noted that "a given increase in fecal volume occurred at one dose level lower with lactulose compared with [oligofructose]."

Rumessen and Gudmand-Hoyer (1998) investigated the intestinal transport and fermentation of long-chain chicory inulin (DP ≥ 10) and oligofructose in a single-blind crossover study of healthy adults; this study was discussed in Section 5.2.2.1 on pharmacokinetics. Five healthy men and 5 women aged 18-25 years received a lactulose challenge followed by 9 further single-dose tests in random order, separated by 48 hours or more: 20 g lactulose; 10, 20, and 30 g oligofructose; 20 g long-chain inulin; 20 and 50 g fructose; 20 g sorbitol; and 20 g sorbitol + 20 g glucose. Breath samples were taken every 30 minutes for 12 hours after each test and analyzed for H_2 and CH_4. Blood was sampled via catheter every 30 minutes for 3 hours for measurement of glucose and acetate. Urine was collected after administration of short-chain fructans for assessment of the presence of fructans.

One volunteer failed to complete the tests due to a non-related condition. All participants showed a significant rise in H_2 after lactulose challenge. Average orocecal transit times for both shorter- and longer-chain fructans, lactulose, sorbitol, and fructose ranged from 30 to 105 minutes, with considerable overlap across all tested carbohydrates, but 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 and were similar to responses to lactulose. All volunteers showed malabsorption of 50 g fructose, resulting in significantly more symptoms than 20 g fructose. Ingestion of sorbitol with equimolar amounts of glucose did not reduce malabsorption or abdominal distress. Abdominal symptoms after fructan ingestion increased with increasing dose and decreasing chain length.

Castiglia-Delavaud et al. (1998) assigned 9 healthy young men with an average age of 21.5 years to receive 1 of 3 diets for a total of 28 days: control diet, control diet + 50 g sugar-beet fiber/day, or control diet + 50 g inulin/day. Each 28-day test period included 2 days on the control diet, 14 days with increasing content of inulin or sugar-beet fiber, and 12 days with 50 g/day of inulin or sugar-beet fiber. The tested inulin product included 62% inulin derived from chicory; other components were not reported but may have included FOS. During the last 8 days of each experimental period, feces and urine were collected for analysis of energy, nitrogen, total lipids and SCFA, starch, cellulose and hemicelluloses, glucose, uronic acids, and bacterial counts. Energy expenditure was measured by whole-body indirect calorimetry.

Ingestion of sugar-beet fiber or 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 among diets were not significantly different. The fermentability of sugar-beet fiber averaged about 89% while that of inulin approached 100%. The calculated energy contents of the two non-starch polysaccharides were 2.8 kcal/g for sugar-beet fiber and 1.2 kcal/g for inulin.

In a randomized, double-blind, crossover study, van den Heuvel et al. (1999) gave 12 healthy male adolescents aged 14–16 years (mean age = 15.3 years) 15 g/day of oligofructose or sucrose (control) daily over 3 main meals for 9 days separated by a 19-day washout period. A dose of ^{44}Ca was given orally followed by an intravenous dose of ^{48}Ca on day 8 of each intervention period. Calcium absorption was determined in 36-hour urine samples based on relative isotope levels.

All of the young men completed the study with no apparent tolerance issues; reports of GI complaints did not differ between interventions. Calcium absorption was significantly higher during oligofructose intervention than during the control period.

A randomized, double-blind, controlled study by van Dokkum et al. (1999) investigated the effect of daily intake of 15 g nondigestible poly- or oligosaccharides on large-bowel function, blood lipid concentrations, and glucose absorption. Twelve healthy men (mean age = 23 years) received 4 successive interventions of 3 weeks each: chicory inulin, oligofructose, GOS, and control, apparently with no washout periods. The daily 15-g dose of non-digestible oligosaccharide was consumed in 3 doses of 5 g each. During the final week of each intervention, analyses were conducted of stool weight, intestinal transit, fecal pH, SCFA, bile acids, fecal enzymes, blood lipids, glucose absorption, and reported GI symptoms.

All participants completed the study with no significant differences between interventions in GI tolerance outcomes. The interventions 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 concentrations of fecal deoxycholic acid and inulin and GOS lowered β-glucuronidase activity, a marker for potential release of conjugated bile acids. No differences in blood lipids reached significance.

In a non-randomized single-blinded cross-over study, Kruse et al. (1999) studied the effects of ingestion of chicory inulin with DP ≥ 2 on healthy adults. Six male and 5 female healthy adults aged 26-53 years consumed a “typical Western diet” providing 45% of the energy from fat for 8 days, then switched to a 30%-energy-from-fat diet containing from 22 to 34 g inulin/day for 64 days. The experiment was then repeated with the inulin replaced by rice, potatoes, or bread. Fasting blood and stool samples were collected at the end of run-in (day 8), day 27, and day 64. All participants were asked about GI symptoms at the end of run-in and intervention phases.

Two participants withdrew due to GI discomfort and 1 for non-study-related reasons, leaving 8 subjects providing complete datasets. No effect was seen on blood lipids (TC, HDL and LDL cholesterol, and TAG). Bifidobacteria counts increased significantly during the inulin phase and decreased again during the control phase, but total bacteria counts were not changed, consistent with selective stimulation of bifidobacteria. Concentrations of SCFA showed great interindividual variability and no significant differences were seen between diets. The most common complaints were mild to moderate flatulence and bloating, which tended to lessen over time; participants did not report being greatly affected by GI symptoms or discomfort even at high doses of inulin and there were no complaints about nausea or loose stools.

In a randomized, unblinded crossover study, the effects of daily consumption of 50 g of a breakfast cereal containing 0 or 9 g chicory inulin on blood lipids and the colonic ecosystem was studied in 12 healthy normolipidemic young men with a mean age of 23.3 years (Brighenti et al. 1999). The participants consumed the cereal without inulin (control diet) for 4 weeks, then the cereal with added inulin for 4 weeks, and the control diet for a final 4 weeks. The authors claimed that the cereals with or without inulin were indistinguishable by the participants; if this is true, then the study was single-blinded. The men provided fasting blood samples at study

initiation and after each 4-week feeding period, which was analyzed for TC, HDL cholesterol, and TAG. Participants also provided fecal samples for assessment of pH, SCFA, lactic acid, bile acids, and microbial counts; underwent physical examinations; and completed semiquantitative food-frequency and bowel-habit questionnaires. At 3 time points, the men also provided breath samples at hourly intervals after cereal consumption for analysis of expired H_2. A side sample of 7 healthy volunteers (3 males and 4 females with average age = 28.8 years) consumed test or control cereal and submitted to finger pricks at 15, 30, 45, 60, 90, 120, 150, and 180 minutes for glucose measurement.

No changes in body weight, dietary habits, fecal pH, bile acid output, or SCFA were observed, and the glucose tolerance tests obtained the same incremental areas under the curve for both cereals. However, plasma TC and TAG were significantly lowered by ingestion of inulin, while fecal concentration of lactic acid and breath H_2 were significantly increased. Inulin significantly lowered counts of total facultative anaerobes and significantly increased the proportion of bifidobacteria. Apart from occasional reports of gas, which was apparently well tolerated, GI symptoms and bowel function did not differ significantly between the test and placebo groups.

During a controlled feeding study to assess the comparative bifidogenic effect of chicory-derived oligofructose (Menne et al. 2000), 8 healthy adults (5 females and 3 males aged 20-50 years) consumed diets providing 8 g oligofructose/day for up to 5 weeks. Fecal samples were collected prior to the intervention and after 2 and 5 weeks of ingestion of oligofructose and analyzed for total anaerobes, bifidobacteria, lactobacilli, bacteroides, coliforms and *Clostridium perfringens*.

Both 2 and 5 weeks of oligofructose feeding resulted in a significant increase in bifidobacteria with no change in 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 and defecation frequency increased 12% (not tested for statistical significance), and reported side-effects were infrequent and mild. The authors concluded that oligofructose functions as a bifidogenic prebiotic and is unlikely to cause significant intestinal discomfort.

The prebiotic effects of inulin and partially-hydrolyzed guar gum were investigated in a double-blind randomized placebo-controlled crossover study (Tuohy et al. 2001), in which 31 healthy adults (14 males and 17 females aged 18-50 years) daily consumed either 3 placebo biscuits or 3 biscuits with a total of 6.6 g inulin and 3.4 g guar gum/day for 21 days; there was no washout between interventions. The inulin was not described. Participants kept daily diaries of stool frequency and consistency and any GI side effects. Stool samples were taken at study initiation, on the last 3 days of each intervention, and weekly for 3 weeks thereafter.

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 the inulin/guar-gum biscuits. Bifidobacteria levels returned to pre-intervention levels within 7 days of cessation of the intervention. No significant changes were observed in fecal pH or in stool frequency or consistency, but volunteers' reports of intestinal effects such as flatulence, abdominal pain, and bloating increased during prebiotic ingestion.

A non-randomized placebo-controlled crossover study (Rao 2001) was designed to investigate the effect on the fecal microbiota of daily ingestion of 5 g oligofructose by healthy adults. Four men and 4 women aged 24-48 years (mean age = 28 years) took single doses of 5 g sucrose/day for 3 weeks and then 5 g oligofructose daily for 3 weeks. Fecal samples were collected before study initiation and after 11 and 21 days of each feeding period and enumerated for bifidobacteria, bacteroides, coliforms, total anaerobes, and total aerobes.

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 ingestion which was not increased after 21 days, but which declined nearly to 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 seen. The authors concluded that these data confirm that oligofructose provides a prebiotic effect even at low doses.

The effects of inulin and a wheat-derived pentosan concentrate (hemicellulose preparation containing arabinoxylan) on the metabolic activity of the intestinal microbiota were compared using fecal markers in a prospective, randomized, double-blind, parallel study (Grasten et al. 2003). Fourteen healthy adults (3 males and 11 females) with mean age = 34 years were randomly assigned to consume wheat bread supplemented with 7.7% chicory inulin (DP ≥ 2) or 7.6% wheat pentosan for 4 days. They were instructed to consume 5 slices of bread, resulting in daily intakes of 15 g inulin or pentosan concentrate. Participants kept daily records of food consumption, defecation frequency and stool consistency, and intestinal symptoms, and provided fecal specimens at baseline and completion. Feces were analyzed for activities of the bacterial enzymes β-glucuronidase, β-glucosidase, and urease, SCFA, phenol, and p-cresol.

Neither intervention significantly affected fecal phenol or p-cresol or affected enzyme activity when measured as change from baseline, but β-glucuronidase was significantly higher in the pentosan group than the inulin group at termination. Both interventions increased concentrations of SCFA, especially acetate and propionate; pentosan, but not inulin, also increased butyrate levels significantly. Non-significant increases were seen in defecation frequency, stool softness, and GI discomfort, although ingestion of 15 g pentosan/day appeared to result in greater discomfort than 15 g inulin/day. No increase was seen in the incidence of diarrhea.

A randomized, double-blind, placebo-controlled crossover study was conducted by Letexier et al. (2003) to determine whether the addition of chicory-derived long-chain inulin to a high-carbohydrate diet would decrease hepatic lipogenesis and plasma TAG concentrations and have a cholesterol-lowering action in healthy humans. Eight 23-32-year-old volunteers, 4 of each sex, ingested 10 g/day of either long-chain inulin or maltodextrin placebo (in 2 5-g doses) for 6 weeks. During the last 3 weeks, they consumed a controlled high-carbohydrate, low-fat diet. Following a 4-month washout period, all subjects consumed the other supplement. At the end of each diet period, fasting hepatic lipogenesis and cholesterol synthesis, plasma lipid concentrations, fatty acid synthase, acetyl-CoA carboxylase 1, and sterol responsive element binding protein 1c messenger RNA concentrations were measured in adipose tissue.

There were no significant differences in intake of total energy, fat, carbohydrate, protein, fiber, fructose, or cholesterol, and no differences were seen in blood levels of glucose, insulin,

glucagon, TC, or HDL and LDL cholesterol; however, TAG concentrations were significantly lowered by consumption of inulin. Hepatic lipogenesis was also significantly lower after inulin, but cholesterol synthesis was not significantly different and none of the adipose tissue messenger RNA concentrations changed significantly after inulin ingestion.

In an open-label study (Langlands et al. 2004), 14 healthy but high-cancer risk pre-colonoscopy patients (8 men and 6 women aged 35-72 years; mean age = 59 years) ingested 15 g/day of a 1:1 combination of oligofructose and long-chain chicory inulin (DP ≥ 10), in 3 daily doses for 2 weeks while a similar group of patients were given no supplement. Patients maintained a diary of supplement use and any side effects. During colonoscopy, multiple biopsies were taken from the cecum, transverse and descending colon, and rectum and cultured to determine the bacterial microbiome while similar biopsies were analyzed for epithelial cell proliferation markers minichromosome maintenance protein 2, Ki67, and proliferating cell nuclear antigen.

The 15 g/day of oligofructose-enriched inulin supplement was well tolerated, but all patients reported an increase in flatulence and some reported mild bloating or increased laxation. The only differences between the patients receiving prebiotics and the controls in bacterial counts were significant increases in bifidobacteria, lactobacilli, and eubacteria in both the proximal and distal colon; no differences were seen 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. The authors noted that the effect of the oligofructose-enriched inulin supplementation was a beneficial augmentation of the desirable genera bifidobacteria and lactobacilli with no increase in less desirable genera such as bacteroides, clostridia, and coliforms.

Bartosch et al. (2005) studied the effect on gut bacteria in healthy elderly people consuming a synbiotic containing 2 strains of bifidobacteria and oligofructose-enriched inulin. Eighteen female volunteers aged 63-90 years (mean age = 72 years) enrolled in a prospective, randomized, double-blind, placebo-controlled trial and were assigned to receive twice-a-day supplements providing 6 g prebiotic and 3.5×10^{10} cfu each of *B. bifidum* BB-02 and *B. lactis* BL-01 or 6 g maltooligosaccharide placebo for 4 weeks. Fecal samples were taken at baseline, weekly during the feeding period, and 1 and 3 weeks after feeding cessation.

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 authors concluded that administration of the synbiotic provided significant beneficial modification of the gut microbiome of elderly individuals, with no apparent adverse effects.

In a pilot study, a randomized, single-blinded, placebo-controlled, crossover design was used to assess the effects of oligofructose on satiety and energy intake in humans (Cani et al. 2006). Subjects included 10 healthy individuals (5 men and 5 women aged 21–39 years with a mean of 27.2 years) who received either 8 g oligofructose twice a day (16 g/day total) or placebo (dextrin maltose) for 2 weeks with a 2-week washout period between crossover phases. Food consumption and adverse effects such as thirst, nausea, flatulence, gastric reflux, abdominal pain or rumbling, or loose stools (reported as diarrhea) were monitored daily. Energy intake, hunger, satiety, and fullness were assessed at the end of each experimental phase.

Compliance was described as excellent. There were few reported tolerance problems, and the minor GI effects initially reported disappeared after day 3. Ingestion of 16 g oligofructose/day significantly increased satiety and reduced breakfast, lunch, and total energy intake as compared to the placebo.

Scholtens et al. (2006a) conducted a study to evaluate the effect of oligofructose on fecal cytotoxicity in healthy adults receiving diets with adequate calcium. In a randomized, double-blind, placebo controlled, crossover trial, 12 volunteers (6 men and 6 women aged 18-35 years; mean age = 21.4 years) consumed 25-30 grams of oligofructose or maltodextrin/day for 2 weeks; 5-g sachets were consumed—2 at breakfast, 2 at lunch, and 1 or 2 at dinner (depending on bodyweight). The two intervention periods were separated by a 2-week washout. All participants maintained diaries of stool frequency and consistency and GI effects. After each intervention period, stool samples were collected and measurements were taken of fecal pH, SCFA and lactate, fecal water cytolytic activity, fecal ALP activity, and fecal O-linked oligosaccharides as markers for mucin-type glycoproteins.

All participants completed the study with over 98% compliance. The incidence of reported side effects did not differ between the oligofructose and maltodextrin placebo periods except that flatulence was more frequently reported during the oligofructose period. Ingestion of oligofructose significantly increased defecation frequency but had no effect on consistency. Oligofructose intake resulted in no overall change in SCFA concentration, but the proportion of acetate was significantly higher and that of butyrate significantly lower than when placebo was being ingested. Fecal water cytolytic activity was non-significantly lower during oligofructose consumption than during the control period. No statistically significant differences were seen in fecal ALP activity or O-linked oligosaccharide production, indicating no difference in fecal mucin content. The authors concluded that consumption of up to 25-30 g oligofructose/day had no effect on the cytotoxicity of fecal water, fecal ALP activity, or fecal concentration of mucin-type oligosaccharides in healthy adults consuming a normal diet unrestricted in calcium. Specifically, there was no indication of intestinal epitheliolysis.

Ten Bruggencate et al. (2006) studied gut barrier function in healthy men in a prospective, randomized, double-blind, placebo-controlled, cross-over study with 34 volunteers. Men aged 18-55 years received 3 daily servings of oligofructose in lemonade totaling 20 g oligofructose/day or similar lemonade containing 6 g sucrose (placebo) for 2 weeks, with a 2-week washout period between cross-over interventions. The lemonade also contained chromium EDTA as a marker of intestinal permeability. As in previous research by this group, a calcium-deficient state was induced by prohibiting consumption of dairy products or other calcium sources during the study, resulting in daily calcium intake of only about 300 mg/day compared to a normal intake of 1000-1200 mg/day. On the last 2 days of each intervention period, all participants evaluated their GI symptoms, and feces and urine were collected for measurement of fecal lactic acid and mucins (by fluorometry), bacterial enumeration using real-time PCR, determination of the cytolytic activity of fecal water on erythrocyte permeability, and measurement of urinary chromium EDTA to assess intestinal permeability.

Compliance was good, although complaints of flatulence and intestinal bloating were 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. However, ingestion of 20 g oligofructose/day did not affect the cytolytic activity of fecal water and had no significant effect on intestinal permeability. The authors 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 due to the reduced pH that would be expected to result from ingestion of any fiber.

The reported findings of this study—and by implication other studies published by Ten Bruggencate and her coworkers—were severely disputed in a letter to the editor by Guarner (2006). Among other issues, he criticized the assumption that fecal mucin excretion is a marker of mucosal irritation, pointing out first that mucin secretion and excretion are increased by probiotic lactobacilli and epidermal growth factor, neither of which are mucosal irritants, and second that increased mucin secretion is a beneficial effect that protects against luminal aggressions. Guarner summarized his critique as follows: "The study by Ten Bruggencate and co-workers demonstrates that dietary fructooligosaccharides do not affect intestinal barrier function in healthy men on a low calcium diet, as proven by the CrEDTA test. The authors should consider revision of their conclusion, which is not supported by the data presented in the paper."

De Preter et al. (2007) studied both immediate and long-term effects of probiotics (*L. casei* Shirota and *B. breve* Yakult) and prebiotics (oligofructose-enriched inulin) on colonic nitrogen-protein metabolism in healthy humans. Twenty healthy young adults (10 of each sex, mean age = 21 years) were randomly assigned to 2 intervention groups in a placebo-controlled crossover trial. The trial included 3 ingestion periods of 4 weeks each, with 2-week washout periods in between. During the first ingestion period, half of the participants received 10^9 cfu *B. breve* twice a day along with a maltodextrin placebo resembling inulin while the others received 6.5×10^9 cfu *L. casei* twice a day along with the maltodextrin placebo. During the second ingestion period, all participants received placebo milk product resembling their probiotics as well as 10 g oligofructose-enriched inulin twice a day. During the third ingestion period, all participants received both probiotic and prebiotic placebos twice a day. Half of the participants also had a fourth ingestion period during which they received both 10 g prebiotic and 6.5×10^9 cfu *L. casei* twice a day. Urine and fecal samples were collected for analysis at the beginning and end of each ingestion period.

Oligofructose-enriched inulin significantly increased bifidobacteria and reduced proteolytic activity in the colon as measured by ammonia and p-cresol excretion, 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) investigated whether fructans have a lipid-lowering effect in adults when administered for prolonged periods in a prospective, randomized, double-blind, placebo-controlled design using a combination of long-chain inulin [DP ≥ 10] and oligofructose [DP < 10]). Seventeen healthy adults were randomly assigned to receive 2 daily doses totaling 10 g/day of long-chain inulin + oligofructose (n = 9) or maltodextrin placebo (n = 8) for 6 months. They were interviewed at baseline and at 2, 4, and 6 months regarding compliance and any

adverse effects and underwent metabolic assessment at baseline and 6 months. Fasting cholesterol and lipogenesis rates were measured using deuterated water to label synthesized cholesterol and lipids, and plasma concentrations of glucose, non-esterified fatty acids, TAG, total and free cholesterol, and HDL fraction were assessed. Subjects also took an oral glucose tolerance test and submitted to needle biopsies of abdominal subcutaneous adipose tissue for measurement of messenger RNA concentrations.

There were no differences in dietary intakes or body weights between the prebiotic and placebo groups and there were no effects on fasting plasma glucose, insulin, glucagon, nonesterified fatty acids, or TAG. The authors reported a nonsignificant reduction in TC and LDL and a significant increase in HDL due to ingestion of long-chain inulin + oligofructose. 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. The authors concluded that, while no adverse effects were noted as a result of ingestion of 10 g long-chain inulin + oligofructose/day for 6 months, there were no significant beneficial effects on plasma lipid concentrations.

De Preter et al. (2008) followed up their previous work with a second randomized placebo-controlled study of the effects of pre- and probiotics on intestinal bacterial enzyme activity. In a very complex design, 53 healthy adults (28 males and 25 females aged 19-26 years, mean = 22 years) were assigned to one of 5 intervention groups which were subjected to a total of 16 different 4-week interventions (3 or 4 per group, separated by washout periods). Each intervention condition included varying levels of lactulose, oligofructose-enriched inulin, or maltodextrin placebo, and varying doses of *S. boulardi*, *B. breve*, or *L. casei* administered in milk or a milk placebo. (The prebiotic was administered in 3 of the interventions at 20 g/day, taken in two installments; lactulose was given in 2-daily-doses of either 10 or 15 g/each.) Feces were collected at the beginning and end of each intervention period and tested for activities of β-glucuronidase and β-glucosidase.

Generally speaking, the probiotics had less effect on enzyme activity levels than did the prebiotics; both lactulose and 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 authors concluded that the reduction in β-glucuronidase activity is beneficial and may have important health implications.

In a prospective, randomized, double-blind, placebo-controlled cross-over study, Russo et al. (2008) evaluated the effect of long-chain inulin on lipid profile and lipoprotein(a) in healthy young males. Twenty-two men with a mean age of 18.8 ± 0.7 years consumed a controlled diet including 100 g pasta/day. After a 2-week run-in, the young men consumed control unsupplemented pasta or pasta providing 11 g/long-chain inulin/day for 5 weeks, with an 8-week washout period between interventions. Food intake was monitored and the participants completed dietary surveys during the last week of each assessment period along with questionnaires regarding GI symptoms. Fasting blood samples were taken at baseline and after each intervention for analysis of TC, TAG, HDL, and Lp(a).

Fifteen volunteers completed the study and followed the controlled diets. None of the subjects reported GI side effects related to administration of long-chain inulin, and no change in bowel habit was recorded. 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 levels, but only cholesterol/HDL ratio differed significantly from levels seen at the end of the placebo period. The authors concluded that the intervention exerts "slight but significant effects on the lipid profile and Lp(a) concentration."

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
scFOS					
Bouhnik et al. (1996)	Prospective, randomized, double-blind, placebo-controlled study of the effects of scFOS on micro-biota and stooling	20 healthy adults (10 of each sex) aged 22-39 years	scFOS	0 or12.5 g/day for 12 days	scFOS intake had no effect on fecal pH or total anaerobe counts, but bifidobacteria numbers increased during scFOS feeding, returning to baseline levels within 12 days after cessation. β-fructosidase activity also increased during scFOS ingestion, returning to baseline within 12 days, but other enzyme activity levels and bile acids were not affected. No differences were observed in the weight of stools or in lipid content. The authors concluded that ingestion of 12.5 scFOS/day was well tolerated and produced significant bifidogenesis but no other significant effects.
Bouhnik et al. (1999)	Open-label dose-response study of bifidogenesis to scFOS	40 healthy adults (18 males and 22 females aged 18 to 47 years; mean age = 29.6 years)	scFOS	0, 2.5, 5, 10, or 20 g/day for 7 days	No increase in total anaerobes was 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 more frequently by those receiving 20 g scFOS/day than the other groups, but no other GI symptom differed among groups. The authors 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.”

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
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, soy oligo-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	No differences were 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, soy oligo-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. Although all treatments produced GI side effects, there were no differences across treatments. Diarrhea was not reported in any of the groups.
Bouhnik et al. (2006)	Randomized, double-blind, placebo-controlled trial to study the dose-response relationship between scFOS ingestion and bifidogenesis	40 apparently healthy adults (18 men and 22 women) 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. No differences were found in fecal pH or in 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 participant. The authors 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."

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

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Fukushima et al. (2002) Part 2	Open-label studies of the 2 drinks used in Part 1 to assess safety	9 apparently healthy adults (6 males and 3 females) consuming the canned drink and 10 adults (7 males and 3 females) consuming the powder-type drink	scFOS	5.7 and 4.5 g/day for 7 days	No abnormal changes were observed in clinical chemistry or hematology (total protein, AST, ALT, ALP, GGT, AMY, uric acid, BUN, creatinine, calcium, phosphorus, iron, TC, HDL-cholesterol, neutral lipid, glucose, red and white blood cell counts, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count), or in urinalysis (specific gravity, pH, urobilinogen, bilirubin, ketone bodies, protein, glucose, occult blood), or in the clinical interview, and stooling was normal with no indication of diarrhea.
Garleb et al. (1996)	Randomized, double-blind, controlled study of the effect of the addition of scFOS to enteral feeding formulas	27 apparently healthy male college students	scFOS	0, 5, or 10 g/L for 14 days	The low and high scFOS groups had daily intakes of about 15 and 30 g scFOS. There was no change in body weight or deviations from the normal range of blood chemistry values (glucose, BUN, creatinine, bilirubin, TC, TAG, protein, albumin, globulin, ALP, lactate dehydrogenase, ALT, AST, calcium, sodium, potassium, chloride, iron, phosphorus, and GGT). Fecal acetate, isobutyrate, and isovalerate concentrations were higher among students 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. 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 authors 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.”

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Hess et al. (2011)	Randomized, double-blind, controlled crossover study of the effect of scFOS on hunger and satiety	20 apparently healthy adults aged 18-64 years (10 men aged 28±2 years and 10 women aged 28±4 years)	scFOS	0, 10, or 16 g/day taken in 2 doses over 24 hours	Breath H_2 measured at 0 and 4 hours after scFOS ingestion increased dose-dependently, indicating that scFOS undergoes fermentation within 4 hours. There were no symptoms of intolerance, no consistent effects on hunger or satiety, and no reported adverse effects.
Hidaka et al. (1986) Part 1	Open-label study of the effect of scFOS ingestion on blood glucose and insulin	No information was given regarding the number, sex, or age of subjects	scFOS	25 g in a single dose	There was no effect of scFOS ingestion on either blood glucose or insulin, indicating the nondigestibility of the substance. No adverse effects were reported.
Hidaka et al. (1986) Part 2	Open-label study of the effect of scFOS ingestion on microbiota	Healthy adults (no further information was reported)	scFOS	8 g/day for 2 months	Fecal bifidobacteria counts increased significantly, as did fecal SCFA and urinary phenol and p-cresol. No adverse effects were reported.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Luo et al. (1996)	Prospective, randomized, double-blind crossover study of the effects of scFOS on hepatic glucose production, insulin-mediated glucose metabolism, erythrocyte insulin binding, and blood lipids	12 19-32-year-old males (mean age = 24)	scFOS	0 or 20 g/day for 4 weeks	None of the participants dropped out or complained of any adverse effects, and diet did not affect intakes of energy or proximates or affect body weight. Nor did the dietary interventions cause significant changes in fasting plasma glucose or insulin, total or HDL cholesterol, apolipoprotein A-1 or B, lipoprotein$_a$, or TAG concentrations or change the mean insulin binding to erythrocytes or apparent receptor affinity. However, mean basal hepatic glucose production was significantly lower after scFOS than after sucrose. The authors concluded that 4 weeks of 20 g scFOS/ day "had no detectable adverse metabolic effect" on healthy adults.
Molis et al. (1996)	Open-label study of the digestion and excretion of scFOS	6 healthy adults, 3 of each sex, aged 20-27 years	scFOS	5 g/day at first, in-creased over 3 days to 20.1 g/day for 8 days	None of the ingested scFOS was excreted in stools, and less than 11% was absorbed in the small intestine, indicating that the portion reaching the colon was completely fermented by colonic microbiota. Only 0.12% of ingested scFOS was recovered in urine. scFOS appeared in the distal ileum within 30-60 minutes after ingestion and continued to be recovered for 8 hours. The estimated energy value of the tested scFOS was 2.3 kcal/g. No GI symptoms were reported during either the placebo or scFOS periods.
Ohta et al. (1999) Part 1	Open-label study of the safety of tablets containing scFOS	10 apparently healthy men aged 37±10 years	scFOS	9 g in a single dose	2 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."

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Ohta et al. (1999) Part 2	Randomized, double-blind, placebo-controlled crossover study of the effect of scFOS on calcium absorption and excretion	10 apparently healthy men aged 37±10 years	scFOS	0 or 3 g in a single dose	Calcium excretion was significantly higher at 2, 6, and 8 hours after ingestion when it was accompanied by scFOS rather than sucrose and the calcium/creatinine ratio was significantly higher at all time points. There were no significant differences in urine volume or levels of hydroxyproline, pyridinoline, or deoxypyridinoline relative to creatinine. The authors concluded that "Calcium absorption was stimulated and the calcium supplementary effects of the calcium tablets were improved." No adverse effects were reported.
Oku and Nakamura (2003)	Open-label crossover study of differences in digestibility of scFOS and other oligosaccharides	38 apparently healthy adults (9 men and 29 women) aged 23.7±6.6 years	scFOS (and GOS and IMO)	10 or 20 g in a single dose	No H_2 was detected in breath after administration of either 10 or 20 g IMO. H_2 was seen in greater quantity and more quickly after ingestion of scFOS than GOS (at 100 minutes vs. 180 minutes). Breath H_2 was twice as abundant after ingestion of 20 g scFOS or GOS than after 10 g. The authors 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 participants showed abdominal symptoms such as flatus, distention, and borborygmi after ingestion of 10 or 20 g scFOS and after 20 g, but not 10, GOS, while no GI symptoms were reported after ingestion of 10 or 20 g IMO.
Slevin et al. (2014)	Randomized, double-blind, placebo-controlled trial of effects of Ca supplementation w/ or w/o scFOS	300 non-osteoporotic postmenopausal women	scFOS	3.6 g/day for 24 months	While the calcium supplement had a beneficial effect on markers of bone turnover, scFOS had no significant additional effect. No adverse effects were reported.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Stone-Dorshow and Levitt (1987)	Randomized double-blind, placebo-controlled study to test the effect of scFOS on breath hydrogen excretion	15 healthy adults aged 21-65	scFOS	0 or 15 g/day for 12 days	Peak excretion of H_2 following 10 g scFOS was 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 subjects who consumed 15 g scFOS/day had significantly greater flatulence than did the 5 taking sucrose, but no other adverse effects were reported.
Tahiri et al. (2001)	Randomized, double-blind, placebo-controlled crossover trial of the effect of scFOS on magnesium absorption and excretion	11 apparently healthy postmenopausal women age 59±6 years	scFOS	0 or 5 g/day for the first 4 days, then 0 or 10 g/day for 31 days	The women consumed a mean of 9.8±0.2 g/day of both scFOS and sucrose. Ingestion of scFOS increased magnesium absorption by 12.3%, which resulted in higher levels of both plasma and urine magnesium, and the authors concluded that "ingestion of moderate doses of scFOS did improve intestinal magnesium absorption and status in postmenopausal women." No adverse effects were reported.
Tahiri et al. (2003)	Prospective, randomized, double-blind, crossover study of the effect of scFOS on calcium absorption in postmenopausal women.	12 healthy postmenopausal women aged 50-70 years (mean = 59.8 years)	scFOS	0 or 10 g/day for 5 weeks	The mean calcium absorption and urinary excretion with scFOS treatment and placebo were not significantly different, nor were plasma parathyroid hormone or 1,25-dihydroxyvitamin D concentrations. The authors suggested that scFOS intake may improve calcium absorption by women in late post-menopause. No adverse effects were reported.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Tokunaga et al. (1993)	Open-label study of the effect of scFOS on the intestinal microbiota	27 apparently healthy adults (21 males aged 36.8±9.0 years and 6 females aged 25.2±3.3 years)	scFOS	1, 3, or 5 g/day for 2 weeks	All 3 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, but the increased counts of bifidobacteria disappeared over the 2-week follow-up. All 3 groups also had statistically significantly increased frequency of defecation and softening of the stool, with no apparent dose effect. There were no reports of adverse effects.
Uenishi et al. (2002) Part 1	Randomized, double-blind crossover study of the effect of scFOS on calcium absorption in young women	8 women aged 20-22 years (mean = 20.5±0.8 years)	scFOS	0 or 3.0 g in a single dose	Consumption of scFOS significantly raised total urinary calcium and $^{44}Ca/^{43}Ca$ ratio measured 4 hours and longer after ingestion. The authors concluded that "addition of scFOS enhances absorption of calcium." No adverse effects were reported.
Uenishi et al. (2002) Part 2	Open-label study of the safety of scFOS in adults and children	26 apparently healthy adults aged 31.8±7.5 years and 15 children aged 6.0±2.6 years	scFOS	3.0 g/day for 13 weeks	No change was observed in mean values for biochemistry (total protein, AST, ALT, GPT, ALP, GGT, AMY, uric acid, BUN, creatinine, calcium, phosphorus, iron, TC, HDL cholesterol, neutral fat, and blood glucose), hematology (leucocyte count, erythrocyte count, hemoglobin, hematocrit value, MCV, MCH, MCHC, and platelet count), or urinalysis (specific gravity, pH, urobilinogen, bilirubin, ketone bodies, protein, sugar, and occult blood). No adverse events were reported. There was no diarrhea. The authors 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."

