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Original Submission

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OFFICE OF PREMARKET APPROVAL

Dr. Linda Kahl
Office of Premarket Approval, HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Dear Dr. Kahl:

We wish to notify you that GTC Nutrition Company has determined that short-chain fructooligosaccharide (scFOS) is "generally recognized as safe" ("GRAS") for use as an ingredient in acidophilus milk, baby foods, bars, beverages, biscuits, cakes, confectionery, cookies, crackers, flavord/unflavored milk, hard candy, ice cream, jams and jellies, muffins, ready-to-eat cereals, sorbet and sherbet, soup, and yogurt, at the levels specified in the attached GRAS notification document . Accordingly, scFOS is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act.

We are hereby submitting the attached document, relied upon by GTC Nutrition Company to make its GRAS determination. As directed by the agency, the information is formatted in accordance with proposal 21 CFR 170.36(c) (62 Fed. Reg. 18937 (April 17, 1997)).

The data and information that serve as the basis for this GRAS notification will be sent to FDA upon request or are available for the FDA's review and copying at reasonable times at the office of Claire Kruger, Ph.D., Senior Science Manager, ENVIRON Corporation, 4350 North Fairfax Drive, Suite 300, Arlington, VA. 22203, telephone: (703) 516-2309, facsimile: (703) 516-2393.

Sincerely,

Claire L. Kruger, Ph.D., D.A.B.T.
Senior Science Manager

cc: V. Frankos
P. Karabell

4350 North Fairfax Drive • Suite 300 • Arlington, Virginia 22203 • USA • Tel· (703) 516-2300 • Fax: (703) 516-2345

GENERALLY RECOGNIZED AS SAFE NOTIFICATION FOR SHORT-CHAIN FRUCTOOLIGOSACCHARIDE

Prepared for

GTC Nutrition Company
Golden, Colorado

Prepared by

Environ International Corporation
Arlington, Virginia

April 17, 2000

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I. GRAS EXEMPTION CLAIM

A. Name and Address of Notifier

GTC Nutrition Company
14252 W. 44th Ave., Unit F
Golden, CO 80403

Contact: Ms. Paula Karabell
Telephone: 303-216-2489
Facsimile: 303-216-2477

B. Name of GRAS Substance

The substance that is the subject of this Generally Recognized as Safe (GRAS) determination is short-chain fructooligosaccharide (scFOS or FOS), also referred to as Neosugar. FOS is marketed and sold under the trade names NutraFlora®, Meioligo®, and Actilight®.

C. Intended Use

FOS is a carbohydrate that is not hydrolyzed in the human intestinal tract and thus is reduced in caloric value compared to other digestible carbohydrates. It can be used in food products as a bulking agent, in place of sugar, with a resultant reduction in the energy content of the food. In addition, since it passes unchanged into the colon, it serves as a substrate for colonic bacteria. One beneficial effect that results is a selective fermentation of the FOS by bifidobacteria. The enhancement of bifidobacterial growth may alter the colonic flora in favor of a normal healthy composition.

The manufacturer intends to add FOS to acidophilus milk, nutritional bars, baby food, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup, and yogurt at the levels specified in Table 1.

TABLE 1

Proposed Food Use Categories and Use Levels of FOS

Proposed Food Use Categories	Foods included in Category	Proposed FOS Use Level	% weight of FOS in food	Serving Size
Acidophilus milk Milk with culture	Acidophilus-milk (1% fat and 2% fat milk)	5.0 g/serving	2.0%	240 ml [a, g] *(approx 244 g)*
Bars Nutritional bars	Meal replacement bars, granola bars, and high energy sports bars	3.0 g/bar	4.6 –13.6%	22-65 g [b]
Baby Foods	Baby foods, including cereals (dry and prepared) and ready-to-serve mixed dishes, desserts, fruits, vegetables, meats, and yogurt drinks	0.25 mg/serving	0.1-3.6%	7 - 125 g [a, c]
Beverages Nutritional Beverage	Instant breakfast beverages, diet beverages, meal replacements, nutrient supplements, protein beverage powders, and electrolyte-replacement beverages (includes ready-to-drink and powders)	3.0 g/ serving (244 g beverage, various amount of powders make one 244g serving)	1.2%	240 ml [a, g] *(approx 244 g)*
Biscuits	Zwieback and teething cookies	0.25 g/ serving	3.6%	7 g [a]
Cakes	Cakes, coffee cakes, croissants, breakfast tarts, scones, sweet breads, sweet rolls, pastries, and doughnuts	2.0 g/serving	1.6-3.6%	55 - 125 g [a, d, g]
Confectionery	Candy bars and confectionery	2.0 g/serving	5.0%	40 g [a]
Cookies	All types of cookies and brownies	1.0 g/serving	2.5-3.3%	30 - 40 g [a, c]
Crackers	Animal, graham, saltine, snack, cheese, and sandwich-type crackers, crispbread, and rice cakes	0.5 g/serving	1.7-3.3%	15 - 30 g [a, f]