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
van den Heuvel et al. (2009)	Randomized, double-blind, placebo-controlled crossover trial of the effect of scFOS on calcium absorption	14 healthy 12-14-year-old girls (mean age = 13±1 years) with chronically low calcium intake	scFOS	0 or 10 g/day for 36 days	No short-term (8 days) or long-term (36 days) increase in calcium absorption was observed in response to scFOS intake, but long-term magnesium absorption increased significantly. The authors stated that "consumption of scFOS did not lead to an increased number of adverse events as compared to the control treatment."
Williams et al. (1994)	Open-label study of the effect of scFOS on the intestinal microbiota	10 apparently healthy adults, 5 of each sex, aged 20-40 years	scFOS	4 g/day for 14 days	Nine of the 10 individuals showed increased bacterial counts; the largest change was the increase in total anaerobes. Smaller increases in total aerobes, bifidobacteria, and lactobacilli were statistically significant, while a nonsignificant decrease was seen in *Enterobacteriaceae*. The authors 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.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Other Fructans					
Alles et al. (1996)	Balanced multiple crossover trial using an orthogonal Latin-square design to investigate the metabolic fate of two different levels of oligofructose	24 non-smoking non-overweight healthy males aged 19-28 years (mean = 22.1 years)	Oligofructose from chicory	5 or 15 g/day for 7 days each	Increased flatulence during ingestion of 15 g oligofructose/day, but no other differences in GI effects, defecation frequency, stool wet or dry weight, stool pH, or stool form. 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 authors concluded that oligofructose added to the diet of young Western subjects is fully metabolized in the large intestine. No adverse effects were reported other than increased flatulence at the high dose.
Bartosch et al. (2005)	Randomized double-blind, placebo-controlled trial of the effect of a synbiotic on gut bacteria in healthy elderly people	18 healthy elderly women aged 63-90 years (mean age = 72 years)	1:1 combination of long-chain inulin and oligofructose from chicory and 3.5×10^{10} cfu each of *B. bifidum* BB-02 and *B. lactis* BL-01	0 or 6 g/day for 4 weeks	The synbiotic intervention had no significant effect on total bacterial counts, but both bifidobacteria and lactobacilli were increased significantly; the increase in bifidobacteria included both the species administered in the synbiotic and other bifidobacterial species. The authors concluded that administration of the synbiotic provided significant beneficial modification of the gut microbiome of elderly individuals with no apparent adverse effects.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Brighenti et al. 1999	Randomized single-blind, placebo-controlled crossover study of the effect of inulin on blood lipids and the colonic ecosystem	12 healthy, normolipidemic young men with a mean age of 23.3 years	Chicory-derived inulin	0 or 9 g/day for 4 weeks	No changes in body weight, dietary habits, fecal pH, bile acid output, or SCFA were observed, and the glucose tolerance tests obtained the same incremental areas under the curve. However, plasma TC and TAG were lowered by ingestion of inulin, while fecal concentration of lactic acid and breath H_2 increased. Inulin lowered counts of total facultative anaerobes and increased the proportion of bifidobacteria. No adverse effects were reported.
Cani et al. 2006	Randomized single-blinded, placebo-controlled, crossover study to assess the effects of oligofructose on satiety and energy intake in humans	10 healthy individuals (5 men and 5 women aged 21–39 years with a mean of 27.2 years)	Cligofructose from chicory	0 or 16 g/day for 2 weeks	Ingestion of 16 g oligofructose/ day significantly increased satiety and reduced breakfast, lunch, and total energy intake as compared to the placebo. There were few tolerance problems, and the minor GI effects reported disappeared after day 3.
Castiglia-Delavaud et al. (1998)	Randomized parallel-group, Latin-square design to study fermentation of inulin	9 healthy young men with an average age of 21.5 years	Chicory inulin	In-creasing dose for 14 days, then 50 g/day for 12 days	Inulin induced significant increases in defecation frequency and stool weight resulting from increases in excretion of water, dry mass, and microbial mass. After deduction of microbial nitrogen, differences in fecal nitrogen excretion between diets were not significantly different. The fermentability of inulin approached 100%. The calculated energy content of inulin was 1.2 kcal/g. No adverse effects were reported.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Clausen et al. (1998)	Randomized cross-over study to assess the induction of diarrhea by ingestion of oligofructose	12 healthy adults (4 males, 8 females) aged 27-56 years	Oligofructose from chicory	Escal-ating dose of 20, 40, 80, and 160 g for 3 days each	Oligofructose produced significant dose-dependent increases in stool weights. Fecal pH decreased; sodium concentration increased while potassium decreased, but there was no change in fecal osmolarity. Little oligofructose appeared in feces until the intake reached 160 g/day, when about 20% of the ingested dose was excreted, about 40% as the free monosaccharide fructose. The authors concluded that a laxation effect was shown, reaching the level of diarrhea induction at large doses. Furthermore, "Fecal volume in carbohydrate-induced diarrhea is proportional to the osmotic force of the malabsorbed saccharide, even though all or the majority of the saccharide is degraded by colonic bacteria." No adverse effects were reported other than the laxation effects.
Coudray et al. (1997)	Randomized cross-over study to investigate the effect of inulin on mineral absorption	9 healthy young men, average age of 21.5 years	Inulin derived from chicory	In-creasing dose for 14 days, then 40 g/day for 12 days	Ingestion of inulin significantly increased the absorption and balance of calcium. Absorption and balance of magnesium, iron, and zinc were not significantly altered. No adverse effects were reported.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
De Preter et al. (2007)	Randomized double-blind, placebo-controlled crossover trial to study prebiotic, probiotic, and synbiotic effects on colonic nitrogen-protein metabolism in healthy humans	20 healthy young adults (10 of each sex, mean age = 21 years)	1:1 blend of long-chain inulin and oligofructose from chicory; *B. breve* and *L. casei*	0 or 10 g/day for 4 weeks with and 4 weeks without probiotics	Oligofructose-enriched inulin significantly reduced proteolytic activity in the colon, while both probiotics had smaller but still statistically significant effects. Both of these effects were temporary and disappeared over the 2-week washout periods. No adverse effects were reported due to the interventions.
De Preter et al. (2008)	Randomized double-blind, placebo-controlled crossover trial of pre-, pro-, and synbiotic effects on intestinal bacterial enzyme activity	53 healthy adults (28 males and 25 females aged 19-26 years, mean = 22 years)	1:1 blend of long-chain inulin and oligofructose from chicory; *S. boulardi, B. breve* and *L. casei*	20 g/day for 4 weeks with and 4 weeks without probiotics	Oligofructose-enriched inulin significantly reduced activity of β-glucuronidase, but not that of β-glucosidase. None of the interventions affected total fecal output or fecal dry mass. The authors concluded that the reduction in β-glucuronidase activity is beneficial and may have important health implications; no adverse effects were reported.
Forcheron and Beylot (2007)	Randomized double-blind, placebo-controlled investigation of fructans and lipid-lowering in adults	17 healthy adults	1:1 blend of long-chain inulin and oligofructose from chicory	0 or 10 g/day for 6 months	There were no differences in dietary intakes, body weights, plasma glucose, insulin, glucagon, nonesterified fatty acids, or TAG between the oligofructose-enriched inulin and placebo groups, but 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 noted from ingestion of 10 g long-chain inulin+FOS/day for 6 months

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Gibson et al. (1995)	Randomized single-blind, parallel-group study of the effects of oligofructose or inulin on colonic microbiota and colonic function	8 healthy volunteers (7 males, 1 female aged 21 to 48 years; mean age = 33.6 years)	Oligofructose or inulin from chicory	0 or 15 g/day for 15 days	Both oligofructose and inulin increased bifidobacteria concentrations in stools, while bacteroides, clostridia, and fusobacteria decreased when subjects were fed oligofructose and gram-positive cocci decreased when subjects were fed inulin; however, total bacterial counts were not different. Fecal wet and dry matter, nitrogen, energy excretion, and breath H_2 increased significantly with both substrates, but SCFA and breath CH_4 were not affected. No adverse effects were reported due to ingestion of 15 g/day of either inulin or oligofructose.
Grasten et al. (2003)	Randomized double-blind, parallel-group study of the effects of inulin on the metabolic activity of intestinal microbiota	14 healthy adults (3 males and 11 females) with mean age = 34 years	Chicory inulin	0 or 15 g/day for 4 days	There was no effect on fecal phenol or *p*-cresol or affected enzyme activity when measured as change from baseline, but β-glucuronidase was significantly lower in the inulin group at termination. Inulin increased concentrations of SCFA, especially acetate and propionate. Non-significant increases were seen in defecation frequency, stool softness, and GI discomfort. No increase was seen in diarrhea.
Kruse et al. (1999)	Non-randomized cross-over study of the effects of inulin on bifidogenesis	11 healthy volunteers (6 male and 5 female) aged 26-53 years	Inulin from chicory roots	0 or 22-34 g/day for 64 days	No effect was seen on TC, HDL or LDL, or TAG. Bifidobacteria counts increased during the inulin phase and decreased again during the control phase, but total bacteria counts were not changed. Concentrations of SCFA were unaffected. Mild flatulence and bloating tended to lessen over time; there were no complaints about nausea or diarrhea.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Langlands et al. (2004)	Open-label study of the effect of inulin+FOS on the bacterial micro-biome and epithelial cell proliferation markers	14 healthy but high-cancer risk pre-colonoscopy patients (8 men and 6 women aged 35-72 years; mean age = 59 years)	1:1 combination of oligofructose and long-chain inulin from chicory	15 g/day for 2 weeks	Increases were noted in bifidobacteria, lactobacilli, and eubacteria in both the proximal and distal colon; no differences were seen in total aerobes or anaerobes, bacteroides, coliforms, or clostridia. The mucosa was macroscopically normal in all participants and prebiotic supplementation had no effect on markers of epithelial cell proliferation. 15 g/day of prebiotic supplement was well tolerated, but all patients reported an increase in flatulence and some reported mild bloating or increased laxation.
Letexier et al. (2003)	Randomized double-blind, placebo-controlled crossover study of the effect of inulin on hepatic lipo-genesis and TAG levels	8 healthy 23-32-year-old volunteers, 4 of each sex	Inulin from chicory	0 or 10 g/day for 6 weeks	There were no significant differences in intake of total energy intake, fat, carbohydrate, protein, fiber, fructose, or cholesterol, and none were seen in blood levels of glucose, insulin, glucagon, TC, or HDL and LDL cholesterol; however, TAG concentrations were significantly lowered by consumption of inulin. Hepatic lipogenesis was also significantly lower after inulin, but cholesterol synthesis was not different and none of the adipose tissue messenger RNA concentrations changed significantly after inulin ingestion. Ingestion of 10 g inulin/day did not produce any reported adverse effects.
Menne et al. (2000)	Randomized single-blinded, crossover study to assess the bifidogenic effect of oligofructose	8 healthy adults (5 females and 3 males) aged 20-50 years	Chicory-derived oligofructose	0 or 8 g/day for up to 5 weeks	Oligofructose ingestion resulted in a significant increase in bifidobacteria with no change in 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 authors concluded that oligofructose functions as a bifidogenic prebiotic and is unlikely to cause significant intestinal discomfort. No adverse effects were reported.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Pedersen et al. (1997)	Randomized double-blind crossover study of the effect of inulin on blood lipids and GI discomfort	72 healthy normolipidemic women aged 20-36 years	Inulin from chicory	0 or 14.4 g/day for 4 weeks	No significant differences were observed in dietary intakes, nor in plasma TC, HDL- or LDL-cholesterol, or TAG concentrations. During the inulin-ingestion period a significantly higher degree of GI discomfort from flatulence and other symptoms was reported, but no serious adverse effects.
Rao (2001)	Non-randomized placebo-controlled crossover study of the effect of oligofructose on the fecal microbiota	4 healthy men and 4 women aged 24-48 years (mean age = 28 years)	Oligofructose from chicory	0 or 5 g/day for 3 weeks	Ingestion of 5 g oligofructose/day significantly increased bifidobacteria numbers over those present at pretest or after sucrose ingestion. Bifidobacteria numbers reached a maximum after 11 days ingestion and declined nearly to 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 seen. The authors concluded that oligofructose has a prebiotic effect at low doses. There were no reported adverse effects.
Rumessen et al. (1990)	Open-label study of intestinal handling of inulin with a high proportion of FOS and its effects on blood glucose, insulin, and C-peptide	8 healthy adults (6 males and 2 females) aged 23-33 years	Inulin with a high proportion of FOS from Jerusalem artichokes	5, 10, or 20 g in single doses	Inulin was apparently completely unabsorbed at any dose; and 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.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Rumessen and Gudmand-Hoyer (1998)	Randomized single-blind crossover study of the intestinal transport and fermentation of long-chain inulin and oligofructose	5 healthy men and 5 women aged 18-25 years	Long-chain chicory inulin or oligofructose	10, 20, or 30 g in single-doses	All participants showed a significant rise in H_2 after lactulose challenge. Average orocecal transit times for both shorter- and longer-chain fructans ranged from 30 to 105 minutes, with long-chain inulin having a significantly longer transit time than oligofructose. Breath H_2 and venous acetate production increased in proportion to increasing fructan dose. Abdominal symptoms after fructan ingestion increased with increasing dose and decreasing chain length, but no other adverse effects were reported.
Russo et al. (2008)	Randomized double-blind, placebo-controlled cross-over study of the effect of long-chain inulin on lipid profile and lipoprotein(a)	22 healthy men with a mean age of 18.8 ± 0.7	Long-chain inulin from chicory	0 or 11 g/day for 5 weeks	The period of ingestion of long-chain inulin showed significant improvement in levels of HDL, TAG, Lp(a), and TC/HDL ratio compared to baseline, but only cholesterol/HDL ratio differed significantly from placebo. The authors 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 subject reported GI side effects related to administration of long-chain inulin.
Scholtens et al. (2006a)	Randomized double-blind, placebo controlled, crossover trial to evaluate the effect of oligofructose on fecal water cytotoxicity in healthy adults with adequate calcium status	12 volunteers (6 men and 6 women) aged 18-35 years; mean age = 21.4 years	Oligofructose from chicory	0 or 25-30 g/day for 2 weeks	Oligofructose increased defecation frequency but had no effect on consistency. Oligofructose intake resulted in no overall change in SCFA level, but acetate increased and butyrate decreased. Fecal water cytotoxicity was lower during oligofructose intake than during the control period. No differences were seen in fecal ALP activity or O-linked oligosaccharide production, indicating no difference in fecal mucin content. Consumption of 30 g oligofructose/day had no effect on the cytotoxicity of fecal water, fecal ALP activity, or fecal concentration of mucin-type oligosaccharides in healthy adults 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.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Ten Bruggencate et al. (2006)	Randomized double-blind, placebo-controlled, cross-over study of the effect of oligofructose on gut barrier function in healthy men	34 healthy men aged 18-55 years	Oligofructose from chicory	0 or 20 g/day for 2 weeks	Flatulence and intestinal bloating were reportedly more common during oligofructose consumption. Oligofructose ingestion increased fecal wet weight and excretion of mucin and lactic acid and increased counts of bifidobacteria and lactobacilli. Oligofructose did not affect the cytotoxicity of fecal water and had no effect on intestinal permeability. The authors speculated that the increased excretion of mucin reflected oligofructose-induced mucosal irritation in humans, caused by the rapid production of organic acids in the proximal colon. They did not recognize that the artificial low-calcium environment created by the dietary calcium restriction led to poor buffering capacity that may have resulted in mucosal irritation.
Tuohy et al. 2001	Randomized double-blind, placebo-controlled crossover study of the prebiotic effects of inulin and guar gum	31 healthy adults (14 males and 17 females aged 18-50 years)	Inulin (not further described; identified as FOS in the article) + partially hydrolyzed guar gum	0 or 6.6 g inulin/day and 3.4 g guar gum/day for 21 days	No significant differences were found in the numbers of total bacteria, *Bacteroides* spp, *Clostridium* spp, or *Lactobacillus* spp, but *Bifidobacterium* spp increased significantly during ingestion of inulin+guar gum. Bifidobacteria returned to pre-treatment levels within 7 days of cessation of treatment. No changes were observed in fecal pH or in stool frequency or consistency, but reports of intestinal effects such as flatulence, abdominal pain, and bloating increased during prebiotic ingestion. No other adverse effects were reported.

Table 5. Studies of Fructans in Healthy Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
van den Heuvel et al. (1998)	Randomized double-blind, placebo-controlled, crossover study of the effect of inulin, oligofructose, and GOS on absorption of iron and calcium in healthy men	12 healthy men aged 20-30 years	Chicory inulin or oligofructose	0 or 15 g/day for 3 weeks	The treatments resulted in no significant differences in either iron or calcium absorption. The authors concluded that 15 g/day inulin, oligofructose, or GOS had no effect on iron and calcium absorption in young healthy men. All participants completed the study with no reported difficulties and with no reported adverse effects.
van den Heuvel et al. (1999)	Randomized double-blind, placebo-controlled, crossover study of the effect of oligofructose on calcium absorption in healthy male adolescents	12 healthy male adolescents aged 14–16 years (mean age = 15.3 years)	Oligofructose from chicory	0 or 15 g/day for 9 days	Calcium absorption was significantly higher during the oligofructose treatment than during the control period. All of the young men completed the study with no apparent tolerance issues; reports of GI complaints did not differ between treatments.
van Dokkum et al. 1999	Randomized double-blind, diet-controlled study of the effect of inulin, oligofructose, or GOS on large-bowel function, blood lipid concentrations, and glucose absorption	12 healthy men (mean age = 23 years)	Chicory inulin or oligofructose	0 or 15 g/day for 3 weeks	The treatments had no effect on fecal weight, transit time, colonic function, or concentrations of SCFA other than acetic acid, which was significantly higher during ingestion of inulin and GOS, and valeric acid, higher with inulin ingestion. Breath H_2 was significantly higher on the oligofructose diet than the control. Inulin and oligofructose significantly lowered concentration of fecal deoxycholic acid and inulin and GOS lowered β-glucuronidase activity. No differences in blood lipids reached significance. All participants completed the study with no significant differences between treatments in GI tolerance issues.

7.2. Compromised Adults

Studies discussed in this section are summarized in Table 6 at the end of the section.

7.2.1. Studies with scFOS

Yamashita et al. (1984) conducted a prospective, randomized, double-blind, placebo-controlled trial in patients with type 2 diabetes to evaluate the effects of scFOS on blood lipids and glucose. Eighteen patients (8 males, 10 females; mean age = 48.5 ± 7.3 years) consumed a coffee drink providing 8 g scFOS/day for 2 weeks while 10 patients (5 of each sex; mean age = 50.3 ± 6.5 years) received the same coffee drink with 5 g sucrose/day placebo. The scFOS and sucrose provided approximately equal sweetness and the patients all were maintained on identical controlled diets. Fasting blood samples were taken at baseline and at the end of the intervention for analysis of glucose, TC, HDL, TAG, and free fatty acids.

Significant decreases in blood glucose, TC, and LDL were seen after the 2-week intake of scFOS compared to baseline, while these changes did not occur in the sucrose placebo group. The levels of HDL, TAG, and free fatty acids were not significantly affected either by scFOS or placebo.

In a randomized trial, Mitsuoka et al. (1986) enrolled 21 outpatients (7 men, 14 women) suffering from hyperlipemia, diabetes, high blood pressure, and peripheral arterial occlusion and assigned them to receive 1, 2, or 4 g scFOS/day for an average of 8 weeks. The 3 groups had 7, 9, and 5 individuals, respectively, assigned to them. Fasting blood and stool samples were collected every 2 weeks; stool was examined for microbiota and blood was analyzed for TC, TAG, free fatty acids, uric acid, and glucose.

Bifidobacteria counts increased dose-dependently with scFOS intake; counts of total bacteria, lactobacilli, bacteroides, enterobacteria, and streptococci correlated positively with bifidobacteria counts, while total blood cholesterol correlated negatively.

Mitsuoka et al. (1986) enrolled 10 chronic renal failure patients (average age = 54.0 years) and 6 healthy persons (average age = 49.5 years) in an open-label study in which they received 6.1 g scFOS/day for 12 months. Stool samples were collected “several times” during the intervention. The renal-failure patients showed continuous increases in total fecal bacteria counts and bifidobacteria over the entire 12 months; the healthy participants had smaller increases in bifidobacteria and no other changes in fecal microbiota. The authors concluded that “it is considered that … long-term administration does not have any undesirable effects.”

Hidaka et al. (1986) administered 8 g scFOS/day in the diet to 23 senile inpatients aged 50 to 90 years for 2 weeks. Fecal bifidobacteria counts increased significantly while lactobacilli increased and clostridia decreased nonsignificantly. In an attempt to establish dose-response, scFOS was given to 21 senile inpatients aged 54 to 88 years for 8 weeks at doses of 1, 2, or 4 g/day. The authors reported that bifidobacteria increased at all tested doses in a dose-dependent fashion, but no statistical analysis was reported.

In an uncontrolled open-label study, Mitsuoka et al. (1987) added 8 g scFOS/day to the diets of 23 older individuals (mean age = 73±9 years) for 2 weeks, collecting fecal samples at baseline and at the end of the intervention. No adverse effects were reported, and the fecal

enumeration of bifidobacteria increased significantly (by about a factor of 10) with concomitant reduction in fecal pH by about 0.3.

In an open-label study (Hidaka et al. 1991), 10 patients with chronic renal failure (6 males, 4 females; mean age = 45.0 years) and 6 healthy adults (3 of each sex; mean age = 49.5 years) consumed 8 g scFOS/day for 1 year with enumerations of fecal bacteria at baseline and after 1, 3, 6, 8, 9, and 12 months. In both groups, total bacterial numbers and bifidobacteria increased significantly after 1 month and remained elevated throughout the year; significant changes were not seen in other genera.

Hidaka et al. (1991) studied the effects of ingestion of scFOS in two experiments. In the first experiment, a prospective, randomized, double-blind, placebo-controlled trial, 46 hyperlipidemic patients (20 males and 26 females aged 24-86 years) were randomly allocated to receive either 8 g scFOS/day (n = 23) or sucrose placebo (n = 23) incorporated into confectioneries for 5 weeks. Lipid profiles, blood glucose, free fatty acids, and uric acid were measured before and after the study in fasting blood samples, as well as blood pressure and body weight. Thirty-seven patients (20 scFOS, 17 sucrose) completed the protocol. TC and diastolic blood pressure were significantly reduced with the scFOS intervention compared to placebo. No significant differences between the two interventions were observed in body weight, HDL, TAG, blood glucose, free fatty acids, or uric acid.

In the second trial, an open-label study, 9 hypercholesterolemic adults (7 males and 1 female aged 27-67 years) ingested 8 g scFOS/day in syrup form for 1 month. Serum cholesterol, HDL, TAG, and apolipoproteins were measured in fasting blood samples before and after the intervention, as were fecal microbiota and SCFA. The only statistically significant change seen in blood chemistry was an increase in apolipoprotein E. No statistically significant changes were noted in SCFA nor in the numbers of bifidobacteria, lactobacilli, bacteroides, or total fecal bacteria.

In an open label study (Hidaka et al. 1991), 15 functionally constipated patients (5 males and 10 females aged 20-82 years) consumed individually-assigned doses of scFOS ranging from 5 to 20 g/day for 6 weeks. Patients reported defecation frequency every 2 weeks. Ingestion of scFOS significantly increased defecation frequency with no adverse effects other than reports of mild flatulence from 6 patients.

Luo et al. (2000) investigated the effect of scFOS on blood lipids and glucose concentrations, hepatic glucose production, and insulin resistance in 12 patients (8 males and 4 females; mean age = with 57 ± 2 years) with type 2 diabetes. In this prospective, randomized, double-blind, placebo-controlled crossover trial, the participants received 20 g scFOS/day or sucrose placebo for two 4-week periods, separated by a 2-week washout. The scFOS and placebo were supplied as powder in packets to be added as sweeteners to food or beverages several times daily. The subjects completed diet records twice a week during each intervention. Fasting blood samples were collected before and every 2 weeks during the intervention and analyzed for glucose, insulin, TAG, TC, lipoproteins, and free fatty acids. Glycated hemoglobin, fructosamine, erythrocyte insulin binding, glucose turnover, and insulin tolerance were assessed at the end of each intervention period.

Two patients were excluded from the study for reasons unrelated to the intervention. No significant differences between the 2 intervention periods were observed in blood glucose, insulin, TAG, TC, HDL, free fatty acids, apolipoprotein A1 or B, or lipoprotein(a). Glycated hemoglobin, fructosamine, erythrocyte insulin-binding, basal hepatic glucose production and insulin-mediated glucose disposal also did not differ between the 2 interventions. The authors concluded that "4 wk of 20 g/d of FOS had no effect on glucose and lipid metabolism in type 2 diabetics." None of the participants complained of any adverse symptoms during the intervention periods, but there was apparently no formal assessment of patient symptoms or attempt to elicit reports.

In an open-label study of 19 generally healthy and well-nourished elderly nursing-home patients aged 85±6.0 years, Guigoz et al. (2002) administered 2 daily doses of scFOS totaling 8.0 g/day for 3 weeks to assess the treatment's bifidogenesis and consequent impact on non-specific immune outcomes such as phagocytosis and changes in lymphocyte subpopulations. Samples of feces, urine, and fasting blood were taken at the beginning of the 3-week washout period prior to scFOS administration, at the beginning and end of scFOS intake, and at the end of a 3-week follow-up period. Feces were analyzed for pH as well as bifidobacteria, lactobacilli, bacteroides, enterobacteriaceae, enterococci, and *Clostridium perfringens*. Fasting blood samples were analyzed for albumin, prealbumin, C-reactive protein, TC, TAG, phospholipids, α1-acid glycoprotein, folate, cobalamin, mononuclear cells and their phagocytic activity, and IL-6 mRNA expression.

Mean counts of bifidobacteria increased by 2.8±0.57 log10 cfu during the 3 weeks of scFOS intake; counts of bacteroides also significantly increased but other species were unaffected. The increased levels of bifidobacteria and bacteroides seen immediately after scFOS ingestion had decreased by the follow-up testing 3 weeks later. No change was seen in fecal pH, stool frequency, or flatulence. Significant increases were reported in peripheral T lymphocytes and lymphocyte subsets CD4+ and CD8+, but there was no change in total leukocytes, activated T lymphocytes, or natural killer cells. Phagocytic activity of granulocytes and monocytes and IL-6 mRNA levels were significantly decreased by scFOS ingestion, indicating a decrease in inflammatory process. The authors concluded that their results "strongly support [the bifidogenic effects of scFOS in elderly subjects] with a 2-log increase in bifidobacteria counts . . . A diminution in inflammatory process is suggested by the decreased expression of IL-6 mRNA in peripheral blood monocytes."

Giacco et al. (2004) conducted a prospective, randomized, double-blind, placebo-controlled cross-over trial in 30 hyperlipidemic patients (20 males and 10 females) with mean age = 45.5 ± 9.9 years. The participants received either 11 g scFOS or 15 g maltodextrin+ aspartame placebo/day as powders in packets to be added to coffee or tea for 2 months, after which they were switched to the other intervention for an additional 2 months. There was apparently no intervening washout period. At the end of the 2-month run-in and after each of the 2 intervention periods, fasting blood samples were taken for analysis of glucose and lipids and 7-day food records were completed. Fasting measurements of insulin, free fatty acid, apolipoprotein A1, and lipoprotein(a) were assessed at the end of each intervention as well as GI symptoms by questionnaire that requested patients to indicate any symptoms of abdominal pain, flatulence, meteorism, "looser stools," and diarrhea (the distinction between looser stools and diarrhea was not described).

The final analysis included 27 subjects; the 3 withdrawals were not related to the intervention. No significant difference in fasting blood characteristics was detected between scFOS and placebo except a slightly higher Lp(a) level after scFOS compared to placebo. No changes were found in diet composition or body weight during the study. Side-effects were minimal (mostly mild flatulence) and not significantly different during scFOS and placebo intervention. No patients reported abdominal pain or looser stools, but 3 patients reported diarrhea—2 during ingestion of scFOS and one during the placebo period.

Langkamp-Henken et al. (2004) conducted a randomized, double-blind, placebo-controlled, multi-center trial to investigate if consumption of an experimental nutritional formula could reduce days of symptoms of upper respiratory tract infection and affect antibody and lymphocyte proliferative responses to flu vaccine in an elderly population. The experimental formula contained 4.4 g scFOS along with structured TAG, taurine, carnitine, and a variety of vitamins and minerals absent from the control formula. Sixty-six ambulatory men and women >65 years were enrolled and randomly assigned to receive the experimental (n = 34) or control (n = 32) formula for 183 days. At baseline, a non-fasting blood sample was taken for analysis of vitamin E concentration, lymphocyte proliferation, antibody titers to flu vaccine, clinical chemistries, and hematology. Patients kept records of formula consumption, GI tolerance, and symptoms of upper respiratory tract infection; such symptoms were analyzed from study day 15 forward, the day on which patients received a single lot of flu vaccine. Non-fasting blood was collected on day 57 and analyzed for vitamin E, flu antibody titers, and lymphocyte proliferation to vaccine components.

Patients receiving the experimental nutritional formula experienced significantly enhanced immune function and fewer days of upper respiratory tract infection symptoms. Because the experimental and control formula differed in so many respects it is not possible to attribute benefit to scFOS or any other single component. Of significance is that no adverse events were reported potentially caused by ingestion of 4.4 g scFOS/day by this sensitive population. The only GI symptoms reported were one case of cramping in the scFOS group, one case of diarrhea in the control group, once case of flatulence in each group, and one case of vomiting in the scFOS group, and the authors concluded that, "There were no differences in GI intolerance between subjects drinking the control and experimental formulas."

Colecchia et al. (2006) investigated the use of a synbiotic comprising scFOS and *Bifidobacterium longum* W11 in the treatment of patients with constipation-variant IBS. A total of 636 IBS patients, 250 men and 386 women aged 51±16 years (range = 18-80 years), were enrolled in a multi-center open-label study. Patients received 3.0 g scFOS/day for at least 36 days. Patients completed a questionnaire at enrollment and the end of the study to describe stool frequency and abdominal symptoms such as distension and bloating. Patients were reevaluated 30 days after the end of treatment.

All patients completed the study and reported a significant increase in the frequency of bowel movements and a significant reduction in abdominal symptoms. These improvements began to disappear during the 30 days following cessation of treatment. The authors reported that, "Treatment was well tolerated; all patients concluded the study. No significant side effects were observed other than mild transient episodes of diarrhea in 6 patients (0.9%)."

Twelve apparently healthy elderly volunteers aged 69±2 years, 6 of each sex, participated in an open-label study (Bouhnik et al. 2007) of the effect of scFOS ingestion on fecal bifidobacteria counts, stool characteristics, oro-fecal transit time, and clinical tolerance. After a 2-week run-up, participants consumed 2 daily doses totaling 8 g scFOS/day for 4 weeks, followed by a 4-week washout period. Participants kept a daily record of intolerance symptoms (flatulence, borborygmi, bloating, and abdominal pain) and severity, defecation frequency, and stool characteristics. Stool samples were collected at the end of run-up, scFOS intake, and washout for analysis of pH, bile acids, neutral sterols (cholesterol, coprostanol, cholestanol, and ketones), and bacterial counts. Oro-fecal transit time was measured by timing the first appearance in stool of radio-opaque pellets swallowed at breakfast.

Fecal bifidobacteria counts increased significantly with scFOS ingestion, but returned to baseline values during the washout period. Fecal clostridia and total anaerobe counts increased significantly during the washout period. Fecal cholesterol also increased during scFOS ingestion and returned to baseline during the washout; no other sterols showed significant change and there were no significant changes in either primary or secondary bile acids. No effects were reported on stool characteristics or oro-fecal transit time. While flatulence and bloating were significantly more frequent during scFOS intake than during the run-up period, the severity was mild. Borborygmi and abdominal pain did not differ between run-up and scFOS ingestion, and diarrhea was not reported. The authors concluded that "four-week scFOS ingestion, with a dose of 8 g/d, is well tolerated and leads to significant increase in faecal bifidobacteria and cholesterol excretion in healthy elderly."

Karakan et al. (2007) studied the effect of adding scFOS to early enteral nutrition solution for feeding of patients with severe acute pancreatitis in a randomized, double-blind, placebo-controlled trial. Thirty patients aged 46.1±14.0 years with severe acute pancreatitis requiring stoppage of oral feeding were enrolled and assigned to receive nasojejunal feeding at an initial rate of 30 ml/hour and increasing to 100 ml/hour with solution containing 0 or 1.5 g fiber/100 ml, which provided 0 or 24 g fiber/day. Approximately 50% of the fiber was scFOS.

Both enteral feeding solutions were well tolerated. The median durations of enteral feeding and of the hospital stay were significantly shorter in the group receiving the prebiotic. Significant improvement was also seen in pancreatitis severity scores and the authors reported that, "In conclusion, nasojejunal EN with prebiotic fiber supplementation in severe AP improves hospital stay, duration of nutrition therapy, acute phase response and overall complications compared to standard EN therapy."

A total of 105 patients diagnosed with minor functional bowel disorders (15 men and 90 women aged 16 to 75 years; mean age = 38.5±11.4 years) participated in a randomized, double-blind, placebo-controlled, multi-center study of the efficacy of treatment with scFOS (Paineau et al. 2008). An initial questionnaire asked about digestive symptoms—abdominal discomfort, abdominal fullness, bloating, feeling of incomplete bowel movement, urgency, and straining at defecation—as well as quality of life. Patients were assigned to receive 2 daily doses totaling 5 g/day of scFOS (n = 54) or placebo (n = 51) consisting of 50% microcrystalline sucrose and 50% maltodextrin for 6 weeks.

Eight patients, 4 from each group, withdrew prior to study completion. Compliance with the protocol was judged good for 24 patients receiving scFOS and 26 receiving placebo, but less

satisfactory for the other patients. Measures of digestive comfort and quality of life showed significant improvement in patients receiving scFOS. A total of 27 adverse events were recorded, 11 in the scFOS group and 16 in the placebo group, including infectious diseases such as bronchitis or sinusitis, headache, and lower back pain as well as GI symptoms such as diarrhea, constipation, abdominal pain, vomiting, or nausea, possibly related to treatment. The frequency and severity of these events did not differ significantly between groups. "On the basis of these findings," the authors stated, "we conclude that regular consumption of 5 g scFOS/d may improve digestive comfort in subjects with minor functional bowel disorders."

7.2.2. Studies with Other Fructans

Kleessen et al. (1997) investigated the effect of chicory inulin (DP ≥ 2) or lactose on the intestinal microbiota, SCFA and lactate concentrations, pH, β-glucosidase and β-glucuronidase activity, and bowel habits of elderly women suffering from constipation. In a randomized, double-blind, placebo-controlled parallel study, 35 otherwise healthy female patients aged 68-89 years (mean age = 76.4 years) were assigned to receive 20 g/day of either inulin (n = 17) or lactose (n = 18) in a single daily dose for 8 days; the dose was increased stepwise over the next 3 days to 40 g/day in 2 daily doses, which dose was maintained for an additional 8 days. Patients were interviewed daily regarding well-being, tolerance, GI effects, and number and consistency of bowel movements. Stool samples were collected at baseline, or day 7 or 8, and on day 18 or 19 and analyzed for pH, β-glucosidase and β-glucuronidase activity, lactate, SCFA, and microbiota.

Ten patients had to be excluded for non-intervention-related reasons, leaving 10 patients in the inulin group and 15 in the lactose group. Fecal pH, concentrations of SCFA and lactate, and β-glucosidase and β-glucuronidase activities did not differ significantly between groups. Individuals consuming lactose had a significant increase in fecal counts of enterococci and decreases in lactobacilli and clostridia, while those ingesting inulin showed significant increases in bifidobacteria and decreases in enterococci. Total bacterial counts did not differ between groups. Inulin showed a significantly better laxative effect than lactose and reduced functional constipation with only mild reported discomfort, even at 40 g/day; the laxative effect was not related to dose.

Davidson et al. (1998; also reported by Davidson and Maki 1999) conducted a randomized, double-blind, crossover trial to investigate the effect of ingestion of 18 g chicory inulin/day on serum lipids in hypercholesterolemic adults. Twelve men and 13 women with a mean age of 60.2 years and LDL cholesterol levels between 3.36 and 5.17 mmol/L but no other health conditions (other than well controlled hypertension) were randomized to receive low-fat diets providing 0 or 18 g inulin/day, to be ingested in 3 servings, for 6 weeks, followed by a 6-week washout and then 6 weeks on the other diet. The inulin was incorporated into study foods as a replacement for sucrose. Three-day food records were collected at the beginning and end of each intervention period, vital signs and body weight were measured, blood was collected for lipids analysis, and adverse events were documented.

Five participants failed to complete the trial, only 1 for reasons (GI discomfort) believed to be related to the ingestion of 18 g fiber. However, compliance with the diet was significantly better during the control phase than during the inulin phase (97.2% and 93.7%, respectively). Both TC and LDL cholesterol increased nonsignificantly during the control phase and decreased

nonsignificantly during the inulin phase; the difference between the two interventions was statistically significant. Mild GI discomfort was significantly more common during the inulin than the control phase, but no significant adverse effects were observed.

Alles et al. (1999) studied the effects of 15 g/day of oligofructose on blood glucose, lipids, and acetate in 20 patients with type 2 diabetes (9 males and 11 postmenopausal females; mean age = 59 years) in a prospective, randomized, single-blind, placebo-controlled, cross-over study. Participants were randomized to receive yogurt providing either 4 g glucose/day (placebo) or 15 g oligofructose/day (in 2 doses of 7.5 g each) for 20 days, followed by the other intervention with no intervening washout period. Blood was drawn after overnight fasts on days 1, 18, 21, 39, and 42 and patients kept a diary of supplement intake, GI symptoms, and any adverse effects.

Compliance was 100% during both interventions and there were no differences in average daily food intakes or physical activities; no problems of intolerance were reported aside from greater flatulence during the oligofructose intervention. However, the effects of administration of 15 g oligofructose/day on fasting blood concentrations of glucose, acetate, TC, LDL cholesterol, or TAG failed to reach statistical significance.

Jackson et al. (1999) studied the effect of 10 g long-chain inulin/day on fasting blood lipids, glucose, and insulin levels in healthy middle-aged adults with moderate hyperlipidemia. Fifty-four men and women (numbers of each sex not reported) between the ages of 35 and 65 years (mean age = 52.2 years), healthy except for moderately raised levels of plasma TC and TAG, took part in a randomized, double-blind, placebo-controlled parallel study. Participants received either 10 g long-chain inulin or a maltodextrin placebo/day in a single dose at breakfast for 8 weeks, followed by a 4-week follow-up period and then the other intervention condition. Fasting blood samples were taken prior to the intervention and at 4, 8, and 12 weeks; at the end of week 8 all volunteers completed a questionnaire assessing compliance and GI side effects.

All participants completed the study with no significant changes in body weight or levels of fasting plasma glucose, TC, HDL or LDL cholesterol, apoA-1, or apoB; however, fasting plasma insulin and TAG levels were significantly reduced in the long-chain-inulin group as compared to the controls. The authors did not report any adverse GI effects.

In a prospective, randomized, double-blind, placebo-controlled cross-over study with no washout period by Causey et al. (2000), the effect of chicory inulin on serum characteristics and fecal composition of hypercholesterolemic men was examined. Twelve men aged 27-49 years with fasting TC levels > 200 mg/dl received 20 g inulin or sucrose placebo/day for 3 weeks within a controlled diet; the inulin and sucrose were formulated into vanilla ice cream. Fasting blood samples to assess blood lipids and hematological parameters were taken at the end of each intervention. Fecal samples were collected for the final 6 days of each intervention period and analyzed for fecal weight, pH, SCFA, intestinal transit time, and bile acids. Patients received a symptom evaluation survey to complete once during each intervention.

Except for a significant decrease in TAG with inulin compared to placebo, there was no significant change in blood lipids. Hematological outcomes were also unaffected by inulin with the exception of serum ferritin, which was significantly lower after inulin compared to placebo intervention. None of the fecal outcomes differed significantly between the two interventions.

Flatulence (based on the symptom evaluation survey) increased during the inulin phase compared to the placebo phase; the number of bowel movements per day was not affected by inulin intervention.

In a multicenter, prospective, randomized, double-blind, placebo-controlled parallel group study, Olesen and Gudmand-Heyer (2000) studied the effect of regular consumption of oligofructose from chicory on irritable bowel syndrome (IBS). For the 2-week run-in, 114 otherwise healthy IBS patients—about 85% women—aged 18-70 years (mean age = 45.1 years) ingested sachets providing 10 g placebo (dry glucose syrup)/day. Sixteen patients withdrew during this phase and the 98 remaining patients were randomly assigned to receive 10 g oligofructose/day (n = 52) or placebo (n = 46) in single daily doses for 2 weeks, after which the doses were raised to 20 g/day for 10 weeks. Compliance, tolerability, and both beneficial and adverse effects were recorded at 2, 4, 6, 8, and 12 weeks.

Two patients receiving oligofructose had tolerance problems during the first 2 weeks and withdrew from the study. An additional 14 patients from the test groups withdrew during the study, most due to tolerance issues, as well as 9 members of the control group. There were no significant differences between the groups in reports of flatulence, abdominal distension or pain, or defecation frequency, nor in improvements in IBS symptoms. Although significantly fewer infections were seen in the test group than among the controls, the authors concluded that oligofructose did not ameliorate IBS symptoms. It could also be concluded that 20 g oligofructose/day did not increase GI symptoms in patients with particular sensitivity to gut sensations.

In a prospective, randomized, double-blind, placebo-controlled study (Balcazar-Munoz et al. 2003), 12 obese, hypertriglyceridemic and hypercholesterolemic subjects aged 19-32 years ingested 7 g/day of either inulin (not further described in the abstract) or placebo (n = 6/intervention) for 4 weeks. Blood was drawn for testing of biochemical and metabolic profiles, and insulin sensitivity was evaluated at baseline and after the feeding period.

Intake of 7 g inulin/day had no effect on insulin sensitivity, nor on plasma levels of glucose, creatine, bilirubin, uric acid, ALT, AST, lactic dehydrogenase, or sodium/potassium, but significant reductions were seen in total, LDL, and VLDL cholesterol and in TAG.

Since burn patients suffer altered GI permeability, Olguin et al. (2005) undertook a prospective, randomized, double-blind, placebo-controlled study of the effect of chicory-derived oligofructose on gut barrier function of such patients. Forty-one males and females aged 14-70 years with 2nd- or 3rd-degree burn injuries were randomly assigned to receive 2 daily administrations totaling 6 g/day of either oligofructose (n = 21) or sucrose (n = 20) for 15 days. Gut permeability was assessed with lactulose and mannitol at baseline and on days 3, 7, 14, and 21. Eighteen healthy subjects served as a reference group for GI permeability.

Two patients died and 8 left the hospital before study completion, leaving 16 patients receiving oligofructose and 15 receiving placebo. Burns increased intestinal permeability four-fold as compared to the reference group, but no differences were seen in patients receiving oligofructose or sucrose, nor were there significant differences in burn infection rates, complications, need for enteral nutrition, number of surgical interventions, or length of

hospitalization. These data do not suggest an increase of gut permeability with oligofructose in prone patients.

In a double-blind cross-over study, Dahl et al. (2005) investigated the effects of inulin-fortified beverages on bone resorption markers, bowel frequency, and indicators of GI function in institutionalized wheelchair-bound adults. Fifteen institutionalized adults aged 23-57 years (mean age = 43 years) with a variety of health issues (7 with multiple sclerosis, 4 with cerebral palsy, 4 with neuropathies; 10 suffering from dysphagia and 5 without dysphagia) consumed 5 servings/day of modified starch-thickened beverages with or without 3 g chicory inulin/serving, providing a daily total of 0 or 15 g inulin for 3 weeks. Bowel frequency, fecal output, and laxative use were determined by testing or by nursing charts. Bone resorption was measured via urinary excretion of fasting calcium and of cross-linked N-telopeptides of collagen.

Urinary collagen, N-telopeptides, and fasting calcium excretion were not significantly different and did not suggest an increase in bone resorption. Defecation frequency was unaffected, but weighted stool frequency was improved with added inulin. This resulted in significantly lower use of enemas and laxatives. There were no issues with acceptability or tolerance as assessed by questions regarding GI discomfort.

In a small prospective, randomized, double-blind, placebo-controlled, cross-over pilot study of 7 patients with nonalcoholic steatohepatitis, Daubioul et al. (2005) investigated the ability of oligofructose to decrease TAG accumulation in liver tissue. The patients ingested the contents of 2 sachets totaling 16 g/day of either oligofructose or maltodextrin (placebo) for 8 weeks, followed by a 5-week washout before beginning the alternative intervention. Energy intake, body composition, liver steatosis, and fasting blood biochemistry were analyzed on weeks 0, 4, and 8 of each intervention period and 2 weeks after cessation and an abdominal ultrasound was performed to assess liver fatty infiltration at week 8 of each intervention.

Ingestion of oligofructose resulted in a significant decrease in the level of AST and non-significant decreases in ALT and TAG. No reduction was seen in GGT, ALP, bilirubin, uric acid, glucose, insulin, total or HDL cholesterol, or C-peptide concentrations or in liver size with either intervention.

In a prospective, randomized, double-blind, placebo-controlled trial, Casellas et al. (2007) investigated anti-inflammatory effects of long-chain inulin + oligofructose in patients with active ulcerative colitis. Nineteen patients aged 29 to 44 years, 6 men and 13 women, being treated with mesalazine were randomly allocated to receive 3 daily doses totaling 12 g/day of either prebiotic (n = 10) or maltodextrin (placebo; n = 9) for 2 weeks. Stool samples were taken at baseline for measurement of calprotectin and human DNA and rectal dialysis was performed to isolate fluid for measurement of the concentration of the inflammatory mediators interleukins and eicosanoids. The same tests were performed on day 7 and additional stool samples were taken on day 14.

One patient withdrew from the prebiotic group before the study began; 2 more patients (1 from each group) dropped out prior to day 7, neither for intervention-related reasons; and 1 more patient from the test group was lost before day 14 due to worsening of the colitis. Except for 1 placebo-group member, all patients who completed the study showed significant improvement in their condition. While no effect of the intervention was seen in concentrations of inflammatory

mediators or fecal excretion of human DNA, calprotectin concentrations were significantly reduced in patients receiving the prebiotic as compared to the placebo group. The authors concluded that 12 g oligofructose-enriched inulin/day was well tolerated and can mitigate intestinal inflammation.

Lopez Roman et al. (2008) conducted a prospective, randomized, double-blind study with 4 males and 28 females with mean age = 47 years suffering from primary chronic constipation. The patients were randomly assigned to receive 500 ml reduced-fat milk/day or the same milk supplemented with 20 g/day of a mixture of inulin and digestion-resistant maltodextrin (not further described) for 20 days. The authors reported that difficulty of defecation, sensation of incomplete evacuation, and days between bowel movements were significantly reduced by the supplement.

A prospective, randomized, double-blind, placebo-controlled trial by de Luis et al. (2010), evaluated the ingestion of inulin in obese subjects. Thirty-four volunteers were randomized to consume one cookie a day—either a control fiber-free cookie or one providing 3 g chicory inulin—for 4 weeks. Anthropometric and biochemical characteristics were assessed before and at the end of each intervention.

Fifteen of the 17 enrolled subjects completed the study in each intervention group. Members of the inulin group, but not the controls, experienced significant decreases in total and LDL cholesterol. There were no changes in anthropometric data in either group and inulin intake did not produce any reported adverse GI effects.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
scFOS					
Bouhnik et al. (2007)	Open-label study of the effect of scFOS ingestion on fecal bifidobacteria counts, stool characteristics, oro-fecal transit time, and clinical tolerance	12 apparently healthy elderly volunteers, 6 of each sex, aged 69±2 years	scFOS	8 g/day for 4 weeks	Fecal bifidobacteria counts increased with scFOS ingestion, but returned to baseline values during the washout period. Fecal clostridia and total anaerobe counts increased during the washout period. Fecal cholesterol also increased during scFOS ingestion and returned to baseline during the washout; no other sterols showed significant change and there were no significant changes in either primary or secondary bile acids. No effects were reported on stool characteristics or oro-fecal transit time. While flatulence and bloating were more frequent during scFOS intake than during the run-up period, the severity was mild. Borborygmi and abdominal pain did not differ between run-up and scFOS ingestion, and diarrhea was not reported. The authors concluded that "four-week scFOS ingestion, with a dose of 8 g/d, is well tolerated."
Colecchia et al. (2006)	Open-label multi-center study of use of scFOS and *Bifidobacterium longum* W11 in the treatment of patients with constipation-variant IBS	636 IBS patients, 250 men and 386 women, aged 51±16 years (range = 18-80 years)	scFOS	3.0 g/day for at least 36 days	All patients completed the study and reported a significant increase in the frequency of bowel movements and a reduction in abdominal symptoms. These improvements began to disappear during the 30 days following cessation of treatment. The authors reported that, "Treatment was well tolerated; all patients concluded the study. No significant side effects were observed other than mild transient episodes of diarrhea in 6 patients (0.9%)."

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Giacco et al. (2004)	Randomized, double-blind, placebo-controlled cross-over trial of the effect of scFOS on blood characteristics of hyperlipidemic patients	30 hyperlipidemic patients (20 males and 10 females) with mean age = 45.5 ± 9.9 years	scFOS	0 or 11 g/day for 2 months	No significant difference in fasting blood characteristics was detected between scFOS and placebo except a higher Lp(a) level. No changes were found in body weight. Side-effects were minimal (mostly mild flatulence) and not significantly different during scFOS and placebo intervention. No patients reported abdominal pain, but 3 patients reported diarrhea—2 during ingestion of scFOS and one during the placebo period.
Guigoz et al. (2002)	Open-label study of bifidogenesis from scFOS and impact on non-specific immune characteristics such as phagocytosis and changes in lymphocyte subpopulations	19 generally healthy and well-nourished elderly nursing-home patients aged 85±6.0 years	scFOS	8.0 g/day for 3 weeks	Mean counts of bifidobacteria increased by 2.8±0.57 log10 cfu during the 3 weeks of scFOS intake; counts of bacteroides also increased but other species were unaffected. The increased levels of bifidobacteria and bacteroides after scFOS ingestion decreased by the follow-up testing 3 weeks later Increases were reported in peripheral T lymphocytes and lymphocyte subsets CD4+ and CD8+, but there was no change in total leukocytes, activated T lymphocytes, or natural killer cells. Phagocytic activity of granulocytes and monocytes and IL-6 mRNA levels were decreased by scFOS ingestion. The authors concluded that their results "strongly support [the bifidogenic effects of scFOS in elderly subjects] with a 2-log increase in bifidobacteria counts . . . A diminution in inflammatory process is suggested by the decreased expression of IL-6 mRNA in peripheral blood monocytes." No change was seen in fecal pH, stool frequency, or flatulence and no adverse effects were reported.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Hidaka et al. (1986) Part 1	Open-label study of the bifidogenic effect of scFOS in senile elderly	23 senile inpatients aged 50 to 90 years	scFOS	8 g/day for 2 weeks	Fecal bifidobacteria counts increased significantly while lactobacilli increased and clostridia decreased nonsignificantly with no reported adverse effects.
Hidaka et al. (1986) Part 2	Open-label dose-response study of the bifidogenic effect of scFOS in senile elderly	21 senile inpatients aged 54 to 88 years	scFOS	1, 2, or 4 g/day for 8 weeks	Bifidobacteria increased at all tested doses in a dose-dependent fashion. No adverse effects were reported.
Hidaka et al. (1991) Part 1	Randomized, double-blind, placebo-controlled trial	46 hyperlipidemic patients (20 males and 26 females aged 24-86 years)	scFOS	0 or 8 g/day for 5 weeks	TC and diastolic blood pressure were significantly reduced with scFOS compared to placebo. No significant differences between the two interventions were observed in body weight, HDL, TAG, blood glucose, free fatty acids, or uric acid. No adverse effects were reported from ingestion of 8 g scFOS/day for 5 weeks.
Hidaka et al. (1991) Part 2	Open-label study	9 hypercholesterol-emic adults (7 males and 1 female aged 27-67 years)	scFOS	8 g/day for 1 month	The only significant change in blood chemistry was increased apolipoprotein E. No significant changes were seen in SCFA or in numbers of bifidobacteria, lactobacilli, bacteroides, or total fecal bacteria, and no adverse effects were reported.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Hidaka et al. (1991) Part 3	Open-label study	10 patients with chronic renal failure (6 males, 4 females; mean age = 45.0 years) and 6 healthy adults (3 of each sex; mean age = 49.5 years)	scFOS	8 g/day for 1 year	In both groups, total bacterial numbers and bifidobacteria increased significantly after 1 month and remained elevated throughout the year; significant changes were not seen in other genera. There were no reported adverse effects.
Hidaka et al. (1991) Part 4	Open-label study	15 functionally constipated patients (5 males and 10 females aged 20-82 years)	scFOS	5-20 g/ day for 6 weeks	Ingestion of scFOS significantly increased defecation frequency with no adverse effects other than reports of mild flatulence from 6 patients.
Karakan et al. (2007)	Randomized, double-blind, placebo-controlled trial of adding scFOS to early enteral nutrition solution for feeding of patients with severe acute pancreatitis	30 patients aged 46.1±14.0 years with severe acute pancreatitis requiring stoppage of oral feeding	scFOS	0 or 24 g fiber (about 50% scFOS)/ day for 48 hours	The median durations of enteral feeding and of the hospital stay were significantly shorter in the group receiving the prebiotic. Significant improvement was also seen in pancreatitis severity scores and the authors reported that, “In conclusion, nasojejunal EN with prebiotic fiber supplementation in severe AP improves hospital stay, duration of nutrition therapy, acute phase response and overall complications compared to standard EN therapy.” Both enteral feeding solutions were well tolerated with no reported adverse effects.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Langkamp-Henken et al. (2004)	Randomized, double-blind, placebo-controlled, multi-center trial of a nutritional formula to reduce symptoms of upper respiratory tract infection and affect antibody and lymphocyte proliferative responses to flu vaccine in an elderly population	66 ambulatory men and women >65 years	scFOS	0 or 4.4 g/day for 183 days	Patients receiving formula with scFOS experienced enhanced immune function and fewer days of upper respiratory tract infection symptoms. No adverse events were reported potentially caused by ingestion of 4.4 g scFOS/day by this sensitive population. The only GI symptoms reported were one case of cramping in the scFOS group, one case of diarrhea in the control group, one case of flatulence in each group, and one case of vomiting in the scFOS group, and the authors concluded that, "There were no differences in GI intolerance between subjects drinking the control and experimental formulas."
Luo et al. (2000)	Randomized, double-blind, placebo-controlled cross-over trial of the effect of scFOS on blood lipids and glucose concentrations, hepatic glucose production, and insulin resistance in type 2 diabetes	12 patients (8 males and 4 females; mean age = with 57 ± 2 years) with type 2 diabetes	scFOS	0 or 20 g/day for 4 weeks	No significant differences between the 2 intervention periods were observed in blood glucose, insulin, TAG, TC, HDL, free fatty acids, apolipoprotein A_1 or B, or lipoprotein(a). Glycated hemoglobin, fructosamine, erythrocyte insulin-binding, basal hepatic glucose production and insulin-mediated glucose disposal also did not differ between the both interventions. The authors concluded that "4 wk of 20 g/d of FOS had no effect on glucose and lipid metabolism in type 2 diabetics." None of the participants complained of any adverse symptoms.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Mitsuoka et al. (1986)	Randomized unblinded dose-response trial	21 outpatients (7 men, 14 women) suffering from hyperlipemia, diabetes, high blood pressure, and peripheral arterial occlusion	scFOS	1, 2, or 4 g/day for an average of 8 weeks	Bifidobacteria counts increased dose-dependently with scFOS intake; counts of total bacteria, lactobacilli, bacteroides, enterobacteria, and streptococci correlated positively with *Bifidobacterium* counts, while total blood cholesterol correlated negatively. No adverse effects were reported.
Mitsuoka et al. (1986)	Open-label study of the effect of scFOS on bifidobacteria in renal failure patients	10 chronic renal failure patients (average age = 54.0 years) and 6 healthy persons (average age = 49.5 years)	scFOS	6.1 g/day for 12 months	The renal failure patients showed continuous increases in total fecal bacteria counts and bifidobacteria over the entire 12 months; the healthy participants had smaller increases in bifidobacteria and no other changes in fecal microbiota. The authors concluded that "it is considered that … long-term administration does not have any undesirable effects."
Mitsuoka et al. (1987)	Open-label study of the effect of scFOS on bifidobacteria in older individuals	23 older individuals (mean age = 73±9 years)	scFOS	8 g/day for 2 weeks	The fecal enumeration of bifidobacteria increased significantly (by about a factor of 10) with reduction in fecal pH by about 0.3. No adverse effects were reported

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Paineau et al. (2008)	Randomized, double-blind, placebo-controlled, multi-center study of the efficacy of treatment of minor functional bowel disorders with scFOS	105 patients (15 men and 90 women) aged 16 to 75 years; mean age = 38.5±11.4 years diagnosed with minor functional bowel disorders	scFOS	0 or 5 g/day for 6 weeks	Digestive comfort and quality of life improved in patients receiving scFOS. 27 adverse events were recorded, 11 in the scFOS group and 16 in the placebo group, including infectious diseases such as bronchitis or sinusitis, headache, and lower back pain as well as GI symptoms such as diarrhea, constipation, abdominal pain, vomiting, or nausea, possibly related to treatment. The frequency and severity of these events did not differ significantly between groups. "On the basis of these findings," the authors stated, "we conclude that regular consumption of 5 g scFOS/d may improve digestive comfort in subjects with minor functional bowel disorders."
Yamashita et al. (1984)	Randomized, double-blind, placebo-controlled trial of the effects of scFOS on blood lipids and glucose in patients with type 2 diabetes	18 patients (8 males, 10 females; mean age = 48.5 ± 7.3 years) with type 2 diabetes	scFOS	0 or 8 g/day for 2 weeks	Significant decreases in blood glucose, TC, and LDL were seen after the 2-week intake of scFOS compared to baseline, while these changes did not occur in the sucrose placebo group. The levels of HDL, TAG, and free fatty acids were not significantly affected either by scFOS or placebo. Ingestion of 8 g scFOS/day for 2 weeks did not result in any reported adverse effects.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Other Fructans					
Alles et al. (1999)	Randomized, single-blind, placebo-controlled, cross-over study study of the effects of oligofructose on blood glucose, lipids, and acetate in patients with type 2 diabetes	20 patients (9 males and 11 postmenopausal females; mean age = 59 years) with type 2 diabetes	Oligofructose from chicory	0 or 15 g/day for 20 days	Compliance was 100% and there were no differences in average daily food intakes or physical activities. Administration of 15 g oligofructose/day had no significant effect on fasting blood concentrations of glucose, acetate, TC, LDL cholesterol, or TAG, and no problems of intolerance were reported.
Bach Knudsen and Hessov (1995)	Open-label study of digestion of inulin in patients procto-colectomized for ulcerative colitis	7 ileostomy patients (1 male and 6 females aged 22-73 years, median age = 38 years) proctocolectomized for ulcerative colitis	Inulin from Jerusalem artichokes	10 g on day 1 and 30 g on day 2	Ileal dry matter increased in a dose-dependent manner with increasing inulin load due to increased carbohydrate excretion. Inulin excretion increased rapidly after ingestion, peaking at 3 hours with a mean transit time of 4.9 hours at an intake of 10 g and 3.4 hours at 30 g. Low-DP inulin fractions were more sensitive to hydrolysis than higher DP fractions. Ileal concentrations of SCFA were reduced by inulin ingestion, while the concentration of lactic acid was increased. The authors concluded that this study demonstrated that, while slight hydrolysis of inulin was seen (due to acids, enzymes, or microbial degradation), “inulin is practically indigestible in the small intestine of man” and that “the transit of inulin through the small intestine was rapid.” They also noted that ingestion of the tested levels of inulin resulted in minor malabsorption of low-molecular-weight sugars. No adverse effects were reported from ingesting 30 g inulin.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Balcazar-Munoz et al. (2003) [Article in Spanish with English abstract]	Randomized, double-blind, placebo-controlled study of the effect of inulin on insulin sensitivity in hyperlipidemics	12 obese hyper-tryglyceridemic and hypercholesterol-emic subjects aged 19-32 years	Inulin (not further described)	0 or 7 g/day for 4 weeks	Intake of 7 g inulin/day had no effect on insulin sensitivity, nor on plasma levels of glucose, creatine, bilirubin, uric acid, ALT, AST, lactic dehydrogenase, or sodium/potassium, but significant reductions were seen in total, LDL, and VLDL cholesterol and in TAG. No adverse effects were reported.
Casellas et al. (2007)	Randomized, double-blind, placebo-controlled trial of the anti-inflammatory effects of inulin + oligofructose	19 active ulcerative colitis patients aged 29 to 44 years, 6 men and 13 women, being treated with mesalazine	1:1 combination of long-chain inulin and oligofructose	0 or 12 g/day for 2 weeks	No withdrawals occurred for treatment-related reasons. Except for 1 placebo-group member, all patients showed significant improvement in their condition. While no effect of treatment was seen in concentrations of inflammatory mediators or fecal excretion of human DNA, calprotectin concentrations were significantly reduced in patients receiving oligofructose-enriched inulin. The authors concluded that 12 g oligofructose-enriched inulin/day can mitigate intestinal inflammation and was well tolerated.
Causey et al. (2000)	Randomized, double-blind, placebo-controlled cross-over study of the effect of chicory inulin on serum characteristics and fecal composition of hypercholes-terolemic men	12 men aged 27-49 years with fasting TC levels > 200 mg/dl	Chicory inulin	0 or 20 g/day for 3 weeks	There was no change in blood lipids with inulin except for a decrease in TAG. Hematological outcomes were unaffected by inulin with the exception of serum ferritin, which was significantly lower after inulin compared to placebo intervention. None of the fecal outcomes differed significantly between the two interventions and the number of bowel movements per day was not affected by inulin intervention. Flatulence increased during the inulin phase compared to the placebo phase, but no other adverse effect was reported.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Dahl et al. (2005)	Randomized, double-blind, crossover study of the effects of inulin on bone resorption markers, bowel frequency, and indicators of GI function in institutionalized wheelchair-bound adults	15 institutionalized wheelchair-bound adults aged 23-57 years (mean age = 43 years) with a variety of health issues (7 with multiple sclerosis, 4 with cerebral palsy, 4 with neuropathies; 10 suffering from dysphagia and 5 without dysphagia)	Inulin derived from chicory	0 or 15 g/day for 3 weeks	No significant difference was found in calcium retention as an indicator of bone resorption or defecation frequency, although use of enemas and laxatives significantly decreased. There were no issues with acceptability or tolerance as assessed by questions regarding GI discomfort.
Daubioul et al. (2005)	Randomized double-blind, placebo-controlled, cross-over pilot study of the ability of oligofructose to decrease TAG accumulation in liver tissue of patients with nonalcoholic steatohepatitis	7 patients with nonalcoholic steatohepatitis	Oligofructose from chicory	0 or 16 g/day for 8 weeks	Ingestion of oligofructose resulted in a significant decrease in the level of AST and non-significant decreases in ALT and TAG. No reduction was seen in GGT, ALP, bilirubin, uric acid, glucose, insulin, total or HDL cholesterol, or C-peptide concentrations or in liver size. No adverse effects were reported

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Davidson et al. (1998); Davidson & Maki (1999)	Randomized, double-blind, crossover trial of the effect of inulin on serum lipids in hypercholesterol-emic adults.	12 men and 13 women with a mean age of 60.2 years and LDL cholesterol levels between 3.36 and 5.17 mmol/L but no other health conditions	Inulin derived from chicory	0 or 18 g/day for 6 weeks	Both TC and LDL cholesterol increased nonsignificantly during the control phase and decreased nonsignificantly during the inulin phase; the difference between the two treatments was statistically significant. Mild GI discomfort was more common during the inulin than the control phase, but no significant adverse effects were observed.
de Luis et al. (2010) [article in Spanish with English abstract]	Randomized, double-blind, placebo-controlled trial of the effect of inulin on biochemistry in obese subjects	34 obese volunteers	Chicory inulin	0 or 3 g/day for 4 weeks	Members of the inulin group, but not the controls, experienced significant decreases in total and LDL cholesterol. There were no changes in anthropometric data in either group and inulin intake did not produce any reported adverse GI effects.
Ellegard et al. (1997)	Randomized, double-blind, crossover study of the effects of inulin or oligofructose on cholesterol absorption and excretion of cholesterol, bile acids, energy, nitrogen, and minerals	10 ulcerative colitis patients (5 of each sex, aged 30-71 with mean age 54 years) with conventional ileostomy	Oligofructose or inulin from chicory	0 or 17 g/day for 3 days	Nearly all of the ingested inulin and oligofructose were recovered in the ileostomy effluents. Dry solid and energy excretion both increased significantly on both inulin and oligofructose. Cholesterol absorption, excretion of cholesterol, bile acids, nitrogen, fat, calcium, magnesium, zinc, and iron were not significantly affected by inulin or oligofructose. Daily ingestion of 17 g/day inulin or oligofructose in 3 daily doses was well tolerated.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Jackson et al. (1999)	Randomized, double-blind, placebo-controlled parallel-group study of the effect of inulin on fasting blood lipids, glucose, and insulin levels in adults with hyperlipidemia	54 men and women between the ages of 35 and 65 years (mean age = 52.2 years) with moderate hyper-lipidemia	Chicory inulin	0 or 10 g/day for 8 weeks	All participants completed the study with no significant changes in body weight or levels of plasma glucose, TC, HDL or LDL cholesterol, apoA-1, or apoB; however, plasma insulin and TAG levels were significantly reduced in the inulin group as compared to the controls. The authors did not report any adverse GI effects.
Kleessen et al. (1997)	Randomized, double-blind, placebo-controlled parallel study of the effect of inulin on microbiota, SCFA and lactate levels, pH, β-glucosidase or β-glucuronidase activity, and bowel habits of elderly women suffering from constipation	35 constipated but otherwise healthy female patients aged 68-89 years (mean age = 76.4 years)	Inulin derived from chicory	0 or 20 g/day for 8 days, then 0 or 40 g/day for 8 days	Fecal pH, concentrations of SCFA and lactate, and β-glucosidase and β-glucuronidase activities did not differ significantly between groups. Individuals consuming inulin showed significant increases in bifidobacteria and decreases in enterococci. Total bacterial counts did not differ between groups. Inulin showed a significantly better laxative effect and reduced functional constipation with only mild reported discomfort, even at 40 g/day; the laxation effect was not related to dose. No adverse effects were reported.
Lopez Roman et al. (2008)	Randomized double-blind study of effects of inulin on primary chronic constipation	4 males and 28 females with mean age = 47 years suffering from primary chronic constipation	Mixture of inulin (not further described) and digestion-resistant maltodextrin	0 or 20 g/day for 20 days	Difficulty of defecation, sensation of incomplete evacuation, and days between bowel movements were significantly reduced by the supplement with no reported adverse events resulting from ingestion of 20 g inulin and maltodextrin/day.

Table 6. Studies of Fructans in Compromised Adults.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Olesen and Gudmand-Heyer (2000)	Randomized, double-blind, placebo-controlled, multi-center parallel group study of the effect of FOS on IBS.	114 otherwise healthy IBS patients—about 85% women—aged 18-70 years (mean age = 45.1 years)	FOS from chicory	0 or 10 g/day for 2 weeks, then 0 or 20 g/day for 10 weeks	Although significantly fewer infections were seen in the test group than among the controls, the authors concluded that FOS did not ameliorate IBS symptoms. There were no significant differences between the groups in improvements in IBS symptoms, defecation frequency, reports of flatulence, or abdominal distension or pain.
Olguin et al. (2005)	Randomized, double-blind, placebo-controlled study of the effect of oligofructose on gut barrier function of burn patients	41 males and females aged 14-70 years with 2nd- or 3rd-degree burn injuries	Oligofructose from chicory	0 or 6 g/day for 15 days	Burns increased intestinal permeability four-fold as compared to the reference group, but no differences were seen in patients receiving oligofructose or sucrose, nor were there significant differences in burn infection rates, complications, need for enteral nutrition, number of surgical interventions, or length of hospitalization. No adverse effects were reported.

7.3. Healthy Children

Studies discussed in this section are summarized in Table 7 at the end of the section.

7.3.1. Studies with scFOS

In an unpublished randomized, double-blind, placebo-controlled study (Abbott 1996) submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula, 283 apparently healthy children aged 10-24 months and attending daycare were enrolled in a study of the effect of scFOS on the incidence of diarrhea in toddlers. Children were randomly assigned to drink a minimum of 500 ml/day of a milk-based beverage containing 0 (n = 144) or 340 mg (n = 139) mg scFOS/100 ml for 16 weeks; the beverage was available ad libitum. Parents and daycare staff recorded daily beverage intake and any symptoms of intolerance—stomach cramps, vomiting, bloating, watery stools, or constipation. Medical staff visited the daycare centers at study initiation and on days 7, 28, 56, 84, and 112 to identify episodes of diarrhea and other events; children's weight and height were recorded at entry and on days 56 and 112. Stool samples were collected at entry and on day 112 and analyzed for lactobacilli and bifidobacteria.

A total of 242 children completed the study, 118 in the scFOS group, and 124 in the control group. The average daily intake of the beverage in the scFOS group was 740 ml, resulting in ingestion of about 2.5 g scFOS/day, equivalent to 220 mg/kg bw/day. No differences were reported in daycare attendance, mean number of feedings, growth, or incidence of stomach cramps or vomiting. The children ingesting scFOS had significantly softer or runnier stools and increased bifidobacteria, but no significant difference was reported for lactobacilli. There was no difference between groups on the incidence of diarrhea, but the scFOS group had significantly shorter duration of diarrheal episodes as well as a significantly reduced incidence of otitis media. No adverse effects due to scFOS treatment were reported.

The dose of scFOS needed to ameliorate constipation in children was assessed in an unpublished randomized, double-blind, placebo-controlled, multi-center, acute-dosage study (Abbott 2000) submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula. Fifty-five male and female children aged 2-5 years (mean = 3.96 years), with a history of constipation but otherwise healthy, were randomly assigned to receive scFOS (n = 33) or sucrose (n = 22). Each child received 200 mg/kg bw of scFOS or sucrose once daily at breakfast; the dose was increased by 200 mg/kg bw every other day until each child produced watery stool or a limit of 800 mg/kg bw/day was reached. Parents were contacted daily to confirm administration of the correct dose, rate the consistency of their children's stools, and report any burping, flatulence, fussiness, or vomiting.

Children's stool was significantly softened at an average dose of 600 mg/kg bw/day, although administration of scFOS up to the maximum dose did not alter the frequency of bowel movements. There were no differences between groups in reported adverse events, and none was serious in either group; the only adverse event attributed to the test article (not described) occurred in the sucrose group. The authors concluded that, "Results from this study indicate that scFOS has good potential for improving the treatment of childhood constipation."

Nakamura et al. (2006) studied the effect of scFOS on diarrhea and weight gain in urban-slum children using a randomized, double-blind, placebo-control design in a 6-month study. A

total of 133 children aged 25-59 months (mean age = 46.4±9.7 months) were enrolled; 64 children were randomized to receive 50 ml/day of an isotonic solution supplemented with 2 g scFOS while 69 children received a similar solution with 1 g glucose. Bodyweight was measured every other day, height and arm circumference were measured monthly, and the children's parents were interviewed daily regarding defecation frequency and stool consistency, any treatment with antibiotics, and abdominal or other side effects.

Compliance with the study protocol was good and the average daily intake of scFOS was 1.90 g throughout the study (147±235 mg/kg bw at the start of the study and 138±222 mg/kg bw at the end). There was no significant difference in growth (bodyweight, height, or arm circumference) between the scFOS and control groups. Children receiving scFOS did not experience fewer incidences of diarrhea than did controls, but the mean duration was significantly shorter. There were no differences in side effects or antibiotic treatment between the groups.

7.3.2. Studies with Other Fructans

Tschernia et al. (1999), in a prospective, randomized, double-blind, placebo-controlled study, investigated the effects of supplementation of a pediatric weaning food with 550 mg FOS (not further characterized) per serving on the health status of the children. A total of 123 healthy infants and toddlers aged 4-24 months (mean age = 11.7 months) were enrolled and completed the study; 63 received prebiotic-supplemented cereal and 60 received unsupplemented cereal for 6 months. Data regarding febrile events, respiratory-tract symptoms, use of antibiotics, and daycare absences were collected weekly and growth indicators (weight and length) were measured monthly.

The average daily consumption of unsupplemented cereal was 35.9 g as compared with 32.6 g for FOS-supplemented cereal; the resulting daily FOS intake was 1190 mg. There were no significant differences in growth, but the FOS group of toddlers had significantly fewer febrile events, respiratory-tract symptoms, periods of antibiotic use, and daycare absences than controls. The authors did not report any adverse effects offsetting the demonstrated benefits.

Using a balanced, randomized, double-blind, crossover design, Griffin et al. (2002) assessed the effect of 8 g/day of oligofructose or a mixture of long-chain inulin and oligofructose on calcium absorption in girls at or near menarche. Fifty-nine healthy girls aged 11 to 14 years (mean age = 12.0 years) with adequate intake of calcium (500 to 1400 mg/day) were randomized to receive, in random order, two 4-g servings of either oligofructose or oligofructose-enriched inulin, or two 4-g servings of placebo (sucrose) daily for 3 weeks, with a 2-week washout period. During the study periods, the girls ingested about 1500 mg calcium/day from fortified orange juice. At the end of each 3-week feeding period, calcium absorption was measured using a dual stable isotope technique.

The groups did not differ in compliance or in calcium intake, and no tolerance problems were observed. Calcium absorption was significantly higher in the group receiving oligofructose-enriched inulin than in the placebo group (38.2 v. 32.3%), but no significant difference was seen between the oligofructose group and the placebo group. The authors concluded that "regular intake of modest amounts of a mixture of inulin+FOS [sic] significantly increases calcium

absorption in girls at or near menarche…without any compensatory increase in urinary calcium excretion."

Griffin et al. (2003) studied the effect of long-chain inulin + oligofructose on calcium absorption of 54 healthy premenarche girls aged 10 to 15 years (mean age = 12.4 years) in a balanced, randomized, double-blind, crossover design. The girls received, in random order, two 4-g servings of oligofructose-enriched inulin or two 4-g servings of placebo (sucrose) daily for 3 weeks, with a 2-week washout period. During the study periods, the girls ingested about 1200 mg calcium/day from fortified orange juice. At the end of each 3-week feeding period, calcium absorption was measured using a dual stable isotope technique.

The addition of 8 g/day of oligofructose-enriched inulin to the diet significantly increased calcium absorption by about 10%; this was not correlated with the girls' age or stage of puberty but was inversely correlated with fractional calcium absorption while on the placebo.

Abrams et al. (2005) studied the effect of long-term administration of long-chain inulin + oligofructose on calcium absorption in a randomized, double-blind, placebo-controlled study. Fifty girls and 50 boys aged 9 to 13 years in good health, selected to approximately match the ethnic distribution of the Houston (TX) area, were randomly assigned to receive 8 g/day of prebiotic or maltodextrin (placebo group) for 1 year. The supplement was consumed daily at breakfast, mixed into 180-240 ml calcium-fortified orange juice. Bone mineral content and density were measured before randomization and after 1 year. Calcium absorption was measured with the use of stable isotopes at baseline, at 8 weeks, and at 1 year.

Two participants withdrew from the prebiotics group, 1 due to increased stool frequency and loose stools and the other for non-fructan-related noncompliance with the study protocol, while 3 control-group participants dropped out, none for reasons related to group assignment. There were no significant differences between the groups in calcium intake, but calcium absorption was significantly greater in the group receiving prebiotics than in the control group at 8 weeks and at 1 year. After 1 year, the prebiotics group had a greater increment in both whole-body bone mineral content and whole-body bone mineral density. No adverse effects on growth or other measures were reported.

A follow-up analysis (Abrams et al. 2007) revealed that the adolescents receiving oligofructose-enriched inulin had a significantly smaller increase in body mass index than did the controls, remaining within the normal rate of body-mass index increase while the control group exceeded it. This beneficial effect was still maintained 1 year after the end of the intervention.

Table 7. Studies of Fructans in Healthy Children.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
scFOS					
Abbott (1996) [unpublished; submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula]	Randomized, double-blind, placebo-controlled study of the effect of scFOS on the incidence of diarrhea in toddlers	283 apparently healthy children aged 10-24 months attending daycare	scFOS	0 or 2.5 g/day for 16 weeks	No differences were reported in daycare attendance, mean number of feedings, growth, or incidence of stomach cramps or vomiting. The children ingesting scFOS had softer or runnier stools and increased bifidobacteria, but no difference was reported for lactobacilli. There was no difference between groups on the incidence of diarrhea, but the scFOS group had shorter duration of diarrheal episodes as well as a reduced incidence of otitis media. No adverse effects due to scFOS treatment were reported.
Abbott (2000) [unpublished; submitted to FSANZ in support of a proposal to allow the addition of scFOS to infant formula]	Randomized, double-blind, placebo-controlled, multi-center, acute-dosage study of the dose of scFOS needed to ameliorate constipation in children	55 male and female children aged 2-5 years (mean = 3.96 years) with a history of constipation but otherwise healthy	scFOS	0 or 200 mg/kg bw/day, increasing by 0 or 200 mg/kg bw/day every 2nd day up to 800 mg/kg bw/day	Children's stool was significantly softened at an average dose of 600 mg/kg bw/day, although administration of scFOS up to the maximum dose did not alter the frequency of bowel movements. The authors concluded that, "Results from this study indicate that scFOS has good potential for improving the treatment of childhood constipation." There were no differences between groups in reported adverse events, and none was serious in either group.

Table 7. Studies of Fructans in Healthy Children.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Nakamura et al. (2006)	Randomized, double-blind, placebo-controlled study of the effect of scFOS on diarrhea and weight gain in urban-slum children	133 children aged 25-59 months (mean age = 46.4±9.7 months)	scFOS	0 or 1.9 g/day for 6 months	There was no significant difference in growth (bodyweight, height, or arm circumference) between the scFOS and control groups. Children receiving scFOS did not experience fewer incidences of diarrhea than did controls, but the mean duration was significantly shorter. There were no differences in side effects or antibiotic treatment between the groups.

Table 7. Studies of Fructans in Healthy Children.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Other Fructans					
Abrams et al. (2005, 2007)	Randomized, double-blind, placebo-controlled study of the long-term effect of fructan intake on calcium absorption	50 healthy girls and 50 healthy boys aged 9 to 13 years	1:1 mixture of long-chain inulin and oligofructose from chicory	0 or 8 g/day for 1 year	Drop-outs did not differ by feeding group, and none were for reasons related to treatment. Calcium absorption was greater in the fructan group at 8 weeks and 1 year. After 1 year, the fructan group had a greater increment in both whole-body bone mineral content and whole-body bone mineral density. No adverse effects on growth or other measures were reported. A follow-up analysis (Abrams et al. 2007) revealed that the adolescents receiving the fructan prebiotic had a significantly smaller increase in body mass index (BMI) than the controls, remaining within the normal rate of BMI increase while the control group exceeded it. This beneficial effect was still maintained 1 year after the end of the intervention.
Griffin et al. (2002)	Randomized, double-blind, crossover study of the effect of fructans on calcium absorption in girls near menarche	59 healthy girls aged 11 to 14 years (mean age = 12.0 years), with adequate intake of calcium	Oligofructose from chicory or a 1:1 mixture of long-chain inulin and oligofructose	0 or 8 g/day for 3 weeks	Calcium absorption was significantly higher in the group receiving the oligofructose-enriched inulin mixture than in the placebo group but no significant difference was seen between the oligofructose group and the placebo group. The groups did not differ in compliance or in calcium intake, and no tolerance problems were observed.

Table 7. Studies of Fructans in Healthy Children.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Griffin et al. (2003)	Randomized, double-blind, crossover study of the effect of fructans on calcium absorption in premenarche girls	54 healthy premenarche girls aged 10 to 15 years (mean age = 12.4 years)	1:1 mixture of long-chain inulin and oligofructose from chicory	0 or 8 g/day for 3 weeks	8 g/day of oligofructose-enriched inulin significantly increased calcium absorption with no reported adverse effects.
Tschernia et al. (1999)	Randomized, double-blind, placebo-controlled study of the effects of FOS on health status	123 infants and toddlers aged 4-24 months (mean age = 11.7 months)	FOS (not further described)	0 or 1.19 g/day for 6 months	There were no significant differences in growth, but the FOS group of toddlers had significantly fewer febrile events, respiratory-tract symptoms, periods of antibiotic use, and daycare absences than the controls. No adverse effects were reported.

7.4. Compromised Children

Studies discussed in this section are summarized in Table 8 at the end of the section.

7.4.1. Studies with scFOS

Juffrie (2002) reported on a randomized placebo-controlled trial in which 93 children aged 1-14 years with acute diarrhea ingested 2.5-5 g scFOS/day (with the dose depending on age) while 25 similar children consumed placebos. Blinding of the study was not reported, nor were further details regarding dose levels by age group. Most of the children receiving scFOS wee under the age of 6 years (55 of 93), while only 14 children were over the age of 10 years. The duration of diarrhea was significantly shortened among the children receiving scFOS compared with controls and the author reported that "No side effects such as an increase of diarrhea or prolonged diarrhea were found."

7.4.2. Studies with Other Fructans

In a prospective, randomized, double-blind, parallel-design, multi-country study (Brazil, Mexico, Spain, and Portugal), 668 malnourished children aged 1-6 years (with weight-for-height between 1 and 3 standard deviations below the median) were assigned to receive nutritional supplements either with or without *B. infantis, L. acidophilus*, and FOS for 4 months (Fisberg et al. 2000). The study was presented as a poster and is available only as an abstract that provided no further description of the FOS or the probiotics. Weight, height, supplement intake, stool frequency and consistency, incidence and duration of sickness, and adverse events were monitored monthly.

A total of 626 children completed the study, 310 in the synbiotic group and 316 in the control group. Due to the nutritional supplementation, both feeding groups experienced catch-up growth, but there were no significant differences between the 2 groups. However, the children aged 3-5 years receiving the synbiotic had significantly fewer sick days than the controls and experienced significantly less constipation. Both nutritional supplements were reported to be well tolerated.

Brunser et al. (2006a) studied the ability of prebiotics to mitigate the adverse effects of antibiotic treatment on the intestinal microbiota of young children. In a prospective, randomized, double-blind, placebo-controlled trial, 140 young children aged 1-2 years who had received 1-week amoxicillin treatment for acute bronchitis were randomly assigned to receive milk-based formula with or without 4500 mg prebiotic 1/100 ml for 3 weeks. The prebiotic was a blend of oligofructose and inulin resulting in about 75% fructans with DP ≤ 9 and 25% with DP ≥ 10. Stool samples were taken at the beginning and end of the antibiotic treatment and on days 7 and 21 of formula administration and analyzed for bacterial populations using fluorescent in situ hybridization (FISH). Formula intake, tolerance, and GI symptoms were recorded daily. If diarrhea was suspected, stool samples were taken for analysis of potential enteropathogens.

Ten children did not successfully complete the antibiotic treatment; the remaining 130 children included 64 assigned to the prebiotic group and 66 assigned to the control group. Of these, 7 children in the prebiotic group and 10 controls failed to complete the protocol, none for reasons apparently related to the intervention. There were no differences between the groups in feeding habits or volume, or in GI symptoms or stool frequency or consistency. Diarrhea

occurred in 4 children in the prebiotic group and 7 controls; all episodes were of short duration. There were no differences between the groups in asymptomatic enteropathogen shedding.

Antibiotic treatment caused a significant drop in total bacterial counts, although *E. coli* counts increased during this period and bacteroides and bifidobacteria were relatively unaffected. After antibiotic treatment ceased, the total bacterial counts rapidly regained their previous level, reaching it by day 7 of the feeding period with no significant differences between the 2 feeding groups. However, bifidobacteria were significantly more prevalent on day 7 in the prebiotic group than in the control group, and lactobacilli and enterococci were non-significantly more prevalent. These differences were maintained through day 21.

The authors concluded: "Prebiotics in a milk formula increase fecal bifidobacteria early after amoxicillin treatment without inducing gastrointestinal symptoms" (Brunser et al. 2006a).

Table 8. Studies of Fructans in Compromised Children.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
scFOS					
Juffrie (2002)	Randomized, placebo-controlled trial of the effect of scFOS on children with acute diarrhea	93 children aged 1-14 years with acute diarrhea	scFOS	0 or 2.5-5 g/day	The duration of diarrhea was significantly shortened among the children receiving scFOS compared with controls and the author reported that "No side effects such as an increase of diarrhea or prolonged diarrhea were found."

Table 8. Studies of Fructans in Compromised Children.					
Reference	**Study Design & Objective**	**Subjects**	**Source & Characteristics of Test Article**	**Dose & Duration**	**Results**
Other Fructans					
Brunser et al. (2006a)	Randomized, double-blind, placebo-controlled trial of prebiotic mitigation of the adverse effects of antibiotic treatment on the intestinal microbiota of young children	140 children aged 1-2 years who had received 1-week amoxicillin treatment for acute bronchitis	Blend of chicory oligofructose and inulin in a 70:30 ratio	0 or 4.5 g/L formula for 3 weeks	Antibiotics caused a drop in total bacterial counts, but they regained their previous level by day 7 of the feeding period in both feeding groups. Bifidobacteria were more prevalent on day 7 in the prebiotic group than in the control group, and lactobacilli and enterococci were non-significantly more prevalent. These differences were maintained through day 21. No withdrawals were for reasons related to treatment. There were no differences between the groups in feeding habits or volume, or in GI symptoms or stool frequency or consistency. Diarrhea occurred in 4 children in the prebiotic group and 7 controls, all of short duration.
Fisberg et al. (2000)	Randomized, double-blind, parallel-design, multi-country study to study the effect on growth recovery of the addition of a synbiotic to nutritional supplements	668 malnourished children aged 1-6 years	FOS (not further described) along with *B. infantis* and *L. acidophilus*	Dose not reported; duration = 4 months	Both feeding groups experienced catch-up growth with no significant differences between the groups. However, the children aged 3-5 years receiving the synbiotic had significantly fewer sick days than the controls and experienced less constipation. Both nutritional supplements were reported to be well tolerated.

8. STUDIES OF FRUCTANS IN ANIMALS

The numerous studies in human infants, children, and adults that demonstrate the safety of ingestion of scFOS and other fructans are supported by research employing a variety of animal models. Many of these studies are discussed in this section and summarized in Table 9 at the end of the section.

8.1. Chicks

8.1.1. Studies with scFOS

In 2 experiments, Fukata et al. (1999) studied the effects of competitive exclusion and ingestion of an undefined preparation of scFOS on colonization of chicks with *Salmonella enteriditis*. Both experiments used one-day-old White Leghorn Hy-Line cockerel chicks caged in battery brooders. In each experiment, 60 chicks were divided into 4 groups of n = 15—a control group, a competitive-exclusion group that received the control diet but was inoculated with an undefined bacterial preparation, an scFOS group for which the feed was supplemented with 0.1% scFOS, and a combination-treatment group that received both interventions. All chicks were inoculated with *S. enteriditis* on day 7 (experiment 1) or day 21 (experiment 2). One day, 1 week, and 2 weeks after inoculation, 5 birds from each group were sacrificed and their ceca evaluated for *Salmonella* spp. as well as *Bifidobacterium, Bacteroides,* and *Lactobacillus* spp., and *Escherichia coli* using plating techniques.

In experiment 1, the enumeration of *S. enteriditis* in the chicks inoculated with the competitive-exclusion preparation was significantly decreased compared with the other three groups while in experiment 2, *S. enteriditis* was significantly decreased in the scFOS group and the combination-treatment group. No significant differences between groups were noted on cecal numbers of total bacteria, *Bifidobacterium, Bacteroides, Lactobacillus*, or *E. coli*. The authors concluded that low-dose feeding of scFOS in the diet of chicks with a competitive-exclusion treatment may not lead to a shift in the intestinal gut microbiota but may result in reduced susceptibility to *Salmonella* colonization.

8.2. Mice

8.2.1. Studies with scFOS

Howard et al. (1995) tested the effects of scFOS, XOS, and gum arabic on cecal and colonic microbiota in rats and mice. In the mouse study, 52 male BALB/c weanling mice weighing an average of 22.3 g (13 mice/group) were housed individually and given free access to mouse chow and water containing 0 or 30 g/L of scFOS, XOS, or gum arabic for 14 days. After sacrifice, the cecum and colon were excised and bacteria were enumerated.

There were no differences in feed and water intake or in weight gain. The mice ingesting scFOS had significantly higher concentrations of bifidobacteria than the other 3 groups, both in absolute numbers and as a percent of total bacteria.

Gaskins et al. (1996) studied the effect of scFOS on the inflammatory response to *Clostridium difficile* in antibiotic-compromised C57BL/6NHsd mice. Twenty-four 12-week-old male mice (body weight not reported) were individually housed with free access to feed and water. After 5 days acclimatization, 12 mice received 100 µg cefoxitin/g body weight; on the following day, 6 treated and 6 untreated mice were randomly assigned to receive 30 g scFOS/L

drinking water for the next 5 days, when all mice were challenged with a *C. difficile* suspension, following which the assigned treatment continued for another 5 days. Feed intake and body weight were assessed daily and feces were collected on days 5, 8, 12, and 15 and evaluated for diarrhea, anaerobic bacteria, total clostridia, and *C. difficile*. After sacrifice on day 16, the small intestine, cecum, and colon were excised and assessed for immunocytochemical analysis.

The mice that had received antibiotic treatment exhibited increased susceptibility to clostridia, but this effect was significantly reduced in mice receiving scFOS. The authors noted that the diminution of toxin A titers in antibiotic-treated mice receiving scFOS was "more striking" than the effects of scFOS on bacterial numbers, and suggested that this indicates either reduced toxin production or direct binding of toxin A by scFOS. No adverse effects of scFOS ingestion were reported.

Pierre et al. (1997) used C57BL/6J-*Min/+* mice, which are heterozygous for a mutation in the *Apc* gene, as a model for colon cancer. Forty mice aged 5-6 weeks were randomly divided into 8 groups and housed 1 group/cage: 4 groups of females (n = 8 mice/group) aged 5 or 6 weeks and 4 groups of males (n = 2 mice/group) aged 5 or 6 weeks. The control mice were fed a low-fiber diet while the 3 test groups received the same diet supplemented with 5.8% retrograded corn starch, starch-free wheat bran, or scFOS; feed and water were available *ad libitum* in all groups. Feeding continued for 42 days and the mice were weighed weekly. After sacrifice, the colon and small intestine were removed and examined for tumors, lymphoid nodules, and aberrant crypt foci.

There were no significant differences in growth across the diet groups and no sex- or age-related differences were noted on tumors. A significant reduction in colon tumors, which were largely associated with the distal colon, was observed in mice receiving scFOS. The beneficial effects of scFOS were limited to the colon, and no differences were seen in the small intestine. The authors concluded that "short-chain fructo-oligosaccharides counteract advanced stages of colon carcinogenesis, possibly via stimulation of antitumoral immunity by modulation of the colonic ecosystem."

Ohta et al. (2002) performed ovarectomies on 32 female 8-week-old ddY mice (weight not reported) while 32 similar mice received sham operations in which the ovaries were not removed. After 7 days, the animals were divided into 4 feeding groups to receive a control diet, control diet with 5.0% scFOS, control diet with 0.5% isoflavones, or control diet with both 5.0% scFOS and 0.5% isoflavones for 6 weeks with free access to feed and water. After sacrifice, blood and cecal contents were collected and femora and uterus were sampled. Blood was assayed for genistein, daidzein, and equol, cecal content was evaluated for β-glucosidase, and femora were analyzed for bone area, total mineral content and density, calcium, magnesium, and phosphorus.

The ovarectomized mice receiving scFOS gained significantly more weight than did those receiving isoflavones and both scFOS and isoflavones prevented femoral bone loss. scFOS also significantly increased cecal β-glucosidase activity and equol production from daidzein. No adverse effects were reported from diet supplementation with 5% scFOS.

In a study of scFOS as a mucosal immunoregulator (Hosono et al. 2003), 6-week-old female BALB/c mice (number and weight not reported) were randomly assigned to 3 groups to

receive diets with 10% sucrose and 0% scFOS, 7.5% sucrose and 2.5% scFOS, or 2.5% sucrose and 7.5% FOS for either 4 or 6 weeks. The mice had free access to feed and water. Serum samples were collected every week for analysis of IgG1 and IgG2a and fecal samples were collected biweekly for analysis of IgA. After sacrifice of the mice, splenocytes and Peyer's patch cells were extracted for preparation of $CD4^+$ T cells and measurement of IgA in the cell culture supernatant; the T cells were cultured and assayed for IL-5, IL-6, IL-10, and IFN-γ.

The 3 groups showed equivalent feed intake and growth. IgA secretion from Peyer's patch cells was significantly increased dose-dependently by scFOS administration, as was production of IL-5, IL-6, IL-10, and IFN-γ from $CD4^+$ T cells. On the other hand, scFOS suppressed serum IgG1 activity. The authors concluded that "this information suggests that scFOS acts as a preventive agent with regard to food allergy" via changes in the intestinal environment.

Nakamura et al. (2004) studied the potential benefits of scFOS to the mucosal immune system in infancy using neonatal BALB/c mice. At 2 days of age, litter sizes were adjusted to 4-6 pups and the pups and their dam were housed together and fed *ad libitum* a diet containing 0 or 5% scFOS. Pups were weaned at 21 days of age and fed the same diets *ad libitum* to age 23, 30, 38, or 44 days. Twenty-four-hour fecal samples were collected on days 28, 36, and 42 and analyzed for IgA level. After sacrifice, the small intestine and colon were removed, luminal contents were flushed and analyzed for SCFA, segments were weighed, and the tissue was homogenized and centrifuged for analysis of IgA.

Feed intake and body weight did not differ between the groups. Mice receiving FOS had significantly higher levels of IgA in the jejunum, ileum, and colon, as well as in the feces, and significantly higher levels of cecal acetate, butyrate, and propionate. No adverse effects were observed, and the authors concluded that scFOS increases IgA levels in the colon and also in the small intestine.

The ability of scFOS to reduce intestinal damage associated with ulcerative colitis was studied in a murine model by Winkler et al. (2007). Sixty 10-week-old female C57BL/6 mice (body weight not reported) were given dextran sulfate sodium (DSS) in their drinking water to induce colitis. The mice were randomly assigned to 6 groups (n = 10 mice/group): control (water only for 14 days), DSS group (water with DSS for 14 days), DSS+scFOS group (water with DSS + scFOS for 14 days), DSS>scFOS (water with DSS for 9 days followed by scFOS for 5 days), water>DSS (water only for 9 days followed by water with DSS for 10 days), and water>DSS+scFOS (water only for 9 days followed by water with DSS and scFOS for 10 days). The scFOS was given by orogastric gavage in 2 daily doses totaling 300 mg/day. The mice were observed daily for weight loss, stool consistency, rectal bleeding, and overall condition. A breath test was given at baseline and at the end of treatment to measure H_2 excretion. After sacrifice, the colon was removed and divided into proximal and distal segments.

Treatment with scFOS significantly increased crypt depth and reduced disease symptoms and damage to the distal colon. No adverse effects were seen attributable to scFOS, and the authors concluded, "This finding supports other studies that have demonstrated that certain fermentable carbohydrates may protect against intestinal inflammation and reduce the symptoms of IBD patients."

As a model for allergic skin disease, Fujiwara et al. (2010) studied the effect of dietary scFOS on contact hypersensitivity in NC/Nga mice in 2 experiments. In the first experiment, 5-week-old female mice were given free access to water and to diets that contained 0 or 5% scFOS (n = 6 mice/group) for 3 weeks, after which 2,4-dinitrofluorobenzene was applied to their ears to induce contact hypersensitivity 5 times at 7-day intervals. The mice were sacrificed 38 days after the first application, blood was taken, cecal contents were collected, and the right ear auricle was excised. In the second experiment, 9-week-old male and 8-week-old female mice were again given free access to water and to diets that contained 0 or 5% scFOS (n = 8 mice/group, 2 males and 6 females). The mice were housed in 2 cages, each with 1 male and 3 females; after mating, pregnant females were housed individually. Pups were separated from their dams on postnatal day 21 and given diets with 0 or 5% scFOS. The result was a 2x2 factorial design with the dam having received 0 or 5% scFOS x pup receiving 0 or 5% scFOS, with 6-9 mice/cell. After 3 weeks, contact hypersensitivity was induced as in the first experiment. The mice were sacrificed on postnatal day 73, blood was taken, and the right ear auricle was excised.

In the first experiment, the mice receiving scFOS exhibited significantly less inflammation than did control mice. In the second experiment, the pups that ingested scFOS had significantly less inflammation than the pups that did not, but maternal scFOS consumption had no effect. The reduction of inflammation was accompanied by altered expression of cytokine genes, with scFOS-fed mice showing significantly lower IL-12, IL-17, and IL10; however, there were no significant differences in IFN-γ, IL-4, IL-6, IL-13, or TGF-β1. No differences were seen in cecal levels of bifidobacteria, and the authors concluded that "it is unlikely that the proliferation of bifidobacteria in the gut is responsible for the reduction" in inflammation.

Takemura et al. (2010) fed 6-week-old female ddY mice one of 3 feeding regimens *ad libitum* (n = 6 mice/group): control chow, chow with 5% chicory inulin, or chow with 5% scFOS. After 2 days and again after 28 days on their assigned diets, the mice were inoculated intragastrically with 10^8 cfu *L. plantarum* strain LP14. Feces were collected after 0, 6, 24, 30, 48, and 54 hours and L. plantarum recovery was enumerated.

Recovery of *L. plantarum* in mice fed control, inulin, or scFOS diets for 2 days did not differ at 0 or 6 hours, but was significantly higher in both the scFOS and inulin groups than in the controls after 24 and 30 hours; by 48 hours, differences were no longer apparent. The same pattern emerged when *L. plantarum* recovery was assessed in mice receiving their assigned diets for 28 days. In both assessments, the area under the recovery curve was highest for mice fed the diet with inulin and lowest for the control mice. The authors concluded that dietary fructans promoted growth of *L. plantarum* in the colon, but did not improve its ability to colonize the mouse gastrointestinal tract.

8.3. Hamsters

8.3.1. Studies with scFOS

Wolf et al. (1997) conducted 2 experiments to assess the effect of scFOS treatment on *Clostridium difficile*-induced colitis in 6-8-week-old female golden Syrian hamsters weighing 80-120 g previously gavaged with ciprofloxacin to sterilize the GI tract. In the first experiment, 41 hamsters were randomized to 6 treatments in a 2x3 factorial design: 0 or 30 g scFOS/L drinking water x 0, 0.5×10^9, or 2.0×10^9 cfu *C. difficile*. In the second experiment, 63 hamsters

were randomly assigned to 8 cells in a 2x2x2 design: 0 or 30 g scFOS/L drinking water x 0 or $2.0x10^9$ cfu *C. difficile* x 0 or 50 mg/kg bw/day vancomycin. In both experiments, survival times were recorded up to a maximum of 24 days.

The average daily intake of water by the hamsters in both experiments was about 8 ml and, thus, the intake of scFOS was about 240 mg/day. Treatment with scFOS significantly increased median survival time of *C. difficile*-challenged hamsters. The authors suggested that the mechanism for this benefit might be either the bifidogenic property of scFOS or through its fermentation and resulting increase in SCFA.

8.4. Rats

8.4.1. Studies with scFOS

Takeda and Niizato (1982; presented in Carabin and Flamm 1999) studied possible laxation effects resulting from oral administration of scFOS. Forty-eight 7-week-old male Sprague–Dawley rats were divided into 8 groups. Fasted test animals received a single oral dose of 3 or 6 g/kg bw of test substance dissolved in 2 ml water; two groups received scFOS; 2 groups each received sorbitol and maltitol as positive controls and 2 groups received glucose as a negative control. Rats were individually housed with no access to food or water and the number and consistency of stools produced were recorded at hours 6 and 24 following dosing. Up to hour 6, no loose stooling was observed in any group. Between hour 6 and hour 24, half of the rats in both groups receiving scFOS exhibited loose stools. Stool liquidity increased dose-dependently, with only 1-2 watery stools in the 3-g/kg bw group and 2-4 watery stools in the 6-g/kg group. With sorbitol, 1-5 watery stools were seen in 4 animals in the 3-g/kg bw group and all 6 animals had watery stools in the 6-g/kg bw group. In the 3-g/kg bw maltitol group, 4 rats had 1-5 watery stools while in the 6-g/kg bw group 5 rats had 1-14 watery stools. Among the animals receiving glucose, 1 watery stool was observed in 1 rat in the 3-g/kg bw group and none in the 6-g/kg bw group. It was concluded that the laxative effect of scFOS, while greater than that of glucose, was less than that of the 2 sugar alcohols tested.

Oku et al. (1984) studied the digestibility of scFOS in one *in vitro* experiment and two animal studies. In the *in vitro* study, scFOS was exposed to pancreatic and small-intestinal homogenates and to purified sucrase-isomaltase complex. In the first animal experiment, group-housed male Wistar rats weighing about 50 g (number not reported) were given *ad libitum* access to water and diets containing either 0 or 20% scFOS for 6 weeks. After sacrifice, the pancreas was removed and mucosa from the duodenum, jejunum, and ileum were scraped and tested for the activities of enzymes that hydrolyze 1-kestose and nystose. In the second experiment, 0.5 ml of isotopically labeled scFOS was injected into the subcardinal vein of male Wistar rats weighing about 230 g (number not reported), which were then placed in metabolic cages; excreted CO_2 was measured and urine and feces were collected.

Results of the *in vitro* study demonstrated that little or no digestion of 1-kestose or nystose occurred; no activity of α-AMY could be observed in pancreatic, liver, or kidney homogenate and only trace levels in duodenal, jejunal, and ileal mucosa—about 0.1% of the activity level produced by maltose—and hydrolysis of nystose was significantly less than that of 1-kestose. It was also found that combinations of scFOS with sucrose or maltose had no effect on the rate of digestion of those free sugars.

The 6-week feeding study was intended to test whether long-term ingestion of scFOS might induce greater activity of hydrolyzing enzymes, but this did not occur. Indeed, it was reported that the activities of sucrase and maltase tended to decrease during long-term feeding of scFOS.

The purpose of the intravenous study was to determine if any small amounts of 1-kestose or nystose that might be absorbed intact could be hydrolyzed by enzymes of some internal organ. Nearly all injected radioactivity was excreted in the urine; less than 1% appeared in expired CO_2 and only 1.24% appeared in feces. Thin-layer chromatography of the excreted radioactivity determined that it was in the form of unchanged scFOS, indicating that no hydrolysis of scFOS occurred.

Hidaka et al. (1986) randomly assigned 24 individually housed male Wistar rats (age not reported) weighing about 170 g to 4 dietary groups (n = 6 rats/group) to receive diets supplemented with 0, 0.4%, 2.0%, or 10.0% scFOS for 2 weeks; feces and urine were collected at the beginning and end of treatment. No effect was observed in production of SCFA at the lower doses of scFOS, but addition of the prebiotic at 10% dietary concentration resulted in a significant increase in SCFA and decrease in fecal pH.

Ohta et al. (1995) studied the effect of scFOS on absorption of calcium and magnesium along the colon and rectum of 7-week-old male Sprague Dawley rats (body weight not reported). Twenty-eight rats were individually housed and divided into 2 groups (n = 14 rats/group) to receive *ad libitum* a control diet containing 10% sucrose or a test diet containing 5% sucrose and 5% scFOS for 8 days. Feces were collected on day 7 and, after sacrifice, the cecum, colon, and rectum were excised and all contents collected.

Feed intake was significantly lower among rats receiving scFOS, but there was no significant difference in bodyweight. Rats fed scFOS had significantly higher wet weight of cecal contents and significantly greater absorption of calcium and magnesium. The authors concluded that, "The addition of [scFOS] to the diet significantly increased the absorption of both calcium and magnesium. Moreover, about half of the increase from [scFOS] consumption takes place in the colon and rectum."

Howard et al. (1995) tested the effects of scFOS, XOS, and gum arabic on cecal and colonic microbiota in rats and mice. In the rat study, 44 male Sprague-Dawley weanling rats weighing an average of 51.7 g were individually housed and given *ad libitum* access to rat chow and water containing 0 or 30 g/L of scFOS, XOS, or gum arabic (n = 11 rats/group) for 14 days. After sacrifice, colons and ceca were examined for morphological change (crypt depth, cell density, and proliferation zone).

There were no differences in feed and water intake or in weight gain. The study found variable morphological effects: cecal crypt depth was significantly greater with ingestion of XOS than the other treatments and significantly less with scFOS ingestion than with the other treatments while in the colon the effects were reversed with scFOS producing the greatest crypt depth and XOS the least; the proliferation zone was significantly less in rats receiving scFOS or XOS than controls or rats receiving gum arabic; and cell density was lower in rats fed scFOS or gum arabic than the other diets. However, none of these differences was regarded by the authors as functionally significant.

In a 2x2 factorial study of the effects of scFOS on magnesium absorption (Baba et al. 1996), 28 5-week-old male Sprague-Dawley rats underwent cecal cannulation before being allocated to 4 experimental groups (n = 7/group) to receive different diets. The rats in 2 groups received magnesium in the diet while the rats in the other 2 groups were fed magnesium-free diets but received cecal infusions of magnesium. One of each of these groups received diets lacking in fructans while the other group received diets containing 5% scFOS. The animals were individually housed and remained on their assigned diets for 15 days; they were subjected to magnesium-balance studies after days 3 and 11, during which all feces and urine were collected. After sacrifice, the cecum was excised and both tissue and contents were weighed.

There were no differences among the 4 groups in feed consumption or body weight. Absorption of magnesium was the same whether it was included in the diet or infused into the cecum; the inclusion of scFOS in the diet significantly increased magnesium absorption in both conditions. The authors suggested that this may result from lowered luminal pH due to fermentation of the scFOS, producing SCFA.

Campbell et al. (1997) randomly assigned 50 individually housed male Sprague-Dawley rats (average weight = 411 g) to receive one of 5 treatments for 14 days (n = 10/treatment): control diet, control diet + 5% microcrystalline cellulose (MCC), control diet + 5% MCC + 6% scFOS, control diet + 5% MCC + 6% oligofructose from chicory, or control diet + 5% MCC + 6% XOS. In the experimental diets, oligosaccharides partially replaced the dextrose component of the control diet. The XOS were derived by enzymatic hydrolysis of birchwood xylan and consisted primarily of xylobiose, -triose, and -tetrose. Feed intake was measured daily and body weight every 3 days. After sacrifice, the cecum and colon were excised and weighed; contents were extracted and analyzed for pH, SCFA and lactate concentrations, and bacterial enumerations; and the thickness of cecal and colonic cell walls was measured.

Diets containing 6% oligofructose or scFOS produced significantly higher cecal butyrate concentrations compared with the control diet, MCC alone, or control + xylooligosaccharide diet. Total cecal pools of SCFA were significantly higher and pH was significantly lower after feeding of the diet containing oligosaccharide. Cecal total weight and wall weight were higher from oligosaccharide ingestion, while colonic wet weight was higher for rats consuming XOS compared to other treatments; colon wall weight was unaffected by any treatment. Cecal bifidobacteria and total anaerobes were higher while total aerobes were lower in rats fed oligosaccharide diets compared with those fed the control diet. Cecal lactobacilli levels were unaffected by treatment. The authors concluded that ”providing these oligosaccharides as ingredients in nutritional formulas could benefit the health of the gastrointestinal tract.”

In order to evaluate the effects of scFOS on plasma lipids and the activity of fatty acid synthase in diabetic patients, Agheli et al. (1998) gave scFOS-supplemented diets to insulin-resistant male Sprague Dawley rats for 3 weeks. Five-week-old rats were assigned to receive standard rat chow (57.5% starch, 5% fat; n = 12 rats), a sucrose-rich diet (57.5% sucrose, 14% fat; n = 7 rats), or the sucrose-rich diet supplemented with 10% scFOS (n = 12 rats). Feed intake was controlled by pair feeding in which rats in the sucrose group were offered feed providing the energy equivalent of the consumption of the scFOS-group rats on the previous day. Feed intake and rats’ body weights were recorded daily; after sacrifice in the fed state blood was collected for measurement of glucose, insulin, TAG, SCFA (acetate, butyrate, and propionate) and plasma

lipids (very low, low, intermediate, and high density lipoprotein) and samples of liver and retroperitoneal adipose tissue were taken for measurement of fatty acid synthase activity.

There was no difference between the sucrose-rich and the scFOS-supplemented groups in final body weights, but the scFOS-supplemented rats exhibited significantly lower liver weights, plasma fatty acids, plasma glucose, and TAG concentrations. No effect was seen on insulin, plasma phospholipids, total cholesterol (TC), cholesterol esters, or lipoproteins. While no differences were evident in plasma butyrate or propionate concentrations, acetate was significantly higher in the plasma of rats given scFOS. Fatty acid synthase activity in the liver was significantly lower in scFOS-supplemented rats than those receiving the sucrose-rich diet, but it was significantly higher in adipose tissue. All of these differences were regarded by the authors as beneficial in normalizing fatty acid synthase activity and reducing plasma levels of glucose, free fatty acids, and TAG in insulin-resistant rats.

In a study comparing the effects on mineral absorption of 1-kestose (GF_2), nystose (GF_3), or a mixture of the two, Ohta et al. (1998a) housed 49 5-week-old male Sprague-Dawley rats in individual metabolic cages and randomized them to receive 1 of 7 experimental diets with n=7 rats/diet. Rats in the control group received a diet containing 10% sucrose while rats in the 6 experimental groups received diets with 5% sucrose and 1-kestose (GF_2), nystose (GF_3), or a mixture of 1-kestose and nystose at either 5% or 10% dietary concentration. The rats were fed the experimental diets *ad libitum* for 6 weeks and feed intake and body weight were measured every 2nd day. Feces and urine were collected and nitrogen, calcium, and magnesium balance were examined after the 2nd and 4th weeks.

Feed intake and body weight gain did not differ significantly among the groups. All oligosaccharide varieties increased calcium and magnesium absorption dose-dependently and increased fecal excretion of nitrogen non-dose-dependently. The different degrees of polymerization of the test oligosaccharides did not differ significantly in their effects, although the authors conceded that comparison of oligosaccharides with DP = 3 vs. DP = 4 may not be sufficient to conclude that chain length is of no nutritional importance.

Ohta et al. (1998b) studied the effect of scFOS on calbindin-D9k concentrations in the small and large intestines. Five-week old male Sprague Dawley rats (n = 27) were housed in individual metabolic cages and fed a diet containing 0, 5, or 10% scFOS (n = 9/treatment). Sucrose was added to the first 2 diets at 10% dietary concentration and to the last at 5%. On the final day of feeding, blood was drawn, the rats were killed, and the mucosa was collected from the proximal and distal small intestine, the cecum, and the distal colon.

Ingestion of scFOS significantly increased absorption of calcium and the levels of calbindin in both large intestinal segments dose dependently while significantly decreasing calbindin in both small intestinal segments. The authors concluded that mucosal calbindin is regulated independently in the small and large intestine and that scFOS stimulates calcium absorption by both the paracellular and transcellular route from the large intestine.

Ohta et al. (1998c) subjected 30 individually housed 4-week-old male Sprague-Dawley rats to surgical removal of the stomach (n = 16) or a sham operation in which the abdominal cavity was opened but a gastrectomy was not performed (n = 14). Two of the gastrectomized rats died prior to initiation of the feeding program. For 4 weeks, half of the rats from each group

received 15 g AIN-93 diet/day as a control while the remainder received the same diet with 7.5% scFOS replacing sucrose. Feces were collected almost daily and calcium and phosphorus contents were measured. After sacrifice, the rats' femurs and tibiae were collected.

Neither gastrectomy nor substitution of scFOS for sucrose had any significant effect on feed intake or weight gain. Gastrectomy significantly increased absorption of phosphorus but reduced calcium absorption and both calcium content and bone mineral density of the femur and tibia, while ingestion of scFOS significantly increased calcium absorption and ameliorated the decrease in calcium content and bone mineral density in gastrectomized rats. The authors speculated that these results suggest that $CaCO_3$ was not dissolved in the absence of gastric acid, thus decreasing the formation of insoluble phosphorus compounds; the role of scFOS was to lower the pH of the colon and thereby enhance calcium absorption.

In a study of the effect of scFOS on calcium absorption, Morohashi et al. (1998) randomly divided 16 45-day-old male Wistar rats into two groups, a control group (n = 8) receiving the AIN-93 diet and a scFOS group (n = 8) with a diet containing 5% scFOS replacing half of the sucrose in the control diet. The rats were pair-fed from the age of 51 days after a 3-day adaptation period and a 3-day free-access period to estimate the amount of food required for pair-feeding. A constant amount of calcium (95 mg/day) was fed to the rats in each group. Body weight was recorded daily. At age 60 days, a 3-day metabolic study was performed by intravenous injection of ^{45}Ca. Both total calcium and ^{45}Ca were measured in feces, urine, and serum.

There were no differences between groups in feed intake or body-weight gain and no adverse effects such as weight reduction or diarrhea were observed. Calcium absorption and urinary calcium were significantly greater in rats fed scFOS. Calcium balance in the scFOS group was also significantly improved.

Ohta et al. (1999) fed 24 gastrectomized rats diets supplemented with either iron citrate or heme iron and with 0 or 7.5% scFOS derived for 4 weeks. Blood was collected every 2 weeks and analyzed for hematocrit and hemoglobin concentrations. The gastrectomized rats exhibited anemia whether they were receiving heme iron or iron citrate, but inclusion of 7.5% scFOS significantly improved iron absorption from both iron sources and reduced or prevented anemia.

In order to test the effects of short-term and long-term ingestion of scFOS in rats, 36 6-week-old male Wistar rats with an average weight of 115 g were fed a basal low-fiber diet or the same diet containing 9% scFOS (replacing corn starch) for 2, 8 or 27 weeks (Le Blay et al. 1999). Rats were individually caged with feed and water available *ad libitum*. Feed intake and body weight were recorded every 2-3 days for the first 2 weeks and then twice a month. One week before sacrifice rats were placed in metabolic cages for measurement of transit time and collection of feces. After sacrifice, ceca were weighed and their contents collected for measurement of lactate, SCFA, dry-matter evaluation, and bacterial enumeration.

There were no differences between groups in feed intake or body weight. Cecal SCFA concentration was significantly higher in rats fed scFOS than in those fed the control diet. Butyrate production was significantly higher in rats fed scFOS at all time periods while lactate concentration was significantly elevated after 2 weeks but then decreased. Ingestion of scFOS significantly increased the concentrations of total lactic acid bacteria and *Lactobacillus* spp. after

2 weeks, but these effects disappeared after 8 and 27 weeks of scFOS consumption. The authors suggested that, over time, the cecal microbiota may adapt to continuous intake of scFOS, promoting the growth or metabolic activity of lactate-utilizing bacteria.

In an investigation of the effect of scFOS on mineral absorption, mineral concentration, and femoral bone structure, Takahara et al. (2000) fed 16 42-day-old male Wistar rats (mean weight 173 g) either a control AIN-93 diet (n = 8) or a diet containing 5% scFOS (n = 8); the scFOS replaced half of the sucrose in the control diet. The rats were individually housed in metabolic cages. After 3 days acclimatization, the rats were fed their assigned diets supplemented with 95 mg calcium and 8 mg magnesium/day for 15 days, and calcium and magnesium absorption was assessed based on feces collected in a 3-day metabolic study. After sacrifice, the right femur of each rat was removed, the distal metaphysis was sagittal-sectioned, and the middle of the diaphysis and neck were cross-sectioned. Calcium, magnesium and phosphorus in the three samples were measured.

Rats in the 2 groups did not differ in bodyweight gain or in feed intake. Absorption of both calcium and magnesium were significantly greater in rats receiving scFOS than in the controls. Trabecular bone volume at the metaphysis and bone volume at the neck of the femur were significantly larger in rats ingesting scFOS and the concentrations of calcium, magnesium, and phosphorus were significantly higher in each region of the bone surface. There was a significant association between absorption of calcium and magnesium and concentrations of those minerals in bone.

Perrin et al. (2001) carried out 2 experiments to assess the effects of three different fibers on SCFA production in 10-week-old male and female BDIX rats. The fibers tested were scFOS, resistant starch, and starch-free wheat bran. In the first experiment, intended to determine the period of adaptation to the diets, 96 rats were given free access to a control diet or a diet containing one of the 3 fibers at a concentration of 8% for 2, 16, 30, or 44 days (6 rats/diet/duration). At the end of the assigned feeding period, the rats were killed and the large intestine was isolated, weighed, and divided into the cecum and proximal and distal colon. Mucosal samples were taken from each section and the digesta were analyzed for SCFA, residual starch, and lactic acid.

Rats' weight gain was not affected by diet. All of the fibers showed significant differences from the control diet in SCFA production, but the specific SCFA produced varied between the different fibers: wheat bran produced primarily acetate and little butyrate, while resistant starch and scFOS produced both acetate and butyrate as well as propionate. Inter-individual variability in SCFA production among the rats in each feeding group was lowest at day 44, suggesting that the effects of fiber ingestion had not stabilized prior to that time.

In the second experiment, 36 10-week-old male BDIX rats were given one of the 4 diets for 44 days and then injected subcutaneously with azoxymethane and retained on their assigned diet for another 30 days. After sacrifice, large intestines were removed and sectioned as in the first experiment and each section was examined for aberrant crypts and aberrant-crypt foci.

Diet had no effect on weight gain, but rats receiving scFOS or resistant starch had significantly fewer aberrant-crypt foci than did control-group rats. The authors concluded that "butyrate is associated with protection against the initial stages of colon carcinogenesis."

In a review article, Oku and Nakamura (2002) reported a study in which ^{14}C-scFOS was orally administered to both conventional and germ-free male Wistar rats weighing about 230 g housed in metabolic cages. In the conventional rats, about 60% of the administered radioactivity was exhaled as CO_2 within 24 hours. The total release of radioactivity was similar to that observed with ^{14}C-sucrose, but was delayed about 3 hours. However, administration of ^{14}C-scFOS to germ-free rats resulted in only trace exhalation of radioactivity, demonstrating that metabolism is provided only through fermentation by intestinal microbes.

Cherbut et al. (2003) studied the effect of scFOS on rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis. Individually housed male Wistar rats (age not reported) weighing 246±6 g, with free access to feed and water, were surgically equipped with gastric catheters and randomly assigned to 3 groups (11-12 rats/group) to receive twice-daily infusions of 1 g scFOS/day, 10^{11} cfu/day of lactic-acid bacteria, or saline. After 2 days on treatment, colitis was induced by intracolonic administration of TNBS. Half of the rats in each group were sacrificed after 7 days and the others after 14 days. The colon was removed and opened and colonic damage and inflammation were assessed while cecal contents were collected for bacterial enumeration and SCFA and lactate analysis.

TNBS administration produced sharply decreased feed intake and weight loss, but these effects were significantly reduced in rats receiving scFOS. While scFOS had little effect on colonic inflammation within 7 days, it significantly reduced inflammation within 14 days. The prebiotic treatment also significantly increased cecal levels of SCFA, particularly butyrate, and lactate.

No adverse effects attributable to scFOS were reported, and the authors concluded that, "oral FOS has a beneficial effect on intestinal inflammation in rats. It diminishes the anorexia and weight loss associated with the onset of inflammation; it then reduces the extent of the damage and promotes epithelial healing."

Ito et al. (2009) studied the effect of diets containing 6% scFOS or a special $DP_{average} = 8$ FOS from sucrose compared to a non-supplemented control diet on gut permeability and bacterial translocation in 8-week-old male F344 rats weighing 149-177 g (14 rats/group). Rats received their assigned diet for 7 days, after which they received TNBS to induce colitis, and continued on their assigned diets for another 10 days. Feces from the last 3 days were analyzed for mucin and IgA. Cecal contents were analyzed for mucin, myeloperoxidase, pH, SCFA, and IgA. Portal endotoxin was determined by the limulus amebocyte lysate (LAL) test. Bacterial translocation was determined in mesenteric lymph nodes using brain-heart infusion agar prior to and after TNBS treatment. In a non-colitis control study, 8 male F344 rats/group were fed the same 3 experimental diets for 7 days, anesthetized, and--without colitis-induction--analyzed for the same outcomes. Bacterial translocation was determined in mesenteric lymph nodes using brain-heart infusion agar prior to and after TNBS treatment.

In the fructan intervention experiments without TNBS treatment, rats fed FOS of both chain lengths showed lower feed intake and body weight gain as well as cecal enlargement, higher cecal mucin and IgA concentration, and lower cecal pH. Mucin and IgA concentrations were significantly correlated with butyrate and lactate concentrations.

In the 7-day period before TNBS treatment, rats fed fructans had lower feed intake and body weight gain, consistent with the control findings. However, following chemical colitis induction, rats fed the fructans recovered more quickly with increased feed intake and weight gain than the control rats. Endotoxins, as well as aerobic and anaerobic bacteria, increased after TNBS treatment, but bacterial translocation and mucosal damage were reduced with the fructans; portal endotoxin was lower and fecal mucin was higher with the DP-8 FOS. Cecal mucin, SCFA, and IgA did not significantly differ among the groups following TNBS treatment. The authors concluded that both chain-length FOS exerted prophylactic effects on the acute phase of TNBS-induced colitis in rats, possibly through reduction of bacterial and endotoxin translocation. They attributed the protective effect partially to greater mucin concentration.

8.4.2. Studies with Other Fructans

Bjorck and Nilsson (1991) studied gastric-acid hydrolysis of inulin derived from dahlia tubers, with DP up to 30, in 10 male Wistar rats (average weight = 101.8 g) receiving diets containing 5% inulin for 5 days. Analysis of the inulin revealed a fructose:glucose ratio of 23:1 and no detectible sucrose or free fructose or glucose. One group of n = 5 rats was given Omeprazol to inhibit gastric-acid secretion while 5 other rats served as controls; both groups received the antibiotic drug Nebacitin to suppress the hindgut microflora. Feed was restricted to 10 g/day, but water was freely available. Feed consumption was measured and feces were collected daily.

There were no significant differences between groups in feed intake or in the dry weight of fecal matter. Inulin recovered in feces from the control group with normal levels of secretion of gastric acid was significantly hydrolyzed, whereas only minor hydrolysis occurred in rats with suppressed gastric-acid secretion, indicating the key role of gastric acid in the hydrolysis of inulin to lower molecular-weight fractions. The authors suggested that this gastric hydrolysis, observed under the conditions of the rat study, may liberate small amounts of free fructose, which would explain the apparent digestibility of a small fraction of ingested inulin. This effect would appear to be dependent on the pH of the gastric contents.

Levrat et al. (1991) investigated the digestive and metabolic effects of chicory-derived inulin with DP $\geq$ 2. Forty-eight 6-week-old male Wistar rats weighing an average of 170 g were divided into 4 groups of n = 12 to receive semi-purified diets containing 0, 5, 10 or 20% inulin (replacing wheat starch) for 21 days. The animals were housed 2/cage. Prior to sacrifice, the rats were anesthetized for measurement of blood flow; after sacrifice, blood was taken and the cecum was removed and weighed both full and empty. Contents were weighed and analyzed for dry weight, bile acids, and SCFA.

Inulin content of the diet did not significantly affect feed intake or body weight gain at concentrations up to 10%, but feed intake and weight gain were significantly depressed in rats receiving 20% inulin. Inulin ingestion resulted in a dose-dependent enlargement of the cecum and increases in cecal wall thickness and blood flow to the cecum, as well as dose-dependent pH reduction. Bile acid concentration was not affected. SCFA production increased with increasing inulin up to 10%, but was sharply reduced at the 20% level. Production of propionate increased with increasing inulin dose, but acetate production increased only up to 10% inulin in the diet and was significantly lower when inulin constituted 20% of the diet. Butyrate was significantly increased in rats fed inulin, but with no apparent dose effect. Cecal concentrations of calcium

and phosphate were increased by inulin. For blood lipids, significant reduction of TAG was seen only at 20% inulin concentration while TC decreased linearly with increasing inulin.

In a follow-up 2x2 factorial study, Levrat et al. (1993) investigated the effects of chicory-derived inulin with DP ≥ 2 on urea and ammonia fluxes in the rat cecum. Forty 6-week-old male Wistar rats weighing an average of 180 g were divided into 4 groups of n = 10 to receive semi-purified diets containing 15 or 45% casein and 0 or 15% inulin (replacing wheat starch) for 21 days. The animals were individually housed in metabolic cages for the entire study period. Prior to sacrifice, the rats were anesthetized for measurement of blood flow; after sacrifice, blood was taken and the cecum was removed and weighed both full and empty. Contents were weighed and analyzed for dry weight, bile acids, and SCFA.

Neither protein level nor inulin significantly affected feed intake or weight gain. Casein level did not affect cecal development, but 15% inulin produced approximately 3-fold cecal enlargement and significant increases in the SCFA pool (especially propionic and butyric acids) and consequent pH reduction. Nitrogen retention and balance were not affected by inulin, but digestibility was reduced with the result that more nitrogen was excreted in feces and less in the urine. Inulin significantly depressed uremia and ammonia concentrations in rats receiving 15% casein, but not those receiving 45%. The authors concluded that "intake of a readily fermented oligosaccharide such as inulin does not adversely affect the nitrogen balance or retention."

In 2 experiments, Delzenne et al. (1995) studied the effect of oligofructose and chicory inulin with DP ≥ 2 on retention of energy, nitrogen and minerals (Ca, Mg, Fe, Zn and Cu) in rats. In the first experiment, male Wistar rats weighing about 100 g were divided into 3 groups (n = 10/group) to receive 15 g basal diet/day providing 4 kcal/g, 16.5 g basal diet with 10% inulin/ day, or 16.5 g basal diet with 10% oligofructose/day. On day 18, the animals were placed into metabolic cages for 8 days during which feces and urine were collected every day, feed and water consumption were measured daily, and blood samples were taken at the end of the 8 days in the metabolic cage. After euthanasia on day 26, the rats' ceca and colons were excised and both the tissues and contents were weighed. The second experiment was similar to the first except that the animals received the diets for 42 days rather than only 18 days before being put in the metabolic cages, again being sacrificed after 8 days in the metabolic cages (on day 50).

In both experiments there were no significant differences in body weight gain among the 3 groups, nor were their differences in nitrogen balance. In both experiments, the fecal mass excreted was significantly higher in the animals fed inulin or oligofructose than among the controls due to increased water content; there was no difference in dry mass weight. However, the addition of inulin or oligofructose to the diet significantly reduced the fecal excretion of calcium, magnesium, iron, and zinc; copper excretion was also apparently reduced but not statistically significantly. No significant differences were seen between oligofructose and inulin in this significant improvement in mineral absorption. Nitrogen excretion in the feces was significantly increased by ingestion of inulin or oligofructose, but was balanced by significantly reduced urinary excretion such that nitrogen balance was unchanged, a finding consistent with Levrat et al. (1993).

In a study of the effect of orally administered oligofructose on blood lipids, Fiordaliso et al. (1995) assigned 85 individually housed male Wistar rats weighing about 100 g to receive *ad libitum* access to water and one of 3 diets for 5-12 weeks: control diet (n = 35), control diet + 5%

sucrose (n = 15), or control diet + 10% oligofructose (n = 35). Feed intake, excretion, and body weight were measured twice a week; TAG was measured weekly on a subset of rats. After sacrifice (the fasting period was not reported), blood was taken and the liver was excised from another subset of rats and hepatocytes were isolated.

There were no differences in feed intake or weight gain between the 3 feeding groups; all animals appeared healthy and exhibited normal behavior. Administration of oligofructose produced a significant reduction in serum TAG levels, seen after 1 week of feeding and maintained up to 16 weeks, as well as phospholipids and TC. The cholesterol reduction was due to reduction of the very-low-density fraction; LDL and HDL levels were unaffected. Synthesis and secretion of TAG in isolated hepatocytes were significantly reduced by inclusion of oligofructose in the diet.

Vanhoof and DeSchrijver (1995) individually housed 48 male Wistar rats weighing an average of 200 g in metabolic cages and fed them diets either with or without cholesterol and cholic acid for 2 weeks. Each of these groups was then divided into 3 subgroups (n = 8 rats/group) and given a continuation of their previous starch diet or the previous diet plus 6% either baked or unbaked (referred to by the authors as "raw") inulin from chicory for 5 weeks. Water and feed were freely available and intakes were recorded daily and feces were collected throughout the experimental period. At sacrifice following a 24-hour fast, blood was collected and the liver was removed.

There were no significant differences in feed or water intake or in daily growth across the 6 groups. Rats consuming diets containing cholesterol showed no effect of either unbaked or baked inulin on blood lipids, but those receiving cholesterol-free diets had significant reductions in total, esterified, and free cholesterol, although no effect was seen on TAG. Inulin ingestion also had no effect on liver lipid concentrations or fecal bile acid output regardless of cholesterol in the diet.

Fontaine et al. (1996) investigated the influence of the intestinal microbiota on mucin in the rat, using germ-free and heteroxenic rats inoculated with human fecal microbiota. Twenty 2-month-old germ-free male inbred F344 rats were acquired and 10 were orally inoculated with diluted human feces to produce heteroxenic rats. Rats received *ad libitum* water and control diet supplemented with either 10% sucrose or inulin extracted from chicory (n = 5 rats/type/diet) for 1 month. After sacrifice, cecal pH was measured, the cecum and colon were removed, and the contents separated from the mucosa.

The cecal and colonic contents of germ-free rats contained significantly more acidic mucins than sulfomucin, which was present in the same proportion (cecum) or higher proportion (colon) as neutral mucin. In germ-free rats, ingestion of inulin significantly increased the amount of neutral mucin in the cecal contents and mucosa as well as mucosa-associated acidic mucins. It also increased luminal colonic sulfated mucins. In heteroxenic but not germ-free rats, in which fermentation could occur, inulin significantly decreased acidic mucins in the cecum and colon and increased colonic neutral and sulfated mucins. Inulin also significantly increased the content of SCFA, thus reducing the luminal pH in heteroxenic rats. The authors suggested that the low amount of mucin that was recovered in the colonic mucosa indicated that the presence of the proliferating bacterial biota associated with inulin resulted in mucin being extensively released

from the mucosa to the colonic lumen, and concluded that "inulin may have a beneficial effect on health by stimulating sulfomucin synthesis."

Kok et al. (1996) randomly assigned 32 individually housed male Wistar rats weighing about 120 g to receive *ad libitum* standard chow or chow supplemented with 10% oligofructose for 30 days. Ten animals from each group were killed during the postprandial period on day 30, blood was collected, and the liver was excised. The remaining rats from each group were used for hepatocyte isolation and determination of TAG synthesis.

Addition of 10% oligofructose to feed did not affect intake or final body weight. The oligofructose-supplemented rats had significantly lower postprandial serum levels of TAG and phospholipids, but TC did not differ. Glycerol-3-phosphate acyltransferase activity level was significantly lowered by oligofructose, while phosphatidate phosphohydrolase activity was unaffected, suggesting that the pathway converting glycerol-3-phosphate to phosphatidate was modulated, a suggestion supported by the finding that liver levels of glycerol-3-phosphate were higher in rats receiving oligofructose. Further, the data indicated that the reduced TAG level resulted from reduced synthesis in liver hepatocytes rather than accelerated catabolism.

Djouzi and Andrieux (1997) used germ-free rats inoculated with a human fecal flora to compare the effects of oligofructose, GOS, and glucooligosaccharides (GLS) on the composition and metabolism of the intestinal microbiota. Twenty-four 2-5-month-old gnotobiotic Fischer 344 rats were individually housed, inoculated with a suspension of human feces, and fed either a control diet or a diet containing 4% oligofructose, GOS or GLS replacing sucrose for 4 weeks. The rats were moved to respiratory chambers for 4 days during which breath H_2 and CH_4 were measured and feces were collected. After sacrifice, blood samples were taken and the cecum removed and weighed.

All three oligosaccharides significantly reduced plasma cholesterol levels as compared to controls, but TAG was unchanged. Oligofructose ingestion resulted in no changes in glycolytic activity levels (α or β-glucosidases, β-galactosidase, or β-glucuronidase). No changes were noted in numbers of bacteroides, clostridia, enterococci, or enterobacteria, but bifidobacteria increased significantly in the feces of rats fed oligofructose or GOS. Cecal SCFA production—particularly acetate and butyrate—was significantly increased by ingestion of oligofructose; ammonia and fecal pH were significantly reduced and cecal weight increased, and H_2 and CH_4 excretion were significantly increased as compared to the controls. The authors suggested that unidentified species of butyrate-producing bacteria, as well as bifidobacteria, may be involved in fermentation of oligofructose, with their butyrate production providing beneficial effects on colonocyte metabolism.

In a follow-up to previous research, Kok et al. (1998) studied the ability of oligofructose to ameliorate postprandial lipidemia in rats fed a high-fat diet. Male Wistar rats weighing about 120 g were housed individually and given *ad libitum* access to standard AO4 rat chow (n = 5) or a high-fat diet containing 10% lard, 4% corn oil, and 0.15% cholesterol (n = 10) for 3 days, after which the high-fat-diet rats were divided into 2 groups of n = 5 to continue on the high-fat diet or to receive high-fat diet with 10% oligofructose for 19 days. Feed intake and body weights were measured weekly. Fecal samples were collected on day 11. After sacrifice during the postprandial period, blood was collected and liver specimens were taken.

Control rats receiving standard chow had significantly greater feed intake than those receiving high-fat diets, but the addition of oligofructose did not affect feed intake. Since the high-fat diets had a higher caloric density than the standard chow, intakes were isocaloric and final attained bodyweights were not different despite the reduced consumption. Rats consuming high-fat diets had significantly greater relative liver weights than those eating standard chow and addition of oligofructose had no effect. There were no differences between rats receiving standard or high-fat chow in serum TAG, phospholipid, TC, free cholesterol, and esterified cholesterol levels, but all of these were significantly reduced by the addition of 10% oligofructose to the high-fat diet. On the other hand, the rats on the high-fat diet had significantly higher levels of TAG, phospholipids, and TC in the liver and this effect was not ameliorated by oligofructose. Fatty liver was confirmed by histological and histochemical examination.

Both wet and dry fecal weights were lower in rats receiving the high-fat diet, but this effect was partially erased by the addition of oligofructose. Oligofructose supplementation also significantly decreased the concentration of lipids in the feces.

In a study of the effects of inulin (DP ≥ 2) and phytic acid on mineral absorption, Lopez et al. (2000) randomized 32 male Wistar rats weighing about 160 g to receive 1 of 4 diets (n = 8 rats/diet) for 21 days. The 4 diets were a fiber-free control diet, a diet with 10% inulin, a diet with 0.7% phytic acid, and a diet containing both 10% inulin and 0.7% phytic acid. Rats were maintained 2/cage for the first 10 days and individually housed in metabolic cages for the remaining period; they had free access to feed and water for the entire study. Feed intake and body weight were recorded daily. During the last 5 days, feces and urine were collected for mineral-balance determination. On the last day, rats were anesthetized for determination of cecal venous blood flow; blood was taken after sacrifice and the cecum was excised and its contents were removed.

Neither inulin nor phytic acid significantly affected the rats' body weight or liver or tibia weights. However, rats fed inulin-containing diets had significantly enlarged ceca with contents of lower pH. These adaptations resulted in significantly increased absorption of calcium, magnesium, iron, and copper. Phytic acid decreased absorption of iron, zinc, and copper, but inulin counteracted this effect by significantly stimulating bacterial hydrolysis of phytic acid.

Daubioul et al. (2000) studied the effect of oligofructose on lipid metabolism in obese male *fa/fa* Zucker rats as a model mimicking metabolic alterations occurring in obese non-insulin-dependent diabetes mellitus patients. Five-week-old genetically obese male Zucker rats (number not reported) weighing an average of 140 g were individually housed and given free access to water and either control diet or control diet with 10% added oligofructose for 10 weeks. Feed intake and body weight were recorded twice a week; after 8 weeks, an oral glucose and lipid tolerance test was performed. After sacrifice in the postprandial phase, blood and liver samples were collected for analysis of TAG, phospholipids, TC, glucose concentrations, total protein, fatty-acid synthase activity, ATP citrate lyase and malic enzyme activities, and phosphatidate phosphohydrolase activity.

No changes were seen in postprandial serum TAG or glucose concentrations, but the hepatic concentration of TAG was significantly reduced by over 50% and the epididymal fat mass was also significantly lower. Among the key enzymes involved in fatty acid synthesis and esterification, only malic enzyme activity was significantly lower in oligofructose-fed rats than

in controls. The addition of 10% oligofructose to the diet significantly slowed the increase in body weight. The authors concluded that the metabolic response to dietary fructans is different in obese Zucker rats than in other animal models, and suggested that fructans, while having a hepatoprotective effect with regard to steatosis, do not counteract the hyperlipidemia associated with insulin resistance due to a leptin-receptor defect.

In an exploration of the effect of degree of polymerization on the ability of fructans to stimulate production of SCFA, Kleessen et al. (2001) inoculated 24 5-week-old male germ-free Wistar rats (average weight = 122 g) with human feces and fed them *ad libitum* a control diet, control diet + 2.5% long-chain inulin (DP ≥ 10), control diet + 2.5% oligofructose, or control diet + 2.5% of a 1:1 combination of long-chain inulin and oligofructose. These diets were fed for 7 days, after which the fructan supplementation was doubled to 5% of the diet for an additional 8 days. Feed intake was recorded daily and body weight every 2 days. Feces were collected on day 7; after sacrifice on day 15, the cecum and colon were removed and their contents extracted for analysis, and the cecal and colonic tissues were weighed.

There were no differences among groups in feed intake or body weight gain, and the rats were in good health throughout the study. The addition of fructans to the diet increased the cecal wall weight and the wet weight of the content as well as the wet weight of colonic contents. All fructans significantly decreased cecal and colonic pH and increased fecal output due to increased water excretion. Supplementation with fructans did not affect total bacterial counts, but the different chain-length fructans had differential effects on certain bacterial groups. Rats fed long-chain inulin or oligofructose-enriched inulin had significantly higher numbers of clostridia and eubacteria, while oligofructose produced higher colonic (but not cecal) levels of bifidobacteria. Rats receiving long-chain inulin actually had significantly fewer cecal bifidobacteria than rats receiving the control diet. The highest levels of lactobacilli in the cecum and colon were in rats fed oligofructose-enriched inulin. No differences were seen between feeding groups in enterobacteriaceae or bacteroides.

SCFA concentrations were invariably higher in the cecal contents than in colonic content. Fructan supplementation significantly increased butyrate levels; long-chain inulin was most effective in increasing levels of propionate while oligofructose was most effective in producing higher levels of acetate.

Daubioul et al. (2002) followed up their earlier research with genetically obese Zucker rats as models for non-insulin-dependent diabetes mellitus by testing the differential effects of dietary fructans (highly fermentable) and microcrystalline cellulose (MCC; non-fermentable). Five-week-old genetically obese male Zucker *fa/fa* rats (number not reported) weighing 90-125 g were individually housed and given free access to water and either control diet, control diet with 10% added MCC, or control diet with 10% added 1:1 blend of oligofructose and long-chain inulin for 8 weeks. Feed intake and body weight were recorded twice a week and serum metabolites were measured every 2 weeks in both the postprandial and postabsorptive states. The abdominal regions of 2-3 rats/group were examined by nuclear magnetic resonance spectroscopy after 6 weeks. After sacrifice at 8 weeks, blood and liver samples were collected for analysis of TAG, phospholipids, TC, glucose concentrations, total protein, fatty-acid synthase activity, ATP citrate lyase and malic enzyme activities, and phosphatidate phosphohydrolase activity.

Rats consuming a diet with 10% oligofructose-enriched inulin had significantly lower energy intake, lower body weight, and less TAG accumulation in the liver compared to control and MCC groups. The high fermentation of fructans compared with MCC was reflected by significantly greater cecal contents and greater propionate concentration in the portal vein of rats fed oligofructose-enriched inulin compared with those fed MCC. Propionate selectively decreased the incorporation of acetate into total lipids in the fructan-fed rats. Although ingestion of fructans had no significant effect on the activities of key enzymes controlling fatty acid synthesis, liver weight and steatosis was significantly reduced as compared to control and MCC-fed rats. The authors concluded that "the beneficial effects of fermentable non-digestible carbohydrates on liver steatosis could be useful in overweight people."

In a follow up study in which rats were again given only low-calcium diets, Bovee-Oudenhoven et al. (2003) tested the effects of oligofructose, lactulose, resistant starch, cellulose, and wheat fiber on colonization and translocation of *Salmonella enteriditis*. Forty specific pathogen- free 8-week-old male Wistar rats (mean weight = 234 g) were fed a diet containing only 0.02 mmol calcium/g diet (as calcium phosphate) and 4% oligofructose, lactulose, resistant starch, cellulose, or wheat fiber for 2 weeks and then infected with *S. enteriditis*. Fecal and urine samples were collected before and after infection. Three additional groups of n = 8 rats were given oligofructose-, lactulose-, or cellulose-supplemented diets, infected with *S. enteriditis*, and killed 2 days later for excision of their spleens, mesenteric lymph nodes, and livers, using plate counts on Brilliant Green agar.

Pre-infection feed intake and weight gain were significantly lower in rats ingesting oligofructose and lactulose than resistant starch, wheat fiber, or cellulose, and these differences remained significant after infection. Oligofructose, lactulose, and resistant starch increased counts of bifidobacteria, lactobacilli, and enterobacteria significantly more than cellulose or wheat fiber, resulting in significantly higher concentrations of lactic acid. The cytolytic activity of fecal water in an erythrocyte assay was directly correlated with lactic acid concentration and pH. Rats ingesting oligofructose or lactulose shed fewer *Salmonella* in feces and had higher levels of urinary NO_x. *Salmonella* translocated to all examined compartments, mesenteric lymph nodes, spleen, and liver. There were no significant differences between the cellulose, oligofructose, or lactulose groups in translocation to the mesenteric lymph nodes, but translocation to the spleen and liver was higher with oligofructose and lactulose than with cellulose. The authors concluded that the soluble fiber-type carbohydrates lactulose and oligofructose improved colonization resistance, as indicated by lower fecal levels, but they also suggested an increased intestinal permeability to *Salmonella* from the organ platings. However, the authors ignored the role of the calcium-deficient diet and the resulting consequences to the mineral balance together with the higher production of lactic acid and SCFA by bacteria in the presence of fermentable substrates. On the contrary, the structurally different carbohydrates lactulose and oligofructose exerted similar responses due to the rapid fermentability that they have in common, which may have stimulated macrophage activity as indicated by the NO_x signals.

Using a rat model, Kruger et al. (2003) studied the impact of fructans with various degrees of polymerization on calcium absorption, bone density, and excretion of collagen cross-links. Forty 7-week-old male Sprague-Dawley rats (weight not reported) were fed basal diet for 1 week and then randomized into 4 groups (n = 10/group) to receive basal diet (containing 0.5%

calcium), basal diet + 5% oligofructose, basal diet + 5% long-chain inulin (DP ≥ 10), or basal diet + 5% of a mixture of 8% oligofructose and 92% long-chain inulin. The rats were individually housed in shoebox cages and had *ad libitum* access to water and diet; they remained on their assigned diets for 4 weeks. Metabolic balance studies were conducted at the end of week 1 and at the end of week 4, in which the animals were placed into single metabolism cages for 72 hours while blood was drawn, feed intake was measured, and urine and feces were collected. After sacrifice, the animals' right femurs and spine were excised, weighed, measured, and ground for mineral analysis.

Feed intakes and final body weights did not differ among the 4 groups. Urinary calcium excretion did not differ, but the rats receiving long-chain inulin had significantly less calcium in their feces than did the other groups. Bone mineral density in the femur and spine was significantly higher in the group fed long-chain inulin. The excretion of fragments of Type 1 collagen decreased in all groups over the 4 weeks of feeding, but the decrease was greatest in the group fed long-chain inulin. The authors concluded that long-chain inulin was significantly superior to shorter-chain oligofructans in increasing calcium bioavailability and retention.

Kleessen et al. (2003) studied the effects of oligofructose and long-chain inulin on the mucosal morphometry and mucosa-associated bifidobacteria in gnotobiotic rats. Thirty-six 5-week-old male germ-free Wistar strain AVN-Ipcv rats weighing an average of 118 g were allocated to 6 groups of n=6 rats. Two groups were inoculated by an oral dose of diluted human feces (characterized as containing $5x10^{10}$ cfu total bacteria including $1x10^{10}$ *Bacteroides* spp. and $8x10^{8}$ bifidobacteria); 2 groups were inoculated with $8x10^{9}$ cfu *Bacteroides vulgatus* and $5x10^{8}$ cfu *Bifidobacterium longum*; and the remaining 2 groups remained germ free. Ten days after inoculation, 1 group from each pair was assigned to receive either standard diet alone or supplemented with 2.5% oligofructose and 2.5% long-chain inulin for 28 days. Rats were caged individually with free access to chow and water; feed intake was measured daily and body weight was recorded every 2 days. After sacrifice, the small intestine and colon were removed and subjected to morphological examination, mucosal histochemistry, and microbiological enumeration of the contents.

The rats remained physically healthy and no differences were seen in feed intake or body-weight gain. Total bacterial numbers and *Bacteroides* counts were unaffected by diet, but counts of bifidobacteria in the colonic contents and of mucosa-associated bifidobacteria were significantly higher in the rats receiving fructans. While fructans did not affect the mucosal morphology of germ-free rats, in rats inoculated with bacteria the effect of fructan ingestion was to significantly increase villus height and crypt depth. Similarly, diet had no effect on intestinal mucins in germ-free rats, while in inoculated rats fructans caused significant increases in epithelial mucus layers and the number of goblet cells in the jejunal and colonic mucosa. The authors concluded that "it is probable that [treatment with fructans] could be of benefit in both the protection of the intact intestinal epithelium and the therapy of a disturbed mucosal barrier."

Ten Bruggencate et al. (2003) studied the potential impact of ingestion of oligofructose on translocation of *Salmonella enteriditis*. Eight-week-old male Wistar rats weighing an average of 239 g were housed individually in metabolic cages and assigned to receive restricted quantities of a low calcium control diet or the control diet supplemented with either 3 or 6% oligofructose. After 2 weeks, rats were infected with $1.7x10^{10}$ cfu *S. enteriditis* and killed on day

9 after infection. Fecal samples were collected both before and after infection and assayed for pH, lactic acid, SCFA, sodium, potassium, ammonia, and mucins, as well as bifidobacteria, lactobacilli, enterobacteria, and *Salmonella* spp. *Salmonella* spp. were also measured in mucosa and the cecum. The cytotoxicity of fecal water and urine NO_x were measured as indicators of mucosal irritation and *Salmonella* translocation, respectively. Cytolytic activity ("cytotoxicity") was assessed by determining potassium release from erythrocytes following incubation with fecal water while NO_x served as a surrogate marker for undefined macrophage activity suggested to relate to infection and translocation; neither marker has been validated outside this single laboratory.

Ingestion of 6% oligofructose significantly increased counts of fecal bifidobacteria, but not lactobacilli, while both 3 and 6% dietary levels of oligofructose increased enterobacteria prior to *Salmonella* infection. The cytolytic activity of fecal water was significantly increased non-dose-dependently by oligofructose ingestion, while lactic acid production was significantly increased dose-dependently. After *Salmonella* infection, the *Salmonella* populations in the mucosa and cecum increased in a dose-dependent manner with increasing levels of oligofructose ingestion. The authors concluded that ingestion of oligofructose in this study increased colonization and translocation of *S. enteriditis*.

This study contained methodological weaknesses that cast doubt on its validity. The rats were placed on low calcium diets for reasons that were never stated but that almost certainly had an influence on gut wall integrity. Additionally, the estimate of cytolytic activity was based on potassium release from erythrocytes as a marker that may or may not be valid. Further, the test did not control for the pH effects to be expected in rats fed a high fiber diet compared to controls and which were aggravated by the poor buffering capacity of the low calcium diet. Yet further, the nitric oxide test is based on the assumption that urinary nitric oxides are due to macrophage activity causally linked to *Salmonella* infection/translocation. Finally, the *Salmonella* assay is non-exclusive for *Salmonella*, but rather for enterobacteriaceae; it is noted that before infection, enterobacteria counts were higher in the rats receiving the oligofructose interventions, and so it is likely that the results after infection were due to commensal enterobacteriaceae. For all these reasons, a cause-and-effect relationship for the effects seen in this study cannot be established.

In a follow-up study (Ten Bruggencate et al. 2004), 8-week-old male Wistar rats with mean weight = 276 g were individually housed in metabolic cages and fed restricted quantities of the same low-calcium diet as was used in the previous study (n = 8), or the same diet supplemented with 6% cellulose, chicory inulin, or oligofructose (n = 8 each). Another 32 rats received one of the same 4 diets but with supplementary calcium phosphate (100 mmol $CaHPO_4 \cdot H_2O$/kg diet). After 2 weeks, the animals were infected with *Salmonella enterica serovar enteriditis* and killed 7 days later. Feces were collected both before and after infection and assayed for bifidobacteria, enterobacteria, and lactobacilli, as well as lactic acid, calcium, phosphate, and mucin. The cytotoxicity of fecal water was assessed using an unvalidated test of potassium release from erythrocytes, and urine samples were analyzed for NO_x as an unvalidated marker of macrophage activity. After sacrifice, the cecum was excised and its contents removed and analyzed for pH and bacterial counts.

There were no differences between groups in growth prior to infection; after infection, growth was significantly depressed in the low-calcium oligofructose group. In the low-calcium

condition, ingestion of both inulin and oligofructose significantly lowered cecal pH. Diet had no effect on bifidobacteria in the low-calcium condition; results from the normo-calcium condition were not reported but would likely have shown a bifidogenic effect. Fecal lactobacilli and enterobacteria were significantly increased by both fructans in the low-calcium condition; none of these bacterial counts was affected by *Salmonella* infection. The combination of low-calcium and fructan ingestion significantly increased the cytolytic activity of fecal water and excretion of mucin. Both fructans significantly increased apparent *Salmonella* enumerations (which likely included enterobacteria) in the cecum, unaffected by calcium, and increased translocation only in the low-calcium condition—in the high-calcium condition, no more translocation was seen in the fructan groups than in the controls. Urinary NO_x was higher only in the low-calcium condition. The authors concluded that oligofructose and inulin impair resistance to *Salmonella* infections in rats due to damage to the mucosal barrier, although they recognized that "dietary calcium phosphate inhibited the adverse effects of acidic fermentation and largely prevented damage to the intestinal barrier." In other words, no adverse effects were evident in the presence of adequate calcium. Additionally, this study was plagued by the same methodological weaknesses as was its predecessor (Ten Bruggencate et al. 2003).

Juskiewicz and Zdunczyk (2004) compared the effect of diets containing long-chain inulin (DP ≥10), cellulose, or carboxymethylcellulose (CMC) as single supplements or in dietary combination on the cecal physiology of rats. Sixty 4-week-old male Wistar rats weighing an average of 76 g were divided into 5 groups of 12 rats each. The study lasted for 4 weeks, during which the animals had free access to feed and water, supplemented with 4% long-chain inulin, cellulose, or CMC or with 4% long-chain inulin + 4% cellulose or with 4% long-chain inulin + 4% CMC. At the end of the study, feed consumption and body weight gain were determined and feed conversion efficiency was calculated. After laparotomy, the cecum with contents was removed and weighed and the cecal pH measured. The cecal wall was flushed clean and weighed as the cecal tissue mass while samples of the digesta were analyzed for dry matter, ammonia, and SCFA.

Weight gain was highest in the rats receiving long-chain inulin or cellulose, which did not significantly differ from each other but were significantly higher than the other 3 groups, but there were no significant differences in feed conversion efficiency. Rats receiving CMC, alone or in combination with long-chain inulin, had significantly higher cecal masses, and those receiving cellulose alone had significantly the lowest; rats receiving long-chain inulin, with or without added cellulose, were in between the other groups. The animals fed long-chain inulin alone were generally between the other groups in measures of digesta mass, dry matter, pH of digesta, and ammonia. The long-chain-inulin group showed a significantly higher level of β-glucuronidase and α-glucosidase activities than did the other groups. The highest concentration of protein (Lowry method) in the cecal digesta was found with long-chain-inulin feeding, indicating that only long-chain inulin increased bacterial density in the cecum and thus fecal nitrogen excretion. Finally, long-chain inulin significantly enhanced production of propionate and butyrate compared to the other treatments. No adverse effects were noted in response to any of the treatments. The rats consumed an average of 458 g feed over 28 days, or 16.36 g/day. Since long-chain inulin constituted 4% of the diet, the daily intake of long-chain inulin was about 654 mg. For an average-weight male Wistar rat of 180 g, the intake of long-chain inulin was equivalent to about 3600 mg/kg bw/day.

In another follow-up study (Ten Bruggencate et al. 2005), twenty 8-week-old male Wistar rats with mean weight = 226 g, individually housed in metabolic cages, were fed the same low-calcium restricted diet as was used in previous studies supplemented with 6% cellulose or oligofructose. In this study, an intestinal permeability marker, chromium EDTA, was added to the diets. No diet containing normal levels of calcium was tested. After 2 weeks, the animals were infected with *Salmonella enteriditis* and killed 10 days later. Feces were collected both before and after infection and assayed for bifidobacteria (using PCR), enterobacteria (using Levine EMB agar), and lactobacilli (using Rogosa agar and PCR), as well as lactic acid, calcium, phosphate, and mucin. The cytotoxicity of fecal water was assessed using an unvalidated cytolytic test on potassium release from erythrocytes, and urine samples were analyzed for NO_x as an indicator of macrophage activity.

There were no significant differences between groups in feed intake or weight gain either before or after *Salmonella* infection. Prior to infection, ingestion of oligofructose resulted in significantly larger counts of fecal bifidobacteria, lactobacilli, and enterobacteria, as well as increased fecal lactate and fecal-water toxicity compared with cellulose. *Salmonella* infection caused no changes in fecal bacteria, pH, or cytolytic activity on human erythrocytes. Urinary excretion of chromium EDTA was significantly higher in the rats ingesting fructan than those ingesting cellulose both before and after infection, as was the concentration after infection of urinary NO_x, although this level began to decline after day 8. However, the absence of a test group receiving normal calcium levels limits the ability of this study to lead to greater understanding of the role of fructans in an intestinal milieu with adequate buffering capacity.

In a second experiment reported in the same publication (Ten Bruggencate et al. 2005), eight 8-week-old male Wistar rats with mean weight = 261 g, individually housed in metabolic cages, were fed the same low-calcium restricted diet supplemented with either 6% cellulose or oligofructose. After sacrifice on day 14, the distal ileum, cecum, and proximal colon were excised and their contents removed and analyzed for bacterial counts, SCFA, calcium, phosphate, and mucins.

There were no differences in cecal concentrations of SCFA, although the total pool was significantly increased by oligofructose ingestion, as were cecal concentrations of calcium and phosphate. Ingestion of oligofructose, as compared to cellulose, stimulated growth of bifidobacteria, lactobacilli, and enterobacteria in both cecal and colonic mucosa, although not in the ileal mucosa.

Nzeusseu et al. (2006) compared the effects of chicory-derived inulin (DP $\geq$ 2) with partially hydrolyzed inulin enriched in FOS. Six-week-old male Wistar rats weighing 150-175 g were randomly assigned to 1 of 3 diets (n = 13/diet): basal AO4 diet, basal diet with 5% inulin, or basal diet with 5% partially hydrolyzed inulin. The rats were individually housed and given feed and water *ad libitum*. Animals remained on the assigned diets for 90 days. At baseline and termination, the rats were given whole-body dual-energy X-ray absorptiometry tests; after sacrifice, blood was centrifuged and the serum frozen for analysis; the cecum was removed, weighed, and analyzed for calbinding-9 K; and femurs, tibiae, and lumbar vertebral bodies were removed and analyzed for mineral content.

Body weight, lean body mass, and appendicular bone length did not differ among the 3 groups. Rats fed either intact or partially hydrolyzed inulin had similar increases of about 30% in

cecal wall weight, but the relative increase in cecal levels of calbindin-9 K was twice as high in the inulin group as in the group receiving partially hydrolyzed inulin. Further, a significant decrease in serum levels of type I collagen C-telopeptide was greater in the inulin group (30%) than in the partially-hydrolyzed inulin group (16%). The increase in whole-body bone mineral content was significantly greater in the inulin group. Further, intact inulin, but not partially hydrolyzed inulin, significantly enhanced bone mineral density of the cortical bone in both appendicular and peripheral sites as well as the polar stress/strain index of femurs. The authors concluded that these results suggest that the primary advantage of inulin over shorter chain fructans in increasing bone mineral density is due to its higher ability to reduce bone resorption.

Coudray et al. (2006) investigated the effect of ingestion of chicory inulin on absorption of zinc and copper in male Wistar rats. Eighty rats aged 2, 5, 10, or 20 months (20 rats of each age) were randomly assigned to a control group or a group administered 3.75% inulin in their diet for 4 days followed by 7.5% inulin for 26 days. All diets provided 40 µg Zn and 9 µg Cu/g chow; both feed and water were freely available. Rats were maintained 2/cage in wire-bottomed cages and feed consumption and body weight were recorded weekly. On day 21, the rats were moved to individual metabolic cages; after 3 days they were gavaged with a solution providing ^{67}Zn and ^{65}Cu isotopes and then fed. Urine and feces were collected for 4 days and analyzed for zinc and copper. After sacrifice, blood was collected for mineral analysis of plasma; the cecum was removed and weighed both full and empty and the contents analyzed for pH and mineral analysis. The liver and one tibia were also tested for zinc and copper.

Rats receiving 7.5% inulin consumed significantly less feed and had non-significantly less growth in body weight than control rats. Ingestion of 7.5% inulin resulted in significantly higher cecal wall and content weights and reduced cecal content pH due to significantly increased production of SCFA. Absorption of both zinc and copper was significantly lower in 10- and 20-month-old rats than in 2- and 5 month-old rats. Incorporation of inulin in the diet significantly increased absorption of both zinc and copper in rats of all ages.

The effects of fructans from yacon roots on cecum mucosal morphometry and mineral balance and retention were studied in growing male Wistar rats by Lobo et al. (2007). Twenty-four 5-week-old male Wistar rats weighing 65-70 g were individually housed and fed *ad libitum* diets containing 0, 5, or 7.5% yacon flour for 27 days. The yacon flour contained 55.3% oligofructose with DP ≤ 10, along with 13.5% fructose, 13.4% sucrose, and 8.9% glucose. Feed intake was measured daily and body weight every 2 days. Feces and urine were collected for three 5-day periods beginning on days 4, 10, and 16. After sacrifice, the rats' livers, spleens, and kidneys were excised; ceca were removed along with their contents; and hind limbs were removed and the bones defleshed.

There were no differences in feed intake, weight gain, or feed efficiency among the 3 feeding groups, or in absolute or relative weights of the spleen, liver, or kidney, and no gastrointestinal disturbances were noted. Ingestion of yacon flour resulted in a significant non-dose-dependent increase in dry feces weight and a significant dose-dependent increase in the moisture content of feces. Cecal wall thickness, crypt depth, and the moisture content of cecal contents increased significantly and dose-dependently with yacon flour ingestion. Calcium absorption and both calcium and magnesium balance were significantly enhanced by inclusion of

yacon flour, but there were no differences in bone density although the calcium content of bone increased and resulted in stronger bone structural properties.

Rodenburg et al. (2008) gave 36 specific pathogen-free 8-week-old male Wistar rats (mean weight = 253 g), housed individually in metabolic cages, limited restricted low-calcium diets of 14 g/day with 6% supplementary cellulose or oligofructose. Calcium was omitted from the mineral mix and instead provided at 0.020 mmol/g feed by calcium phosphate. Six of the 18 rats in each group also received chromium EDTA to test gut permeability. mRNA was collected from colonic mucosae and changes in gene expression were assessed for each rat.

There were no differences in feed intake or bodyweight gain between the 2 diets, but increased chromium-EDTA excretion in urine indicated increased intestinal permeability in the oligofructose group. The gene expression response of colonic mucosa to oligofructose was small in magnitude. Oligofructose did not induce changes in tight junction genes and only 8 genes related to mucosal defense were induced by oligofructose, conceded by the authors to be insufficient to explain changes in intestinal permeability, which they acknowledged is prevented by ingestion of adequate calcium to provide normal buffering. Oligofructose significantly increased expression of 177 mitochondria-related genes, indicating that the acidity resulting from its fermentation and the use as fuels of the resulting short-chain fatty acids in a calcium-deficient milieu influences intestinal mucosal energy metabolism.

8.5. Rabbits

8.5.1. Studies with Other Fructans

Juskiewicz et al (2008) tested the physiological effects of a diet supplemented with flour from chicory root, which contained 77% inulin. Twenty-four individually caged 54-day-old male New Zealand white rabbits weighing an average of 2.43 kg were assigned to receive ad libitum control diet or diet supplemented with 2.5 or 5% chicory flour (replacing dried sugar beet pulp) for 36 days. Feed intake and body weight were recorded periodically; after sacrifice, blood was taken; the stomach, small intestine, cecum, and colon were removed and weighed; and samples of digesta were taken for analysis of pH, dry matter, viscosity, ammonia, protein, SCFA, and glycolytic activity.

There were no significant differences in feed intake or weight gain, or in the weight of ileal, cecal, or colonic tissue. Supplementation of the diet with chicory flour resulted in significant increases in the mass of ileal and cecal, although not colonic, digesta. Ileal and cecal digesta also had significantly reduced pH and viscosity as well as significantly lower glycolytic enzyme activity. The total cecal pool of SCFA, especially acetic acid, was significantly increased in the rabbits receiving chicory flour although no dose-related effect was evident. Diet had no effect on blood glucose level, but chicory flour significantly reduced concentrations of TAG, TC and HDL, and urea. The authors concluded that replacement of dried sugar beet pulp by chicory flour was beneficial to ileal metabolism, cecal and colonic fermentation, and plasma lipid profiles.

8.6. Dogs

8.6.1. Studies with Other Fructans

Pouteau et al. (2005) studied the effect of inulin ingestion on acetate turnover in dogs. Nine healthy adult beagle dogs, 5 males and 4 females weighing 13.8-17.5 kg, were fed a control diet for 17 days before being switched to a diet supplemented with 6% chicory, providing 3% chicory-derived inulin, for 21 days. Kinetic studies were performed at the end of the control-diet feeding period and after 4 and 21 days on the chicory-enriched diet. During these studies, labeled acetate was infused intravenously for 8 hours and both peripheral and colonic acetate were monitored and breath samples were taken.

Peripheral acetate concentration and turnover were not significantly changed by 4 days of chicory ingestion, but after 21 days, whole-body acetate turnover increased significantly with no change in concentration. No acetate tracer was recovered in the peripheral circulation. The fraction of oxidized tracer was significantly higher in the colon than in peripheral circulation. The authors concluded that colonic fermentation of inulin occurred and indirectly stimulated whole-body acetate turnover in dogs fed a chicory-enriched diet for 21 days.

8.7. Calves

8.7.1. Studies with scFOS

A total of 112 3-week-old male Prim'Holstein calves were given a calf-milk replacer with a supplement of 0, 3, or 6 g scFOS/day until the age of 24 weeks (Grand et al. 2013). With no reported adverse effects, calves receiving scFOS supplements showed significantly improved feed conversion and carcass weight and tended to have increased levels of SCFA. The authors suggested that "inclusion of scFOS in the calf milk replacer allowed the growth performance of preruminant calves to be enhanced, possibly via a modification of the activities of microbial fermentation."

8.8. Pigs

8.8.1. Studies with scFOS

Xu et al. (2002) assigned 128 growing Jiaxing Black/Duroc/Landrace barrows weighing an average of 20.8 kg to 4 treatments (n = 32 pigs/treatment) for 42 days, with basal diet supplemented with 0, 2, 4, or 6 g scFOS/kg feed; pigs had free access to feed and water. Feed intake and weight gain were measured daily. Eight pigs/treatment were sacrificed at the end of treatment and eviscerated to collect intestinal samples, including specimens of tissue and contents, and pancreas. Supernatants from small intestine and pancreas were analyzed for protease, trypsin, chymotrypsin, AMY, and lipase activity, and intestinal contents were assessed for microbial counts and characterization. Tissue samples were analyzed for villus height and crypt depth.

Feed intake did not differ among the treatment groups, but supplementation with scFOS significantly increased feed conversion and average daily weight gain. Viable bifidobacteria and lactobacilli were significantly increased by scFOS while clostridia and *E. coli* were reduced. The activities of the enzymes protease, trypsin, and AMY were significantly increased in the intestine but not in the pancreas; activities of chymotrypsin and lipase were not affected. Finally, scFOS significantly increased villus height in jejunal mucosa. The authors concluded that the beneficial

effects of scFOS "are due to [scFOS's] ability to improve the intestinal microflora and are not a direct action of [scFOS] on the intestinal tissue."

Tsukahara et al. (2003) housed 6 40-day-old crossbred castrated male piglets weighing approximately 12 kg in individual metabolic cages. After a 7-day adaptation period, the feed of 3 pigs was supplemented with 10% scFOS and all of the pigs received *ad libitum* access to feed and water for 10-11 days. The animals were slaughtered by exsanguination and the intestines were excised and separated into the cecum, gyri centripetales, gyri centrifugales, and rectum; luminal contents were collected and the morphology of the mucosa was examined.

The authors did not report feed or scFOS intake; however, according to Carr (1998), pigs of the age studied would be expected to consume about 700-800 g feed/day, suggesting a daily intake of scFOS of about 70-80 g. The digesta of the pigs given scFOS had significantly higher water content and concentrations of SCFA, especially butyrate, than that of control pigs. Additionally, the mucosa was significantly thicker in the scFOS-fed pigs and the crypt depth was significantly greater. Attributing the effects of scFOS to bacterial fermentation, the authors concluded that "the beneficial roles of scFOS in the physiology of the large intestine rely on the activity of intestinal microbiota."

To investigate the effect of addition of butyrate to parenteral nutrition following small bowel resection in infants, Barnes et al. (2012) installed jugular catheters in 87 48-hour-old male and female neonatal piglets weighing 1.76±0.07 kg and performed 80% jejunoileal resection, then randomized them into 4 treatment groups (21-22 piglets/group). Piglets in each treatment group were individually housed and received 20% standard enteral nutrition and 80% standard parenteral nutrition; the prebiotic group also received 10 g scFOS/L, the probiotic group received 10^9 cfu *Lactobacillus rhamnosus* GG (LGG), and the synbiotic group received both scFOS and LGG. Each treatment group was further randomized to receive treatment for 24 hours, 3 days, or 7 days (7-8 piglets/group).

The authors noted that scFOS was selected for study rather than GOS, despite the prevalence of the use of GOS in infant formula, because scFOS is known to produce appropriate butyrate concentrations, while the extended fermentation profile of GOS "renders it less useful in short-bowel syndrome wherein transit time is greatly reduced."

Pigs were clinically assessed each morning for body weight, body temperature, respiration rate, activity level, healing of the surgical site, edema, and posture. Blood was taken daily for analysis of glucagon-like peptide-2 (GLP-2). After euthanasia, the small and large intestines were excised, segmented, and weighed, and mucosal scrapings were taken for analysis of weight, DNA, RNA, protein, and disaccharidase activity; sections were subjected to morphometric analysis, assessment of mucosal ion and nutrient transport, and measurement of epithelial cell proliferation, apoptosis, and differentiation.

The treatments did not have differential effects on growth: final body weight and the weights of stomach, pancreas, liver, and kidney did not differ among the treatments at each time point. However, prebiotic and synbiotic treatments significantly increased both ileal and jejunal mass as well as ileal mucosal mass; no differences were seen in morphology. Prebiotic treatment also significantly increased jejunal mucosal DNA concentration and both ileal and jejunal protein abundance, as well as ileal, jejunal, and duodenal villus length, but with no effect on crypt depth.

Epithelial cell proliferation was significantly increased while apoptosis was decreased throughout the intestine in response to prebiotic treatment.

No adverse effects were noted from administration of 10 g scFOS/L in the parenteral formula, and the prebiotic was regarded as "highly effective at inducing adaptation in the residual jejunum, ileum, and colon."

8.8.2. Studies with Other Fructans

Correa-Matos et al. (2003) studied the effects of fermentable nondigestible carbohydrates in piglets infected with *Salmonella typhimurium*. The neonatal piglet model was chosen due to its similarity in nutritional requirements, intestinal physiology, and metabolism to the human infant, and its body composition is similar to that of the premature human infant. Forty-eight 2-day-old colostrum-fed piglets (12 piglets/treatment) were randomly assigned to receive sow's-milk replacer formula alone (control) or control formula supplemented with 7.5 g/L of methylcellulose, soy polysaccharides (soy fiber), or an undefined FOS for 14 days. The source and composition of the supplements were not described. On day 7, half of the piglets in each treatment group received an oral gavage of *S. typhimurium* 798 (originally isolated from a pig) or saline. Bodyweight, physical activity level, and stool consistency were assessed daily and body temperature every other day. The piglets were killed on day 14 and the small intestine and colon were removed, weighed, and measured. The jejunum and ileum and a mid-colon section were isolated for analysis of the SCFA content of the contents, histomorphological analysis, and measurement of disaccharidase activity.

S. typhimurium infection produced diarrhea in controls and methylcellulose groups, but not in the soy polysaccharides or FOS groups. Ileal lactase activity and physical activity were significantly lower in the controls than in other groups after infection. Ileal mucosal barrier function was significantly impaired by *S. typhimurium* infection in the control and soy polysaccharide groups, but was unaltered in the jejunum and colon. Overall, consumption of FOS shortened recovery time and improved infection-associated symptoms in piglets infected with *S. typhimurium*. The authors concluded that, "because fermentable fiber enhances intestinal function and reduces the severity of *S. typhimurium* infection–associated symptoms, it may be a cost-effective way in which to reduce the severity of pathogenic infection–associated symptoms in infants."

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
scFOS					
Agheli et al. (1998)	Paired feeding of a high-fat and high sucrose diet w/ or w/o scFOS to test the effects of scFOS on plasma lipids and the activity of fatty acid synthase in diabetics	31 5-week-old insulin-resistant male Sprague Dawley rats	scFOS	0 or 10% dietary concentration for 3 weeks	There was no difference between the sucrose-rich and the scFOS-supplemented groups in final body weights, but the scFOS-supplemented rats exhibited significantly lower liver weights, plasma fatty acids, plasma glucose, and TAG concentrations. No effect was seen on insulin, plasma phospholipids, TC, cholesterol esters, or lipoproteins. While no differences were evident in butyrate or propionate concentrations, acetate was significantly higher in the plasma of rats given scFOS. Fatty acid synthase activity in the liver was significantly lower in scFOS-supplemented rats than those receiving the sucrose-rich diet, but it was significantly higher in adipose tissue. All of these differences were regarded by the authors as beneficial in normalizing fatty acid synthase activity and reducing plasma levels of glucose, free fatty acids, and TAG in insulin-resistant rats. There were no reported adverse effects.
Baba et al. (1996)	2x2 factorial study of the effects of scFOS on Mg absorption	28 5-week-old cecal-cannulized male Sprague-Dawley rats	scFOS	0 or 5% dietary concentration for 15 days	Absorption of Mg was the same whether it was included in the diet or infused into the cecum, and the inclusion of scFOS in the diet significantly increased Mg absorption in both conditions. The authors suggested that this may result from lowered luminal pH due to fermentation of the scFOS and SCFA production. There were no differences in feed consumption or body weight and no adverse effects were reported.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Barnes et al. (2012)	Investigate the effect of addition of butyrate to parenteral nutrition following small bowel resection in infants	87 48-hour-old male and female neonatal piglets weighing 1.76±0.07 kg	scFOS	0 or 10 g/L enteral and parenteral feed for up to 7 days	Final body weight and the weights of stomach, pancreas, liver, and kidney did not differ among the treatments at each time point. However, prebiotic and synbiotic treatment increased ileal and jejunal mass as well as ileal mucosal mass; no differences were seen in morphology. Prebiotic treatment also increased jejunal mucosal DNA concentration and ileal and jejunal protein abundance, as well as ileal, jejunal, and duodenal villus length, but with no effect on crypt depth. Epithelial cell proliferation was increased while apoptosis was decreased throughout the intestine in response to prebiotic treatment. The prebiotic was regarded as “highly effective at inducing adaptation in the residual jejunum, ileum, and colon,” and no adverse effects were noted from administration of 10 g scFOS/L in the parenteral formula
Campbell et al. (1997)	Compare the effects of oligofructose from inulin, scFOS, XOS, and micro-crystalline cellulose	50 male Sprague-Dawley rats (average weight = 411 g)	scFOS or oligofructose produced by the enzymatic hydrolysis of inulin	0 or 6% dietary concentration for 14 days	The diets containing 6% scFOS or oligofructose produced significantly higher cecal butyrate concentrations compared with the control diet, MCC alone, or control + XOS diet. Total cecal pools of SCFA were significantly higher and pH was significantly lower after feeding of the diets containing fructans. Cecal total weight and wall weight were higher from fructan ingestion, while colonic wet weight was higher for rats consuming XOS compared with other treatments; colon wall weight was unaffected by any treatment. Cecal bifidobacteria and total anaerobes were higher while total aerobes were lower in rats fed fructan diets compared with those fed the control diet. Cecal lactobacilli levels were unaffected by treatment. The authors concluded that ”providing these oligosaccharides as ingredients in nutritional formulas could benefit the health of the GI tract,” and did not report any adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Cherbut et al. (2003)	Study the effect of scFOS on rats with TNBS-induced colitis	~35 male Wistar rats weighing 246±6 g with TNBS-induced colitis	scFOS	1.0 g/day for 9 or 16 days	TNBS administration produced decreased feed intake and weight loss, but these effects were reduced in rats receiving scFOS. While scFOS had little effect on colonic inflammation within 7 days, it significantly reduced inflammation within 14 days. The prebiotic treatment also increased cecal levels of SCFA, particularly butyrate, and lactate. The authors concluded that, "oral FOS has a beneficial effect on intestinal inflammation in rats. It diminishes the anorexia and weight loss associated with the onset of inflammation; it then reduces the extent of the damage and promotes epithelial healing." No adverse effects attributable to scFOS were reported.
Fujiwara et al. (2010) Part 1	Study the effect of dietary scFOS on dermal contact hyper-sensitivity	12 5-week-old female NC/Nga mice	scFOS	0 or 5% dietary concentration for 59 days	The mice receiving scFOS exhibited significantly less inflammation than did control mice. No differences were seen in cecal levels of bifidobacteria, and the authors concluded that "it is unlikely that the proliferation of bifidobacteria in the gut is responsible for the reduction" in inflammation. No adverse effects were reported.
Fujiwara et al. (2010) Part 2	Study the effect of dietary scFOS on dermal contact hyper-sensitivity	4 9-week-old male and 12 8-week-old female NC/Nga mice and their pups	scFOS	0 or 5% dietary concentration through gesta-tion for dams and to day 73 for pups	The pups that ingested scFOS had significantly less inflammation than the pups that did not, but maternal scFOS consumption had no effect. The reduction of inflammation was accompanied by altered expression of cytokine genes, with scFOS-fed mice showing significantly lower IL-12, IL-17, and IL-10; however, there were no significant differences in IFN-γ, IL-4, IL-6, IL-13, or TGF-β1. No differences were seen in cecal levels of bifidobacteria, and the authors concluded that "it is unlikely that the proliferation of bifidobacteria in the gut is responsible for the reduction" in inflammation. As in the first experiment, no adverse effects were reported.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Fukata et al. (1999)	2 experiments to study the effects of competitive exclusion and ingestion of scFOS on colonization of chicks with *Salmonella enteriditis*.	60 1-day-old White Leghorn Hy-Line cockerel chicks in each experiment	scFOS	0 or 0.1% dietary concentration for 21, 28, or 35 days	In experiment 1, the enumeration of *S. enteriditis* in the chicks inoculated with the competitive-exclusion preparation was significantly decreased compared with the other three groups while in experiment 2, *S. enteriditis* was significantly decreased in the scFOS group and the combination treatment group. No significant differences between groups were noted in numbers of cecal total bacteria, *Bifidobacterium, Bacteroides* or *Lactobacillus*spp., or *E. coli*. The authors concluded that low-dose feeding of scFOS in the diet of chicks with a competitive-exclusion treatment may not lead to a shift in the intestinal gut microbiota but may result in reduced susceptibility to *Salmonella* colonization. They also reported no adverse effects from the treatment.
Gaskins et al. (1996)	Study the effect of scFOS on the inflammatory response to *Clostridium difficile*	24 12-week-old antibiotic-compromised C57BL/6NHsd mice	scFOS	30 g/L drinking water for 10 days	Mice that had received antibiotic treatment exhibited increased susceptibility to clostridia, but this effect was significantly reduced in mice receiving scFOS. The authors noted that the diminution of toxin A titers in antibiotic-treated mice receiving scFOS was “more striking” than the effects of scFOS on bacterial numbers, and suggested that this indicates either reduced toxin production or direct binding of toxin A by scFOS. No adverse effects of scFOS ingestion were reported.
Grand et al. (2013)	Assess the effect of scFOS on the growth and carcass weight of calves	112 3-week-old male Prim’Holstein calves	scFOS	21 weeks	Calves receiving scFOS supplements showed significantly improved feed conversion and carcass weight and tended to have increased levels of SCFA. The authors suggested that “inclusion of scFOS in the calf milk replacer allowed the growth performance of preruminant calves to be enhanced, possibly via a modification of the activities of microbial fermentation.” No adverse effects were reported.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Hidaka et al. (1986)	Study SCFA production after ingestion of different levels of scFOS	24 male Wistar rats weighing about 170 g	scFOS	0, 0.4%, 2.0%, or 10.0% dietary concentration for 2 weeks	No effect was observed in production of SCFA at the lower doses of scFOS, but addition of the prebiotic at 10% dietary concentration resulted in a significant increase in SCFA and decrease in fecal pH. No adverse effects were reported from scFOS even at the level of 10% dietary concentration.
Hosono et al. (2003)	Study scFOS as a mucosal immune-regulator	6-week-old female BALB/c mice	scFOS	0 or 7.5% dietary concentration for either 4 or 6 weeks	IgA secretion from Peyer's patch cells was significantly increased dose-dependently by scFOS administration, as was production of IL-5, IL-6, IL-10, and IFN-γ from $CD4^+$ T cells, but scFOS suppressed serum IgG1 activity. The authors concluded that "this information suggests that scFOS acts as a preventive agent with regard to food allergy" via changes in the intestinal environment. The groups showed equivalent feed intake and growth and no adverse effects were reported.
Howard et al. (1995) Part 1 [mouse]	Test the effects of scFOS, XOS, and gum arabic on cecal and colonic microbiota	52 male BALB/c weanling mice weighing an average of 22.3 g	scFOS	0 or 30 g/L drinking water for 14 days	The mice ingesting scFOS had significantly higher concentrations of bifidobacteria than the other 3 groups, both in absolute numbers and as a percent of total bacteria. There were no differences in feed and water intake or in weight gain.
Howard et al. (1995) Part 2 [rat]	Test the effects of scFOS, XOS, and gum arabic on cecal and colonic microbiota	44 male Sprague-Dawley weanling rats weighing an average of 51.7 g	scFOS	0 o 30 g/L drinking water for 14 days	Cecal crypt depth was significantly greater with ingestion of XOS than the other treatments and significantly less with scFOS ingestion than with the other treatments while in the colon, the effects were reversed with scFOS producing the greatest crypt depth and XOS the least; the proliferation zone was significantly less in rats receiving scFOS or XOS than controls or rats receiving gum arabic; and cell density was lower in rats fed scFOS or gum arabic than the other diets. However, none of these differences was regarded by the authors as functionally significant. There were no differences in feed and water intake or in weight gain and no reported adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Ito et al. (2009) Part 1	Test the effect of scFOS or $DP_{average}$ = 8 FOS on gut permeability and bacterial translocation in a rat colitis model	24 8-week-old male F344 rats weighing 149-177 g	scFOS or FOS	0 or 6% dietary concentration for 7 days	Rats fed FOS of both chain lengths showed lower feed intake and body weight gain as well as cecal enlargement, higher cecal mucin and IgA concentration, and lower cecal pH. Mucin and IgA concentrations were significantly correlated with butyrate and lactate concentrations. No adverse effects were reported due to ingestion of either fructan.
Ito et al. (2009) Part 2	Test the effect of scFOS or $DP_{average}$ = 8 FOS on gut permeability and bacterial translocation in a rat colitis model	42 8-week-old male F344 rats weighing 149-177 g with TNBS-induced colitis	scFOS or FOS	0 or 6% dietary concentration for 7 days before and 10 days after TNBS inoculation	In the 7-day period before TNBS treatment, rats fed fructans had lower feed intake and body weight gain, consistent with the control findings. However, following chemical colitis induction, rats fed the fructans recovered more quickly with increased feed intake and weight gain than the control rats. Endotoxins, as well as aerobic and anaerobic bacteria, increased after TNBS treatment, but bacterial translocation and mucosal damage were reduced with the fructans; portal endotoxin was lower and fecal mucin was higher with the DP-8 FOS. Cecal mucin, SCFA, and IgA did not differ among the groups following TNBS treatment. The authors concluded that both chain-length FOS exerted prophylactic effects on the acute phase of TNBS-induced colitis in rats, possibly through reduction of bacterial and endotoxin translocation. They attributed the protective effect partially to greater mucin concentration. No adverse effects were reported due to ingestion of either fructan.
Le Blay et al. (1999)	Study the effects of short-term and long-term ingestion of scFOS	36 6-week-old male Wistar rats with an average weight of 115 g	scFOS	0 or 9% dietary concentration for 2, 8, or 27 weeks	Cecal SCFA concentration was significantly higher in rats fed scFOS than in those fed the control diet. Butyrate production was significantly higher in rats fed scFOS at all time periods while lactate concentration was significantly elevated after 2 weeks but then decreased. Ingestion of scFOS significantly increased the concentrations of total lactic acid bacteria and *Lactobacillus* spp. after 2 weeks, but these effects disappeared after 8 and 27 weeks of scFOS consumption. The authors suggested that, over time, the cecal microbiota may adapt to continuous intake of scFOS, promoting the growth or metabolic activity of lactate-utilizing bacteria. There were no differences between groups in feed intake or body weight and no reported adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Morohashi et al. (1998)	Study the effect of scFOS on Ca absorption	16 45-day-old male Wistar rats	scFOS	0 or 5% dietary concentration for 12 days	Ca absorption and urinary Ca were significantly greater in rats fed scFOS. Ca balance in the scFOS group was also significantly improved. There were no differences between groups in feed intake or body-weight gain and no adverse effects such as weight reduction or diarrhea were observed.
Nakamura et al. (2004)	Study the potential benefits of scFOS to the mucosal immune system in infancy	Neonatal BALB/c mice, 4-6 pups/litter	scFOS	0 or 5% dietary concentration for 23, 30, 38, or 44 days	Mice receiving scFOS had significantly higher levels of IgA in the jejunum, ileum, and colon, as well as in the feces, and significantly higher levels of cecal acetate, butyrate, and propionate. The authors concluded that scFOS increases IgA levels in the colon and also in the small intestine. Feed intake and body weight did not differ between the groups and no adverse effects were observed.
Ohta et al. (1995)	Study the effect of scFOS on absorption of calcium and magnesium along the colon and rectum	28 7-week-old male Sprague Dawley rats	scFOS	0 or 5% dietary concentration for 8 days	Feed intake was significantly lower among rats receiving scFOS, but there was no significant difference in bodyweight. Rats fed scFOS had higher wet weight of cecal contents and greater absorption of calcium and magnesium. The authors concluded that, “The addition of [scFOS] to the diet significantly increased the absorption of both calcium and magnesium. Moreover, about half of the increase from [scFOS] consumption takes place in the colon and rectum.” No adverse effects were reported.
Ohta et al. (1998a)	Study the effect of scFOS with slightly different DP on mineral absorption	49 5-week-old male Sprague-Dawley rats	scFOS	0, 5, or 10% dietary concentration for 6 weeks	All scFOS varieties increased Ca and Mg absorption dose-dependently and increased fecal excretion of nitrogen non-dose-dependently. The different degrees of polymerization of the test scFOS did not differ significantly in their effects. While the authors conceded that comparison of scFOS with DP = 3 or 4 may not be sufficient to conclude that chain length is of no nutritional importance, they suggested that the results indicate “that the osmotic effect of indigestible carbohydrates is not the main reason for their stimulatory effect on mineral absorption.” Feed intake and body weight gain did not differ significantly among the groups and there were no reported adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Ohta et al. (1998b)	Study the effect of scFOS on calbindin-D9k concen-trations in the small and large intestines	27 5-week old male Sprague Dawley rats	scFOS	0, 5, or 10% dietary concentration	Ingestion of scFOS significantly increased absorption of Ca and the levels of calbindin in both large intestinal segments dose dependently while significantly decreasing calbindin in both small intestinal segments. The authors concluded that mucosal calbindin is regulated independently in the small and large intestine and that scFOS stimulates Ca absorption by both the paracellular and transcellular route from the large intestine. The authors did not report any adverse effects from ingestion of a 10% dietary concentration of scFOS.
Ohta et al. (1998c)	Study the effect of scFOS on mineral absorption and bone density w/ or w/o gastrec-tomy	30 4-week-old male Sprague-Dawley rats w/ or w/o gastrec-tomy	scFOS	0 or 7.5% dietary concentration for 4 weeks	Neither gastrectomy nor substitution of scFOS for sucrose had any significant effect on feed intake or weight gain. Gastrectomy significantly increased absorption of P but reduced Ca absorption and both Ca content and bone mineral density of the femur and tibia, while ingestion of scFOS significantly increased Ca absorption and ameliorated the decrease in Ca content and bone mineral density in gastrectomized rats. The authors speculated that these results suggest that $CaCO_3$ was not dissolved in the absence of gastric acid, thus decreasing the formation of insoluble P compounds; the role of scFOS was to lower the pH of the colon and thereby enhance Ca absorption. No adverse effects were reported from ingestion of scFOS.
Ohta et al. (1999)	Study the effect of scFOS on Fe absorption after gastrectomy	24 gastrec-tomized rats	scFOS	0 or 7.5% dietary concentration for 4 weeks	The gastrectomized rats exhibited anemia whether they were receiving heme iron or iron citrate, but inclusion of 7.5% scFOS significantly improved iron absorption from both iron sources and reduced or prevented anemia. No adverse effects attributable to scFOS were reported.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Ohta et al. (2002)	Study the effects of scFOS on femoral bone loss in ovarectomized mice	32 ovarectomized female 8-week-old ddY mice and 32 similar non-ovarectomized mice	scFOS	0 or 5.0% dietary concentration for 6 weeks	The ovarectomized mice receiving scFOS gained significantly more weight than did those receiving isoflavones and both scFOS and isoflavones prevented femoral bone loss. scFOS also significantly increased cecal β-glucosidase activity and equol production from daidzein. No adverse effects were reported from diet supplementation with 5% scFOS.
Oku et al. (1984) Part 1	Study the digestibility of scFOS and effects on enzymes	Male Wistar rats weighing about 50 g	scFOS	0 or 20% dietary concentration for 6 weeks	The 6-week feeding study was intended to test whether long-term ingestion of scFOS might induce greater activity of hydrolyzing enzymes, but this did not occur; the activities of sucrase and maltase tended to decrease during long-term feeding of scFOS. No adverse effects from a diet containing 20% scFOS were reported.
Oku et al. (1984) Part 2	Study the potential for hydrolysis of scFOS after absorption	Male Wistar rats weighing about 230 g	scFOS	Administered intravenously in a single dose of unreported size	The purpose of the intravenous study was to determine if any small amounts of 1-kestose or nystose that might be absorbed intact could be hydrolyzed by enzymes of some internal organ. Nearly all injected radioactivity was excreted in the urine; less than 1% appeared in expired CO_2 and only 1.24% appeared in the feces. Thin-layer chromatography of the excreted radioactivity determined that it was in the form of unchanged scFOS, indicating that no hydrolysis occurred. No adverse effects were reported.
Oku and Nakamura (2002)	Study the role of intestinal microbiota in hydrolysis of scFOS	Conventional and germ-free male Wistar rats weighing about 230 g	^{14}C-scFOS	Dose not reported; single oral administration	In the conventional rats, about 60% of the administered radioactivity was exhaled as CO_2 within 24 hours. The total release of radioactivity was similar to that observed with ^{14}C-sucrose, but was delayed about 3 hours. Administration of ^{14}C-scFOS to germ-free rats resulted in only trace exhalation of radioactivity, demonstrating that hydrolysis is provided only by intestinal microbes, and caused no reported adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Perrin et al. (2001) Part 1	Determine the time required for the effects of a high-fiber diet to stabilize production of SCFA	96 10-week-old male and female BDIX rats	scFOS (or wheat bran or resistant starch)	2, 16, 30, or 44 days	Rats' weight gain was not affected by diet. All of the fibers showed significant differences from the control diet in SCFA production, but the specific SCFA produced varied among the different fibers: wheat bran produced primarily acetate and little butyrate, while resistant starch and scFOS produced both acetate and butyrate as well as propionate. Interindividual variability in SCFA production among the rats in each feeding group was lowest at day 44, suggesting that the effects of fiber ingestion had not stabilized prior to that time. There were no reported adverse effects.
Perrin et al. (2001) Part 2	Assess the effects of different fibers on SCFA production and aberrant-crypt foci	36 10-week-old male BDIX rats	scFOS (or wheat bran or resistant starch)	44 days before inoculation with azoxy-methane and 30 days after	Diet had no effect on weight gain, but rats receiving scFOS or resistant starch had significantly fewer aberrant-crypt foci than did control-group rats. The authors concluded that "butyrate is associated with protection against the initial stages of colon carcinogenesis." No adverse effects were reported.
Pierre et al. (1997)	Study the effects of scFOS on the development of tumors in C57BL/6J-*Min/+* mice as a model for colon cancer	40 C57BL/6J-*Min/+* mice, hetero-zygous for a mutation in the *Apc* gene, aged 5-6 weeks	scFOS	0 or 5.8% dietary concentration for 42 days	There were no significant differences in growth across the diet groups and no sex- or age-related differences were noted on tumors. A reduction in colon tumors, which were largely associated with the distal colon, was observed in mice receiving scFOS. The beneficial effects of scFOS were limited to the colon, and no differences were seen in the small intestine. The authors concluded that "short-chain fructo-oligosaccharides counteract advanced stages of colon carcinogenesis, possibly via stimulation of antitumoral immunity by modulation of the colonic ecosystem." No adverse effects were reported attributable to ingestion of scFOS.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Takahara et al. (2000)	Study the effect of scFOS on mineral absorption, mineral concentration, and femoral bone structure	16 42-day-old male Wistar rats (mean weight 173 g)	scFOS	0 or 5% dietary concentration for 15 days	Rats in the 2 groups did not differ in bodyweight gain, or in feed intake. Absorption of both Ca and Mg were significantly greater in rats receiving scFOS than in the controls. Trabecular bone volume at the metaphysis and bone volume at the neck of the femur were significantly larger in rats ingesting scFOS and the concentrations of Ca, Mg, and P were significantly higher in each region of the bone surface. There was a significant association between absorption of Ca and Mg and concentrations of those minerals in bone. No adverse effects were reported.
Takeda and Niizato (1982)	Study laxation effects of scFOS	48 7-week-old male Sprague–Dawley rats	scFOS	3000 or 6000 mg/kg bw in a single dose	Up to hour 6 after dosing, no diarrhea was observed in any group. Between hour 6 and hour 24, half of the rats in both groups receiving scFOS exhibited diarrhea. Diarrhea increased dose-dependently, with only 1-2 diarrheal stools in the 3-g/kg bw group and 2-4 diarrheal stools in the 6-g/kg group. With sorbitol, 1-5 diarrheal stools were seen in 4 animals in the 3-g/kg bw group and all 6 animals had diarrheal stools in the 6-g/kg bw group. In the 3-g/kg bw maltitol group, 4 rats had 1-5 diarrheal stools while in the 6-g/kg bw group 5 rats had 1-14 diarrheal stools. Among the animals receiving glucose, 1 diarrheal stool was observed in 1 rat in the 3-g/kg bw group and none in the 6-g/kg bw group. It was concluded that the diarrheal effect of scFOS, while greater than that of glucose, was less than that of the 2 sugar alcohols tested.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Takemura et al. (2010)	Test the effect of scFOS and inulin on growth and colonization ability of *L. plantarum* strain LP14	18 6-week-old female ddY mice	scFOS or inulin from chicory	0 or 5% dietary concentration for 28 days	Recovery of *L. plantarum* in mice fed control, inulin, or scFOS diets for 2 days did not differ at 0 or 6 hours, but was significantly higher in both the scFOS and inulin groups than in the controls after 24 and 30 hours; by 48 hours, differences were no longer apparent. The same pattern emerged when *L. plantarum* recovery was assessed in mice receiving their assigned diets for 28 days. In both assessments, the area under the recovery curve was highest for mice fed the diet with inulin and lowest for the control mice. The authors concluded that dietary fructans promoted growth of *L. plantarum* in the colon, but did not improve its ability to colonize the mouse GI tract. No adverse effects were reported due to ingestion of either scFOS or inulin.
Tsukahara et al. (2003)	Test the effect of scFOS on production of SCFA and on intestinal morphology	6 40-day-old crossbred castrated male pigs weighing about 12 kg	scFOS	0 or 10% dietary concentration for 10-11 days	The daily intake of scFOS was about 70-80 g. The digesta of the pigs given scFOS had significantly higher water content and concentrations of SCFA, especially butyrate, than that of control pigs; the mucosa was thicker and the crypt depth was greater. Attributing the effects of scFOS to bacterial fermentation, the authors concluded that “the beneficial roles of scFOS in the physiology of the large intestine rely on the activity of intestinal microbiota.” 10% scFOS in the diet caused no reported adverse effects.
Winkler et al. (2007)	Assess the ability of scFOS to reduce intestinal damage associated with ulcerative colitis	60 10-week-old female C57BL/6 mice	scFOS	0 or 300 mg/day for 10 or 14 days	Treatment with scFOS significantly increased crypt depth and reduced disease symptoms and damage to the distal colon. No adverse effects were seen attributable to scFOS, and the authors concluded, “This finding supports other studies that have demonstrated that certain fermentable carbohydrates may protect against intestinal inflammation and reduce the symptoms of IBD patients.”

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Wolf et al. (1997)	Evaluate the effect of scFOS treatment on *Clostridium difficile*-induced colitis	6-8-week-old female golden Syrian hamsters weighing 80-120 g given cip-rofloxacin	scFOS	0 or 30 g/L drinking water (0 or 240 mg/ day) for up to 24 days	Treatment with scFOS significantly increased median survival time of *C. difficile*-challenged hamsters. The authors suggested that the mechanism for this benefit might be either the bifidogenic property of scFOS or through its fermentation and resulting increase in SCFA. No adverse effects were reported.
Xu et al. (2002)	Study the effects of scFOS on digestive enzyme activities, microbiota, and intestinal morphology of growing pigs	128 growing Jiaxing Black/ Duroc/ Landrace barrows weighing an average of 20.8 kg	scFOS	0, 2, 4, or 6 g/ kg feed for 42 days	Feed intake did not differ among the treatment groups, but supplementation with scFOS significantly increased feed conversion and average daily weight gain. Viable bifidobacteria and lactobacilli were significantly increased by scFOS while clostridia and *E. coli* were reduced. The activities of the enzymes protease, trypsin, and AMY were significantly increased in the intestine but not in the pancreas; activities of chymotrypsin and lipase were not affected. scFOS significantly increased villus height in jejunal mucosa. The authors concluded that the beneficial effects of scFOS "are due to [scFOS's] ability to improve the intestinal microflora and are not a direct action of [scFOS] on the intestinal tissue." No adverse effects were reported.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Other Fructans					
Bjorck and Nilsson (1991)	Evaluate the gastric-acid hydrolysis of inulin	10 male Wistar rats (average weight = 101.8 g)	Inulin derived from dahlia tubers	0 or 5% dietary concentration for 5 days	There were no significant differences between groups in feed intake or in the dry weight of fecal matter. The inulin recovered in feces from the control group with normal levels of secretion of gastric acid was significantly hydrolyzed, whereas only minor hydrolysis occurred in rats with suppressed gastric-acid secretion, indicating the key role of gastric acid in the hydrolysis of inulin to lower molecular-weight fractions. The authors suggested that this gastric hydrolysis may liberate small amounts of free fructose, which would explain the apparent digestibility of a small fraction of ingested inulin. N.B. the rats were on a diet restricted to 10 g feed/day, which may have decreased gastric buffering and lowered the pH. No adverse effects were reported.
Bovee-Oudenhoven et al. (2003)	Test the effects of oligofructose, lactulose, resistant starch, cellulose, and wheat fiber on colonization and translocation of *Salmonella enteriditis*.	40 pathogen-free 8-week-old male Wistar rats (mean weight = 234 g)	Oligofructose from chicory	0 or 4% for 2 weeks prior to infection and 2 days after	Pre-infection feed intake and weight gain were significantly lower in rats ingesting oligofructose and lactulose than resistant starch, wheat fiber, or cellulose, and these differences remained significant after infection. Oligofructose, lactulose, and resistant starch increased counts of bifidobacteria, lactobacilli, and enterobacteria significantly more than cellulose or wheat fiber, resulting in significantly higher concentrations of lactic acid. The cytotoxicity of fecal water was directly correlated with lactic acid concentration. Rats ingesting oligofructose or lactulose shed fewer *Salmonella* in feces and had higher levels of urinary NO_x. *Salmonella* translocated to all examined compartments, mesenteric lymph nodes, spleen, and liver. There were no significant differences between the groups in translocation to the mesenteric lymph nodes, but translocation to the spleen and liver was higher with oligofructose than with resistant starch, wheat fiber, or cellulose. The authors concluded that these findings indicated increased intestinal permeability and impaired resistance to *Salmonella* infection. However, they ignored the role of the poorly buffered intestinal milieu resulting from the calcium deficiency (rats were fed diets containing only 0.02 mmol calcium/g diet and the resulting likelihood of damage to the production of lactic acid and SCFA by bacteria in the presence of fermentable substrates, an effect expected with any fiber.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Correa-Matos et al. (2003)	Study the effects of fermentable carbohydrates in piglets infected with *Salmonella typhimurium*.	48 2-day-old colostrum-fed piglets	FOS not further described (or soy poly-saccharides or methyl-cellulose)	7.5 g/L formula for 14 days	*S. typhimurium* infection produced diarrhea in controls and methylcellulose groups, but not in the soy polysaccharides and FOS groups. Ileal lactase activity and physical activity were significantly lower in the controls than in other groups after infection. Ileal mucosal barrier function was impaired by *S. typhimurium* infection in the control and soy polysaccharide groups, but was unaltered in the jejunum and colon. Ingestion of FOS shortened recovery time and improved infection-associated symptoms in piglets infected with *S. typhimurium.* The authors concluded that, "because fermentable fiber enhances intestinal function and reduces the severity of *S. typhimurium* infection–associated symptoms, it may be a cost-effective way in which to reduce the severity of pathogenic infection–associated symptoms in infants." No adverse effects were reported due to any of the tested fermentable carbohydrates.
Coudray et al. (2006)	Investigate the effect of chicory inulin ingestion on absorption of Zn and Cu	80 male Wistar rats aged 2, 5, 10, or 20 months	Inulin from chicory	3.75% dietary concentration for 4 days followed by 7.5% for 26 days	Rats receiving 7.5% inulin consumed significantly less feed and had nonsignificantly less growth in body weight than control rats. Ingestion of 7.5% inulin resulted in significantly higher cecal wall and content weights and reduced cecal content pH due to significantly increased production of SCFA. Absorption of both Zn and Cu was significantly lower in 10- and 20-month-old rats than in 2- and 5 month-old rats. Incorporation of inulin in the diet significantly increased absorption of both Zn and Cu in rats of all ages. There were no reported adverse effects.
Daubioul et al. (2000)	Explore the effect of oligofructose on lipid metabolism in obese non-insulin-dependent diabetes mellitus patients	5-week-old genetically obese male Zucker rats weighing an average of 140 g	Oligofructose from chicory	0 or 10% dietary concentration for 10 weeks	No changes were seen in serum TAG or glucose concentrations, but the hepatic concentration of TAG was significantly reduced and the epididymal fat mass was significantly lower. Among the key enzymes involved in fatty acid synthesis and esterification, only malic enzyme activity was significantly lower in oligofructose-fed rats than in controls. The addition of 10 g/100 g oligofructose to the diet significantly slowed the increase in body weight. The authors concluded that the metabolic response to dietary fructans is different in obese Zucker rats than in other animal models, and suggested that fructans, while having a hepatoprotective effect with regard to steatosis, do not counteract the hyperlipidemia associated with insulin resistance due to a leptin-receptor defect. There were no reported adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Daubioul et al. (2002)	Explore the effect of oligo-fructose-enriched inulin on lipid metabolism in obese non-insulin-dependent diabetes mellitus patients	5-week-old genetically obese male Zucker *fa/fa* rats weighing 90-125 g	1:1 blend of long-chain chicory inulin and oligofructose	0 or 10% dietary concentration for 8 weeks	Rats consuming a diet with 10% oligofructose-enriched inulin had significantly lower energy intake, lower body weight, and less TAG accumulation in the liver compared with the control and MCC groups. The high fermentation of fructans compared with MCC was reflected by significantly greater cecal contents and greater propionate concentration in rats fed oligofructose-enriched inulin compared with those fed MCC. Propionate selectively decreased the incorporation of acetate into total lipids in the fructan-fed rats. Although ingestion of fructans had no significant effect on the activities of key enzymes controlling fatty acid synthesis, liver weight and steatosis was significantly reduced as compared to control and MCC rats. The authors concluded that "the beneficial effects of fermentable non-digestible carbohydrates on liver steatosis could be useful in overweight people." No adverse effects were reported due to ingestion of 10% long-chain inulin and oligofructose in the diet.
Delzenne et al. (1995) Part 1	Study the effect of oligofructose and inulin on retention of energy, nitrogen, and minerals (Ca, Mg, Fe, Zn, and Cu)	30 male Wistar rats weighing about 100 g	Oligofructose or inulin from chicory	0 or 10% dietary concentration for 26 days	There were no significant differences in body weight gain or in nitrogen balance. The fecal mass excreted was significantly higher in the animals fed inulin or oligofructose than among the controls due to increased water content; there was no difference in dry mass weight. Addition of inulin or oligofructose to the diet significantly reduced the fecal excretion of Ca, Mg, Fe, and Zn; Cu excretion was also apparently reduced but not statistically significantly. No significant differences were seen between oligofructose and inulin in this significant improvement in mineral absorption. Nitrogen excretion in the feces was significantly increased by ingestion of inulin or oligofructose, but was balanced by significantly reduced urinary excretion such that nitrogen balance was unchanged. 10% oligofructose or inulin in the diet produced no reported adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Delzenne et al. (1995) Part 2	Study the effect of oligofructose and inulin on retention of energy, nitrogen and minerals (Ca, Mg, Fe, Zn and Cu)	30 male Wistar rats weighing about 100 g	Oligofructose or inulin from chicory	0 or 10% dietary concentration for 50 days	The results were the same after 50 days as they had been after 26 days in the first experiment. No reported adverse effects resulted from 10% oligofructose or inulin in the diet.
Djouzi and Andrieux (1997)	Compare the effects of oligofructose, GOS, and glucooligo-saccharides on the composition and metabolism of the intestinal microbiota	24 2-5-month-old gnotobiotic Fischer 344 rats inoculated with a human fecal flora	Oligofructose from chicory	4% dietary concentration for 4 weeks	All 3 oligosaccharides significantly reduced plasma cholesterol levels as compared to controls, but TAG was unchanged. Oligofructose ingestion resulted in no changes in glycolytic activity levels (α or β-glucosidases, β-galactosidase, or β-glucuronidase). No changes were noted in numbers of bacteroides, clostridia, enterococci, or enterobacteria, but bifidobacteria increased significantly in the feces of rats fed oligofructose or GOS. Cecal SCFA production—particularly acetate and butyrate—was significantly increased by ingestion of oligofructose; ammonia and fecal pH were significantly reduced and cecal weight increased, and H_2 and CH_4 excretion were significantly increased as compared to the controls. The authors suggested that unidentified species of butyrate-producing bacteria, as well as bifidobacteria, may ferment oligofructose, with their butyrate production providing beneficial effects on colonocyte metabolism. No adverse effects were reported.
Fiordaliso et al. (1995)	Study the effect of oligofructose on blood lipids	85 male Wistar rats weighing about 100 g	Oligofructose from chicory	0 or10% dietary concentration for 5-12 weeks	Administration of oligofructose produced a significant reduction in serum TAG levels, seen after 1 week of feeding and maintained up to 16 weeks, as well as phospholipids and TC. The cholesterol reduction was due to reduction of the very-low-density fraction; LDL and HDL levels were unaffected. Synthesis and secretion of TAG in isolated hepatocytes were significantly reduced by oligofructose. There were no differences in feed intake or weight gain between the feeding groups; all animals appeared healthy and exhibited normal behavior.

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Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Fontaine et al. (1996)	Investigate the influence of the intestinal microbiota on mucin	20 2-month-old germ-free male inbred F344 rats	Inulin from chicory	0 or 10% dietary concentration for 1 month	The cecal and colonic contents of germ-free rats contained significantly more acidic mucins than sulfomucin, which was present in the same proportion (cecum) or higher proportion (colon) as neutral mucin. Ingestion of inulin significantly increased the amount of neutral mucin and decreased acidic mucins in the cecal contents and increased neutral and sulfated mucins in the colonic contents and significantly increased the amounts of neutral and acidic mucins in the cecal mucosa. Inulin also significantly increased the content of SCFA, thus reducing the luminal pH. The authors suggested that the low amount of mucin that was recovered in the colonic mucosa indicated that the presence of the proliferating bacterial biota associated with inulin resulted in mucin being extensively released from the mucosa to the colonic lumen, and concluded that "inulin may have a beneficial effect on health by stimulating sulfomucin synthesis." No adverse effects were reported from ingestion of inulin at a 10% dietary concentration.
Juskiewicz and Zdunczyk (2004)	Compare the effect of long-chain inulin, cellulose, or CMC on cecal physiology	60 4-week-old male Wistar rats weighing an average of 76 g	Long-chain inulin from chicory root	0 or 4% dietary concentration for 4 weeks (daily intake = 654 mg, or about 3600 mg/kg bw/day for a 180-g rat)	Weight gain was highest in the rats receiving long-chain inulin or cellulose, which did not significantly differ from each other, but there were no significant differences in feed conversion efficiency. Rats receiving CMC, alone or in combination, had significantly higher cecal masses, and those receiving cellulose alone had significantly the lowest; rats receiving long-chain inulin, with or without added cellulose, were in between the other groups. The animals fed long-chain inulin alone were generally between the other groups in measures of digesta mass, dry matter, pH of digesta, and ammonia. The long-chain-inulin group showed a significantly higher level of β-glucuronidase and α-glucosidase activities than did the other groups. The highest concentration of Lowry's protein in the cecal digesta was found with long-chain-inulin feeding, indicating that only long-chain inulin increased bacterial density in the cecum. Finally, long-chain inulin significantly enhanced production of propionate and butyrate compared to the other treatments. No adverse effects were noted in response to any of the treatments.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Juskiewicz et al (2008)	Test the physiological effects of flour containing 77% inulin from chicory root	24 54-day-old male New Zealand white rabbits weighing an average of 2.4 kg	Flour containing 77% inulin from chicory root	0, 2.5, or 5% dietary concentration of chicory flour (1.9 or 3.8% inulin) for 36 days	There were no significant differences in feed intake or weight gain, or in the weight of ileal, cecal, or colonic tissue. Supplementation of the diet with chicory flour resulted in significant increases in the mass of ileal and cecal, although not colonic, digesta. Ileal and cecal digesta also had significantly reduced pH and viscosity as well as significantly lower glycolytic enzyme activity. The total cecal pool of SCFA, especially acetic acid, was significantly increased in the rabbits receiving chicory flour although no dose-related effect was evident. Diet had no effect on blood glucose level, but chicory flour significantly reduced concentrations of TAG, TC and HDL, and urea. No adverse effects were reported.
Kleessen et al. (2001)	Study the effect of DP on the ability of fructans to stimulate production of SCFA	24 5-week-old germ-free male Wistar rats with average weight = 122 g, inoculated with human feces	Oligofructose, long-chain inulin, or a 1:1 combination of long-chain inulin and oligofructose from chicory	0 or 2.5% dietary concentration for 7 days, then 0 or 5% for 8 days	Fructans increased the cecal wall weight and wet weights of cecal and colonic contents. Both fructans significantly decreased cecal and colonic pH and increased fecal output due to increased water excretion. Fructans did not affect total bacterial counts, but the different chain-length fructans had differential effects on certain bacterial groups. Rats fed long-chain inulin or oligofructose-enriched inulin had significantly higher numbers of clostridia and eubacteria, while oligofructose produced higher colonic (but not cecal) levels of bifidobacteria. Rats receiving long-chain inulin actually had significantly fewer cecal bifidobacteria than rats receiving the control diet. The highest levels of lactobacilli in the cecum and colon were in rats fed the combination of oligofructose-enriched inulin. No differences were seen between feeding groups in enterobacteriaceae or bacteroides. SCFA concentrations were invariably higher in the cecal contents than in colonic contents. Fructans significantly increased butyrate levels; long-chain inulin was most effective in increasing levels of propionate while oligofructose was most effective in producing higher levels of acetate. There were no differences between groups in feed intake or body weight gain, and the rats were in good health throughout the study with no reported adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Kleessen et al. (2003)	Study the effects of oligofructose and long-chain inulin on mucosal morphometry and mucosa-associated bifidobacteria	36 5-week-old male germ-free Wistar rats weighing 118 g on average, inoculated with human feces	Oligofructose and long-chain inulin from chicory	0 or 2.5% dietary concentration each of oligofructose and long-chain inulin (5% total dietary concentration of fructans) for 28 days	Total bacterial numbers and *Bacteroides* counts were unaffected by diet, but counts of bifidobacteria in the colonic contents and of mucosa-associated bifidobacteria were significantly higher in the rats receiving fructans. While fructans did not affect the mucosal morphology of germ-free rats, in rats inoculated with bacteria the effect of fructan ingestion was to significantly increase villus height and crypt depth. Diet had no effect on intestinal mucins in germ-free rats, while in inoculated rats fructans caused significant increases in epithelial mucus layers and the number of goblet cells in the jejunal and colonic mucosa. The rats remained healthy and no differences were seen in feed intake or body-weight gain; no adverse effects were reported.
Kok et al. (1996)	Study the effect of oligofructose on synthesis of TAG	32 male Wistar rats weighing about 120 g	Oligofructose from chicory	0 or 10% dietary concentration for 30 days	Addition of 10% oligofructose to feed did not affect intake or final body weight. The oligofructose-supplemented rats had significantly lower serum levels of TAG and phospholipids, but TC did not differ. Glycerol-3-phosphate acyltransferase activity level was significantly lowered by oligofructose, while phosphatidate phosphohydrolase activity was unaffected, suggesting that the pathway converting glycerol-3-phosphate to phosphatidate was modulated, a suggestion supported by the finding that liver levels of glycerol-3-phosphate were higher in the rats receiving oligofructose. Reduced TAG level apparently resulted from reduced synthesis in liver hepatocytes rather than accelerated catabolism. No adverse effects were reported from 10% oligofructose in the diet.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Kok et al. (1998)	Study the effect of oligofructose on triacylgly-cerol synthesis and the ability of oligofructose to ameliorate postprandial lipidemia	15 male Wistar rats weighing about 120 g	Oligofructose from chicory	0 or 10% dietary concentration for 19 days	Rats receiving standard chow had significantly greater feed intake than those receiving high-fat diets, but the addition of oligofructose did not affect feed intake. Intakes were isocaloric and final bodyweights were not different. Rats consuming high-fat diets had greater relative liver weights than those eating standard chow and addition of oligofructose had no effect. There were no differences between rats receiving standard or high-fat chow in serum TAG, phospholipid, TC, free cholesterol, or esterified cholesterol levels, but all of these were significantly reduced by the addition of oligofructose to the high-fat diet. Rats on the high-fat diet had higher levels of TAG, phospholipids, and TC in the liver and this effect was not ameliorated by oligofructose. Fatty liver was confirmed by histological and histochemical examination. Both wet and dry feces weights were lower in rats receiving the high-fat diet, but this effect was partially erased by the addition of oligofructose. Oligofructose decreased the concentration of lipids in the feces. No adverse effects were reported from 10% oligofructose in the diet.
Kruger et al. (2003)	Study effects of fructans with various DP on Ca absorption, bone density, and excretion of collagen cross-links	40 7-week-old male Sprague-Dawley rats	Oligofructose, long-chain inulin, or a combination of 8% oligo-fructose and 92% long-chain inulin from chicory	0 or 5% dietary concentration for 4 weeks	Feed intakes and final body weights did not differ among the 4 groups. Urinary Ca excretion did not differ, but the rats receiving long-chain inulin had significantly less Ca in their feces than did the other groups. Bone mineral density in the femur and spine was significantly higher in the group fed long-chain inulin. The excretion of fragments of Type 1 collagen decreased in all groups over the 4 weeks of feeding, but the decrease was greatest in the group fed long-chain inulin. The authors concluded that long-chain inulin was significantly superior to shorter-chain oligofructans in increasing Ca bioavailability and retention, but no adverse effects were reported from either treatment.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Levrat et al. (1991)	Investigate the digestive and metabolic effects of different dietary con-centrations of inulin	48 6-week-old male Wistar rats weighing an average of 170 g	Chicory inulin	0, 5, 10 or 20% dietary concentration for 21 days	Inulin did not significantly affect feed intake or body weight gain at concentrations up to 10%, but feed intake and weight gain were significantly depressed in rats receiving 20% inulin. Inulin ingestion resulted in a dose-dependent enlargement of the cecum and increases in cecal wall thickness and blood flow to the cecum, as well as dose-dependent pH reduction. Bile acid concentration was not affected. SCFA production increased with increasing inulin up to 10%, but was sharply reduced at the 20% level. Production of propionate increased with increasing inulin dose, but acetate production increased only up to 10% inulin in the diet and was significantly lower when inulin constituted 20% of the diet. Butyrate was significantly increased in rats fed inulin, but with no apparent dose effect. Cecal concentrations of Ca and phosphate were increased by inulin. Significant reduction of TAG was seen only at 20% inulin concentration while TC decreased linearly with increasing inulin.
Levrat et al. (1993)	2x2 factorial design to study the effects of inulin on urea and ammonia fluxes in the cecum	40 6-week-old male Wistar rats weighing an average of 180 g	Chicory inulin	0 or 15% dietary concentration for 21 days	Neither protein level nor inulin significantly affected feed intake or weight gain. Casein level did not affect cecal development, but 15% inulin produced a 3-fold cecal enlargement and a significant increase in the SCFA pool (especially propionic and butyric acids) and consequent pH reduction. Nitrogen retention and balance were not affected by inulin, but digestibility was reduced with the result that more nitrogen was excreted fecally and less in the urine. Inulin significantly depressed uremia and ammonia concentrations in rats receiving 15% casein, but not those receiving 45%. The authors concluded that “intake of a readily fermented oligosaccharide such as inulin does not adversely affect the nitrogen balance or retention.”

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Lobo et al. (2007)	Study the effects of fructans from yacon roots on cecum mucosal morphometry and mineral balance and retention	24 5-week-old male Wistar rats weighing 65-70 g	Yacon flour containing 55.3% oligo-fructose	0, 5, or 7.5% dietary concentration of yacon flour; 0, 2.8, or 4.1% dietary con-centration of oligofructose for 27 days	Ingestion of yacon flour resulted in a significant non-dose-dependent increase in dry feces weight and a significant dose-dependent increase in the moisture content of feces. Cecal wall thickness and crypt depth and the moisture content of cecal contents increased significantly and dose-dependently with yacon flour ingestion. Ca absorption and both Ca and Mg balance were significantly enhanced by inclusion of yacon flour, but there were no differences in bone density although the Ca content of bone increased and resulted in stronger bone structural properties. There were no differences in feed intake, weight gain, or feed efficiency among the 3 feeding groups, nor in absolute or relative weights of the spleen, liver, or kidney, and no GI disturbances were noted.
Lopez et al. (2000)	Study the effects of inulin and phytic acid on mineral absorption	32 male Wistar rats weighing about 160 g	Chicory Inulin w/ or w/o phytic acid	0 or 10% dietary concentration for 21 days	Neither inulin nor phytic acid significantly affected the rats' body weight or liver or tibia weights. However, rats fed inulin-containing diets had significantly enlarged ceca with contents of lower pH. These adaptations resulted in significantly increased absorption of Ca, Mg, Fe, and Cu. Phytic acid decreased absorption of Fe, Zn, and Cu, but inulin counteracted this effect by significantly stimulating bacterial hydrolysis of phytic acid.
Nzeusseu et al. (2006)	Compare the effects of partially-hydrolyzed and intact inulin	39 6-week-old male Wistar rats weighing 150-175 g	Chicory inulin, intact or partially hydrolyzed	0 or 5% dietary concentration for 90 days	Body weight, lean body mass, and appendicular bone length did not differ among the 3 groups. Rats fed either intact or partially hydrolyzed inulin had similar increases of about 30% in cecal wall weight, but the relative increase in cecal levels of calbindin-9 K was twice as high in the intact inulin group as in the partially hydrolyzed inulin group. Further, a significant decrease in serum levels of type I collagen C-telopeptide was greater in the intact inulin group. The increase in whole-body bone mineral content was significantly greater in the intact inulin group than in the partially hydrolyzed inulin group. Further, intact, but not partially hydrolyzed inulin, significantly enhanced the bone mineral density of the cortical bone in both appendicular and peripheral sites as well as the polar stress/strain index of femurs. Neither intact nor partially hydrolyzed inulin produced any reported adverse effects.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Pouteau et al. (2005)	Study the effect of inulin ingestion on acetate turnover	5 male and 4 female adult Beagle dogs weighing 14-18 kg	Chicory inulin	3% dietary concentration for 21 days	Peripheral acetate concentration and turnover were not significantly changed by 4 days of inulin ingestion, but after 21 days, whole-body acetate turnover increased significantly with no change in concentration. No acetate tracer was recovered in the peripheral circulation. The fraction of oxidized tracer was significantly higher in the colon than in peripheral circulation. The authors concluded that colonic fermentation of inulin occurred and indirectly stimulated whole-body acetate turnover in dogs fed an inulin-enriched diet. They did not report any adverse effects.
Rodenburg et al. (2008)	Study the effect of oligofructose on intestinal mucosa in a low-Ca milieu	36 specific pathogen-free 8-week-old male Wistar rats (mean weight = 253 g)	Oligofructose from chicory	0 or 6% dietary concentration	There were no differences in feed intake or bodyweight gain between the 2 diets, but Cr-EDTA excretion in urine indicated increased intestinal permeability in the oligofructose group. The gene expression response of colonic mucosa to oligofructose was small in magnitude. Oligofructose did not induce changes in tight junction genes and only 8 genes related to mucosal defense were induced by oligofructose, conceded by the authors to be insufficient to explain changes in intestinal permeability, which they acknowledge is prevented by ingestion of adequate Ca to provide normal buffering. Oligofructose significantly increased expression of 177 mitochondria-related genes, indicating that oligofructose or the acidity resulting from its fermentation in a Ca-deficient milieu influences intestinal mucosal energy metabolism.
Ten Bruggencate et al. (2003)	Study the impact of oligofructose on translocation of *Salmonella enteriditis* under low-Ca conditions	8-week-old male Wistar rats weighing an average of 239 g	Oligofructose from chicory	0, 3, or 6% dietary concentration for 23 days	6% oligofructose significantly increased counts of fecal bifidobacteria, but not lactobacilli, while both 3 and 6% oligofructose increased enterobacteria prior to *Salmonella* infection. The cytolytic activity of fecal water was significantly increased non-dose-dependently by oligofructose, while lactic acid production was significantly increased dose-dependently. After *Salmonella* infection, the *Salmonella* populations in the mucosa and cecum increased in a dose-dependent manner with increasing levels of oligofructose. The authors concluded that ingestion of oligofructose in this study increased colonization and translocation of *S. enteriditis*. They attributed this effect to rapid production of acidic fermentation metabolites and resulting impairment of the mucosal barrier.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Ten Bruggencate et al. (2004)	Study the impact of oligofructose or inulin on translocation of *S. enteriditis* under normal or low-Ca conditions	64 8-week-old male Wistar rats with mean weight = 276 g	Oligofructose or chicory inulin	0 or 6% dietary concentration for 3 weeks	There were no differences between groups in growth prior to infection; after infection, growth was significantly depressed in the low-Ca oligofructose group. In the low-Ca condition, ingestion of both inulin and oligofructose significantly lowered cecal pH. Diet had no effect on bifidobacteria, but fecal lactobacilli and enterobacteria were significantly increased by both fructans; none of these bacterial counts was affected by *Salmonella* infection. The combination of low-Ca and fructan ingestion significantly increased the cytotoxicity of fecal water and excretion of mucin. Both fructans significantly increased *Salmonella* enumerations in the cecum, unaffected by Ca, and increased translocation only in the low-Ca condition—in the normal-Ca condition, no more translocation was seen in the fructan groups than in the controls. The authors concluded that oligofructose and inulin impair resistance to *Salmonella* infections in rats due to damage to the mucosal barrier, although they recognized that "dietary calcium phosphate inhibited the adverse effects of acidic fermentation and largely prevented damage to the intestinal barrier." In sum, no adverse effects were evident in the presence of adequate Ca.
Ten Bruggencate et al. (2005) Part 1	Study the impact of oligofructose on intestinal permeability and *S. enteriditis* translocation under low-Ca conditions	20 8-week-old male Wistar rats with mean weight = 226 g	Oligofructose from chicory	0 or 6% dietary concentration for 24 days	There were no significant differences between groups in feed intake or weight gain either before or after *Salmonella* infection. Prior to infection, ingestion of oligofructose resulted in significant larger counts of fecal bifidobacteria, lactobacilli, and enterobacteria, as well as increased fecal lactate and fecal-water toxicity compared with cellulose. *Salmonella* infection caused no changes in fecal bacteria, pH, or cytotoxicity. Urinary excretion of CrEDTA was significantly higher in the rats ingesting fructan than those ingesting cellulose both before and after infection, as was the concentration after infection of urinary NO_x, although this level began to decline after day 8.

Table 9. Studies of Fructans in Animals.					
Reference	**Research Objective**	**Animal Model**	**Source & Description of Test Article**	**Dose & Duration of Feeding**	**Findings**
Ten Bruggencate et al. (2005) Part 2	Study the impact of oligofructose on intestinal bacteria, SCFA, Ca, P, and mucins	8 8-week-old male Wistar rats with mean weight = 261 g	Oligofructose from chicory	0 or 6% dietary concentration for 14 days	There were no differences in cecal concentrations of SCFA, although the total pool was significantly increased by oligofructose ingestion, as were cecal concentrations of Ca and P. Ingestion of oligofructose, as compared to cellulose, stimulated the growth of bifidobacteria, lactobacilli, and enterobacteria in both cecal and colonic mucosa, although not in the ileal mucosa.
Vanhoof and DeSchrijver (1995)	Study the effect of inulin on blood lipids of rats with high or low choles-terol or cholic acid intakes	48 male Wistar rats weighing an average of 200 g	Chicory inulin, either baked or unbaked	0 or 6% dietary concentration for 5 weeks	There were no significant differences in feed or water intake or in daily growth across the 6 groups. The rats consuming diets containing cholesterol showed no effect of either baked or unbaked inulin on blood lipids, but those receiving cholesterol-free diets had significant reductions in total, esterified, and free cholesterol, although no effect was seen on TAG. Inulin ingestion also had no effect on liver lipid concentrations or fecal bile acid output regardless of cholesterol in the diet. No adverse effects were reported.

9. Safety Assessment and GRAS Determination

9.1. Introduction

This section presents an assessment that demonstrates that scFOS is safe, and is also GRAS under the Federal Food, Drug, and Cosmetic Act for direct addition to non-exempt infant formula as a prebiotic ingredient at levels up 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed. This safety assessment and GRAS determination entail two steps. In step one, the safety of the intended use of scFOS is demonstrated. In the second step, the intended use of scFOS is determined to be GRAS by demonstrating that its safety is based on generally available information and generally recognized among qualified scientific experts.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the FFDCA is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under 21 CFR §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under 21 CFR §170.30(c). This GRAS determination employs scientific procedures established under 21 CFR §170.30(b).

In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components: 1) the data and information relied upon to establish the scientific element of safety must be generally available; and 2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific procedures GRAS determination are applied below in an analysis of whether scFOS is safe and GRAS for the uses and at the use levels intended.

9.2. Safety of the Intended Use of scFOS

A scientific procedures GRAS determination requires first that information about the material establish that the intended use of the material is safe. The FDA has defined "safe" or "safety" for food additives under 21 CFR §170.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use." This same regulation specifies that three factors must be considered in determining safety. These three factors are:

1) The probable consumption of the substance and of any substance formed in or on food because of its use;

2) The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet; and

3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

An estimated daily intake (EDI) for the material is derived based on the probable human consumption of the material, taking into account any existing sources of consumption of the material. Finally, the EDI for a substance is compared against a level of consumption that has been shown to be reasonably certain to be without harm. As long as the EDI is less than or approximates this level, the substance can be considered safe for its intended use (FDA 1993).

9.2.1. EDI of scFOS

As indicated above, 21 CFR §170.3(i) requires that, in evaluating the safety of the proposed use of a new food additive, the probable consumption of the substance and of any substance formed in or on food because of its use be considered. Also to be considered is the cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet. Thus, because a scientific procedures GRAS determination requires the same quantity and quality of evidence as is required to obtain approval of the substance as a new food additive, a scientific procedures GRAS determination must also consider the probable consumption and cumulative effect of the substance in the diet. The EDI derivation described below provides a conservative estimate of the intake of scFOS under its intended conditions of use.

Section 4.2 described the derivation of the estimated exposure expected to result from the intended use of scFOS, which calls for its addition to non-exempt infant formula at up to 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed. Based on data regarding infants' energy needs, the 90th percentile of formula intake was determined for the period with the highest level of consumption per kg body weight. This in turn allows determination of the EDI for scFOS representing the 90th percentile of intake during infants' peak period of formula consumption. The EDI for scFOS is 1.035 g/kg bw/day based on consumption of follow-on formula, which is intended to contain scFOS at a higher concentration than starter formula.

This EDI is based on formula consumption per kg body weight between the ages of 14 and 27 days, when it is highest. According to Fomon (1993), infants' energy intake (normalized to body weight), and thus their formula consumption, is lower both prior to this period and following it. This was shown in Figure 4 in Section 4.2. Thus, the EDI is a conservative estimate of long-term exposure.

Additionally, the EDI assumes that the infant receives all of his or her energy from infant formula to which scFOS has been added. Parents may feed their infants a variety of formulas such that this is not the case; further, as the infant grows older and other foods are introduced, formula ceases to provide 100% of energy intake.

9.2.2. Evidence for the Safety of Infant Formula Containing scFOS

9.2.2.1. Toxicity

Extensive toxicological study of scFOS, chicory-derived oligofructose, and yacon-root-flour oligofructose has established that these fructans are non-toxic. In numerous acute, subacute, subchronic, chronic, and developmental studies of oral toxicity, no consistent, statistically significant, dose-dependent effects were reported. NOAELs have invariably been the highest dose levels or dietary concentrations tested. The dose-dependent decrements in weight gain in

animals fed diets containing high concentrations of non-digestible fructans are due to poor caloric utilization of the fructans constituting a significant portion of the animals' diets and not indicative of toxicity. Very large doses of fructans may result in cecal enlargement, a trophic effect and not evidence of toxicity.

The dispositive study for determining an acceptable daily intake of scFOS is the 2-year feeding study of scFOS in male and female Fisher 244 rats reported by Clevenger et al. (1988), which found no evidence of carcinogenicity and a NOAEL of 5% dietary concentration, equivalent to 2170 mg/kg bw/day for males and 2664 mg/kg bw/day for females. No evidence of mutagenicity or clastogenicity were reported in *in vitro* and *in vivo* studies of the genetic toxicity of scFOS and other fructans.

9.2.2.2. Intestinal Microbiota

The effects of scFOS and other fructans on colonic bacteria have been assessed in *in vitro*, animal, and human studies—the last including trials with infants, children, and both healthy and compromised adults. With the exception of bifidogenesis, which has been clearly and consistently demonstrated and is widely regarded as beneficial, the effects of fructan ingestion on overall colonic bacterial populations appear to be small. While infants receiving formula supplemented with fructans showed significant increases in fecal bifidobacteria in nearly all studies that enumerated this genus, lactobacilli increased significantly in fewer than half of the studies that measured fecal lactobacilli; neither significant increases nor decreases have been seen in other genera. Although lactobacilli do not consistently increase in numbers in response to oligofructose ingestion, no study in infants, children, or adults (or in animal models) has ever shown a decrease in these bacteria. Similarly, while numbers of pathogens and potential pathogens are not invariably reduced, they have not been observed to increase in any study. The evidence available at this time does not allow firm conclusions regarding the effects of adding scFOS to infant formula on colonic bacterial colonization. However, the weight of the available evidence indicates that no harm is likely to result to intestinal microbiota populations of infants as a result of the addition of scFOS to infant formula.

9.2.2.3. pH and Production of SCFA

Animal studies in rats (including germ-free rats with human fecal microbiota), rabbits, and dogs have consistently shown a significant increase in cecal and colonic SCFA and a resultant decrease in pH with ingestion of scFOS and other fructans. In human studies, in which analysis of cecal or colonic content is not possible, increased fecal SCFA concentrations have not generally been observed, but this is most likely due to absorption of SCFA during colonic passage. Most animal studies that analyzed individual SCFA found increased levels of acetate and butyrate and, less often, propionate. As a result of the production of SCFA, cecal and colonic pH is reduced, resulting in an environment more conducive to mineral solubility and absorption as well as to acid-tolerant bacteria such as bifidobacteria and lactobacilli. Reduced fecal pH has frequently been observed in human studies, including those in infants given formula supplemented with scFOS or other fructans. The evidence available at this time does not allow firm conclusions on the effects of adding scFOS to infant formula on colonic pH. However, a critical analysis of the published animal and human literature on increased production of SCFA

or reduction in pH suggests that these effects are beneficial; there were no suggestions that they might be harmful.

9.2.2.4. Mineral Absorption

The effect of ingestion of scFOS, as well as other fructans ranging up to long-chain inulin, on mineral absorption has been extensively studied. It is clear that inclusion of fructans in the diet improves calcium absorption in humans and animals, but whether absorption of other minerals such as magnesium, iron, zinc, copper, and selenium is improved by fructans is not fully clear. The 2 animal studies and the single study in children that measured bone density all found significantly increased density with ingestion of inulin or long-chain inulin. While the available evidence suggests that ingestion of fructans generally has beneficial effects on mineral absorption, especially calcium, the evidence available at this time does not allow firm conclusions on the effects of adding scFOS to infant formula on mineral and calcium absorption. However, there is no evidence of an adverse effect on mineral absorption.

9.2.2.5. Intestinal Morphology, Mucosa, and Gut Permeability

Studies in humans, with and without adequate calcium intake, do not suggest adverse effects from fermentation of 20-30 g additional fructans/day. Several experiments from one research group reported an increase in *Salmonella* translocation after inoculation in rats fed oligofructose in a severely low-calcium diet (30 mmol Ca/kg as compared with a standard AIN diet providing 125 mmol Ca/kg). The authors did not explain why they chose to create a calcium-deficient state in the test animals, a situation which raises questions regarding the validity of the model due to the loss of buffering capacity. No explanation for the alleged increase in permeability could be found in the gene expression pattern, which was unchanged in the oligofructose-fed rats compared with controls, and other researchers observed decreases rather than increases in bacterial translocation and endotoxin permeability with fructans. Furthermore, the observed mucin stimulation and acid formation that was also reported is generally regarded as a beneficial rather than detrimental effect in relation to fermentable dietary fiber.

Animal studies in both normal rats and gnotobiotic rats with inoculated human fecal microbiota have shown that inclusion of fructans in the diet results in increased cecal weight and wall thickness, increased villus height and crypt depth, and increased mucin synthesis and mucus-layer thickness. Except in the low-calcium experiments discussed above, ingestion of fructans has not been shown to affect intestinal permeability. The absence of harmful effects such as mucin shedding or increased gut permeability was confirmed in 2 human studies with oligofructose. The available evidence indicates that the morphological and mucosal effects of fructan ingestion are beneficial rather than detrimental, and, although these endpoints have not been studied in human infants, there is no evidence that suggests that addition of scFOS to infant formula results in adverse GI effects. Similarly, while studies in infants have not determined whether small amounts of scFOS may penetrate the relatively permeable mucosa of neonates, no indications of any harmful effects from this source have been reported.

9.2.2.6. Colonic Function, Stooling, and Tolerance

Fructans of all chain lengths from scFOS to long-chain inulin do not differ remarkably in their GI effects from other fiber-type carbohydrates. Intakes of up to 20 g/day were generally well tolerated by both healthy and compromised adults, with more frequently reported and occasionally slightly more severe flatulence. Ingestion of fructans or other fibers tends to increase defecation frequency and stool weight. Extensive research on colonic function has not produced any indications of adverse effects from ingestion of scFOS or other fructans in adults or children, confirming the absence of adverse effects on infants receiving formula supplemented with fructans. In none of these studies, enrolling over 3,100 term and preterm infants, were any serious adverse events observed that could reasonably be associated with feeding. Minor intolerance symptoms such as crying or flatulence were occasionally reported, but generally with no greater frequency or severity in the test groups than in the placebo groups.

In clinical trials of human adults, children, and infants, ingestion of fructans was consistently associated with increased defecation frequency and softer, more loosely formed stools. While some investigators have regarded these effects as evidence of intolerance, it is more generally recognized—especially with regard to formula-fed infants—that such changes in laxation are properly seen as beneficial rather than adverse. Such changes in stooling produce outcomes in formula-fed infants that more closely match the stooling outcomes of breastfed infants. An investigation by Weaver et al. (1988), who observed the stooling behavior of 240 healthy infants aged 2-20 weeks, half of whom were breastfed while the other half received cow's-milk formula, was one of many studies that reported significantly more frequent and significantly softer stools from the breastfed infants. These beneficial alterations of stooling behavior differ from induction of diarrhea, an adverse effect that has not been observed to result from administration of scFOS, other fructans, or other nondigestible oligosaccharides.

9.2.2.7. Liver and Blood Chemistry

Numerous animal and human studies have investigated the effects of scFOS and other fructans on blood chemistry. Significant reductions have frequently been observed in animals and in humans in levels of insulin, glucose, TC, lipoproteins, phospholipids, and TAG, but significant changes in these endpoints have not been observed in numerous other studies. However, no study has ever produced increases in any of these outcomes, indicating a lack of harm even if benefit is absent. Most animal studies that included analysis of liver tissue found significant reductions in production of glucose and lipids, although not in cholesterol, along with lower concentrations of TAG. Only 2 studies examined the effect of fructan ingestion on blood levels of liver enzymes: a study in which inulin was given to hyperlipidemic obese patients found no change in liver enzymes, while a study in which oligofructose was given to patients with steatohepatitis found significant reductions in elevated levels of AST and non-significant decreases in ALT.

A critical analysis of animal and human studies failed to identify any adverse effect of fructan ingestion on liver function or blood chemistry, although these endpoints have not been studied in infants.

9.2.2.8. Water Balance

Infant formula to which scFOS may be added is about 90% water; additional water is derived from the oxidation of protein, fat, and carbohydrate to provide a total of about 95 ml water/100 ml formula (Ziegler and Fomon 1989). Water not used for growth, and not lost in the feces or by evaporation, must be excreted by the kidney. The osmolar concentration of the urine is determined by the renal solute load and the amount of water available for renal excretion. Very high renal osmolarity may increase the risk of hypernatremic dehydration (Ziegler and Fomon 1989; SCF 2001a). It should be noted that excessive fecal water loss, dehydration, and associated failure to thrive have not been observed in the large number of studies in which high doses of scFOS or other fructans were given to animals or in studies of human infants.

When the European Scientific Committee on Food (SCF) was first asked to assess the safety of adding a combination of GOS and fructans to infant formula at a concentration up to 800 mg/100 ml, the Committee concluded that the potential for adverse effects in older infants was "very low," but that insufficient data were available at that time to establish the safe use of the proposed addition in formula intended for neonates (SCF 2001a). In particular, the Committee was concerned that some infants might be at risk of dehydration if the presence of poorly digested carbohydrates significantly increased stool frequency and reduced stool consistency, thus increasing fecal water loss and increasing renal osmolarity. This concern was addressed to the Committee's satisfaction later that same year with the submission of additional data from studies not previously reviewed by the Committee; a new opinion was issued concluding that the Committee then "has no major concerns" about the addition of 800 mg/100 ml of a 9:1 mixture of GOS and fructans to formula intended for neonates (SCF 2001b). This latter judgment was confirmed in the 2003 *Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae* (SCF 2003).

Despite the SCF's acceptance of the safety of adding 800 mg GOS+fructan/100 ml formula, in reviewing an application for the use of only 300 mg oligofructose/100 ml the European Food Safety Authority did not refer to this opinion, but instead concluded (EFSA 2004):

1. "There was an increased prevalence of adverse effects, including loose stools, in infants fed formula with added fructooligosaccharides."
2. "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 formulae."
3. "There is no evidence of benefits to infants from the addition of fructooligosaccharides to infant formula at the conditions specified by the manufacturer while there are reasons for safety concerns."

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. First, as discussed in Section 9.2.2.7, 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. While diarrhea would indeed be properly regarded as an adverse effect, it has not been reported in any of the many controlled studies of ingestion of fructan-supplemented formula by infants (or studies of formula supplemented with GOS or other oligosaccharides). Of particular relevance is the study by Yao et al. (2010), in which stool composition and consistency was a primary outcome measure of feeding infants formula supplemented with 300 or 500 mg oligofructose/100 ml for 8 weeks. No infant consuming these formulas was reported as having diarrhea, and even the incidence of parentally reported watery stools was not increased by the addition of oligofructose, indicating that there was no increase in water loss.

Second, the absence of statistically significant long-term benefit in short-term studies of scFOS or other fructans added at an average of 300 mg/100 ml (compared with an oligosaccharide content of about 800-1200 mg/100 ml in human milk) does not bear upon the safety of the formula and is not relevant to a determination of whether the intended use is GRAS. Few of these studies were designed or powered to detect long-term beneficial effects from the tested interventions.

Parental classification of stool consistency in the studies reviewed by EFSA (2004) relied on the scale 1 = hard, 2 = formed, 3 = mushy, 4 = runny, 5 = watery. The effects of the intervention in the study by Euler et al. (2005) cited by EFSA—addition of oligofructose at either 150 or 300 mg/100 ml—were rather modest; there were no changes in the median scores for stool consistency between baseline and intervention periods for either oligofructose concentration, while the means changed only from 2.6 to 2.7 at 150 mg and from 2.7 to 3.2 at 300 mg oligofructose/100 ml formula. The infants in the human-milk reference group had baseline and intervention-period means of 3.8 and 3.9, respectively. None of the changes was statistically significant, and mean stool consistency of the formula groups remained less watery than that of infants fed human milk, although the addition of oligofructose tended to produce stooling somewhat more similar to that of breastfed infants. Issues of diarrhea and dehydration were also addressed by Bettler and Euler (2006), who compared the effects of formula containing 0, 150, or 300 mg oligofructose/100 ml on stooling patterns of 212 healthy infants and concluded that, “There were no significant differences in incidence of diarrhea, loose stools, dehydration or allergic reaction among the three groups.”

The conclusion that ingestion of fructans by infants does not result in an adverse effect on water balance was further supported by the findings of Lugonja et al. (2010). In this unblinded, non-placebo-controlled study, the water content of the feces of breastfed infants was compared with that of infants consuming formula containing 400 mg/100 ml of a blend of inulin and oligofructose derived from chicory. After 28 days, the mean water content of the stools of infants receiving formula containing inulin+oligofructose was 77.9%, non-significantly lower than the mean water content of breastfed infants’ stools (81.2%).

Food Standards Australia New Zealand (FSANZ), in the final assessment report of the addition of 300 mg/100 ml of inulin/FOS and GOS to food (FSANZ 2008a), noted that “the levels of undigested oligosaccharides in the colon of infants fed GOS and inulin-supplemented infant formula products will be less than half those of breast-fed infants. Therefore, it is unlikely that there is any risk to these very young infants from the presence of inulin-derived substances and GOS in infant formula at the levels suggested” (FSANZ 2008a, p159).

FSANZ (2008a) went on to note that, "It has also been shown that inulin is totally fermented in the large intestine of human adults; although there may be differences between young infants and adults in regard to fermentation capacity, where a substance is entirely fermented in adults, it is likely that some fermentation also occurs in infants" (FSANZ 2008a, p159). FSANZ concluded its discussion with a very strong statement:

> "In regard to the use of other ratios of oligosaccharides, or inulin-derived substances alone in infant formula, FSANZ has considered a concern expressed by EFSA relating to water balance. The available evidence that oligofructose and inulin are fermented by colonic microflora in formula fed infants as described above (presence of SCFA in stool, *in vitro* stool studies, and evidence from adult toleration studies), 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. In addition, a 12-week study in term infants which indicated oligofructose at 3 g/L had no significant effect on growth, blood chemistry, or reported adverse events, supports the safety of inulin-derived substances in infant formula products. It has been shown that 8 g/L of GOS:long-chain inulin is safe, levels as high as 25 g HMOs/L in breast milk is safe, and inulin-derived substances are likely to be fermented to a similar degree to GOS and HMOs, therefore, FSANZ concludes up to 8 g/L added inulin-derived substances will be safe for young infants. This conclusion applies equally to the use of GOS alone or any ratio of GOS:inulin-derived substances so long as the total level of oligosaccharides is no greater than 8 g/L" (FSANZ 2008a, p160).

(Having reached the conclusion that addition of 8 g oligosaccharide/L formula is safe, however, FSANZ set the maximum amount of inulin-derived substances that can be added to infant formula products at 3 g/L "because infant formula manufacturers are not seeking to add inulin-derived substances to infant formula at levels up to 8 g/L at this time; a maximum of 3 g/L will meet their needs" [FSANZ 2008b, p3].)

The 2008 conclusions of Food Standards Australia New Zealand regarding the safety of inulin-supplemented infant formula were repeated in this authoritative body's conclusion that infant formula supplemented with scFOS is equally safe (FSANZ 2013).

9.2.2.9. Infant Growth and Tolerance

The totality of the large body of research into scFOS and other fructans discussed in Sections 6.1 and 6.2 strongly supports the conclusions reached by Srinivasjois et al. (2009) and Rao et al. (2009) that prebiotic supplementation of formula intended for consumption by preterm and term infants is safe and has physiological effects that bring the performance of formula-fed infants closer to that of breastfed infants.

The effects of infant formula containing scFOS or other fructans were investigated in 27 clinical trials enrolling nearly 3,200 infants. Addition levels ranged from <150 to 800 mg prebiotic/100 ml formula, with a median level of 300 mg prebiotic/100 ml formula. Feeding durations were from 1 week to 6 months with a median of 4 weeks. Many studies included a human-milk reference group, and generally found that the stooling performance and colonic microbiota of infants consuming formula with added prebiotics more resembled that of breastfed infants than did that of infants receiving unsupplemented formula.

All studies included some consideration of adverse events; some research included objective measures of endpoints related to safety while other studies included only more subjective assessment, but in no case were any serious adverse events reported that were attributable to feeding or more frequent with the prebiotic formula than with the control formula. This conclusion is supported both by parental responses to questionnaires and objective assessments by trained professionals. While occasional symptoms of intolerance such as crying or flatulence were reported, they were usually no more common in the prebiotic groups than among the controls and were often less common.

Fifteen studies of scFOS and other fructans included measures of growth—most commonly weight, length, and head circumference—and all found that the growth of infants receiving the prebiotic formula was similar to that of infants receiving the control formula.

In summary, 27 studies enrolling over 3,100 term or preterm infants have been conducted in which scFOS or other fructans were administered for periods as long as 6 months. While there has been some variability in the effects seen on stooling, bifidobacteria, and other colonic bacteria, there has been complete consistency in the absence of prebiotic-associated adverse effects and in the ability of the prebiotic-containing formula to support normal growth of both preterm and term infants. It may be concluded that the addition of scFOS to infant formula is beneficial and is unlikely to cause harm.

9.3. General Recognition of the Safety of the Intended Use of scFOS

The intended use of scFOS has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). Furthermore, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of scFOS for direct addition to non-exempt infant formula at up to 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed has been made through the deliberations of an Expert Panel consisting of George C. Fahey, Jr., Ph.D., Ronald E. Kleinman, M.D., Berthold V. Koletzko, M.D., and John A. Thomas, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, and have concluded:

> *Ingestion of scFOS from the proposed use results in intakes by infants that remain within safe limits established by published animal and human studies. Ingredion's scFOS has been sufficiently characterized to ensure that it is a food-grade product. No evidence exists in the available information on scFOS, other fructans, or other oligosaccharides that demonstrates, or suggests reasonable grounds to suspect, a hazard to infants when scFOS is added as a prebiotic ingredient to non-exempt infant formula at levels up to 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed.*

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, scFOS is safe and is GRAS for its proposed use as a prebiotic ingredient to be added to non-exempt infant formula

at up 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed.

9.4. Conclusion of the Expert Panel

We, the undersigned independent Expert Panel members, are qualified by scientific education and experience to evaluate the safety of substances intended to be added to infant formula. We have individually and collectively critically evaluated the information summarized above and other information deemed appropriate, and unanimously conclude that the intended use of short-chain fructooligosaccharides (scFOS), produced as described in accordance with current Good Manufacturing Practice and meeting appropriate human food-grade specifications, to be added to non-exempt infant formulas at up to 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed, is safe.

We further conclude that the intended use of scFOS, produced as described in accordance with current Good Manufacturing Practice and meeting appropriate human food-grade specifications, to be added to non-exempt infant formulas at up to 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed, is Generally Recognized as Safe (GRAS) based on scientific procedures.

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

Signature: _____ Date: _____
George C. Fahey, Jr., Ph.D.
Emeritus Professor of Animal & Nutritional Sciences
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Champaign, Illinois

Signature: _____ Date: _____
Ronald E. Kleinman, M.D.
Chair, Department of Pediatrics
Massachusetts General Hospital, Harvard Medical School
Boston, Massachusetts

Signature: _____ Date: _____
Berthold V. Koletzko, Dr med Dr med habil (M.D. Ph.D.)
Professor of Pediatrics
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Signature: _____ Date: _____
John A. Thomas, Ph.D.
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It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

Signature: _____ Date: 7/11/14
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Signature: _____ Date: _____
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SUBMISSION END