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TABLE 1

Proposed Food Use Categories and Use Levels of FOS

Proposed Food Use Categories	Foods included in Category	Proposed FOS Use Level	% weight of FOS in food	Serving Size
Flavored and unflavored milks	Chocolate milk, hot cocoa, malted milk, chocolate milk prepared with chocolate syrup (includes ready-to-drink and powders); unflavored milk (includes fluid, reconstituted from dry, and dry), soy milk, milk used in the preparation of beverages and milk shakes	1.0 g/ serving (244 g milk; various amounts of powders make one 244 g serving)	0.4%	240 ml [a, g] (approx. 244 g)
Hard candy	Regular and dietetic hard candy	1.0 g/serving	6.7%	15 g [a]
Ice cream Ice cream and frozen yogurt	Ice cream – unspecified flavors, chocolate, non-chocolate flavors, frozen yogurt, ice milk, and frozen milk desserts, including novelties such as bars and cones	1.0 g/serving	1.5%	½ cup [a, g] (approx. 66 g)
Jams and Jellies Fruit preparations	Jam, jellies, marmalades, fruit butters, and fruit in yogurt	0.25 g/ounce	0.9%	1 tbsp [a] (approx. 20 g)
Muffins	All muffin flavors, including cornbread muffins	2.0 g/serving	3.6%	55 g [a]
Ready-to-Eat cereals	Includes all types of ready-to-eat breakfast cereals	2.0 g/serving	3.3 – 15.4%	13 - 61 g [b]
Sorbet and Sherbet	Citrus and non-citrus flavors of sorbet and sherbet	1.0 g/ serving	1.4%	½ cup [a, g] (approx. 74 g)
Soup	Prepared soups – canned (ready-to-eat), condensed, dry mix, and unknown preparation	1.0 g/serving (245 g soup; various amounts of condensed soups or powders make one 245 g servings)	0.4%	245 g [a]

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TABLE 1

Proposed Food Use Categories and Use Levels of FOS

Proposed Food Use Categories	Foods included in Category	Proposed FOS Use Level	% weight of FOS in food	Serving Size
Yogurt	Plain, flavored and fruited yogurt, and yogurt used in dressings	3.0 g/serving	1.3%	225 g [a]

[a] Serving size based on 21 C.F.R. §101.12 CFR "Reference Amounts Customarily Consumed per Eating Occasion"

[b] Serving size based on actual product data

[c] 7 g = other cereal and grain products; 15 g = cereals, dry, instant; 55 g = eggs/egg yolks; 60 g = strained type foods; 70 g = vegetables for toddlers; 110 g = junior type foods or cereals, prepared; 125 g = fruits for toddlers; 170 g = dinners, soups or stews for toddlers; assumed 60 g for unspecified foods

[d] 55 g = light cakes; 80 g = medium weight cakes; 125 g = dense cakes

[e] 30 g = cookies; 40 g = brownies

[f] 15 g = plain crackers; 30 g= snack crackers

[g] Serving weight based on data from the USDA Nutrient Database for Standard Reference, Release 13, 1999.

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D. Basis for GRAS Determination

1. Introduction

In this document, we demonstrate that FOS intake, resulting from its intended use, is safe and is also GRAS, under the Federal Food, Drug, and Cosmetic Act (FDCA or the Act). To accomplish this, first, the safety of FOS intake under its intended conditions of use is established. Then, this intake level is determined to be GRAS by demonstrating that the safety of FOS for its intended use is generally recognized by experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the FDCA 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 through: (1) scientific procedures (§170.30(b)); or (2) experience based on common use in food, in the case of a substance used in food prior to January 1, 1958 (§170.30(c)). A scientific procedures GRAS determination under §170.30(b) requires the same quantity and quality of scientific evidence as is required to obtain approval of the substance as a food additive. This current GRAS determination employs both scientific procedures and common use in food prior to January 1, 1958.

In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted. This so-called "common knowledge" element of a GRAS determination consists of the following 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 a consensus exists among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a GRAS determination based on scientific procedures, supplemented with evidence of safety from common use in food prior to January 1, 1958, are applied below in an assessment of whether FOS used in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup, and yogurt is safe and is GRAS.

Once FOS is determined to be GRAS for its intended use, it is permitted to be used for that purpose because it is not (by definition) a food additive, and therefore does

not require promulgation of a specific food additive regulation under 21 CFR prior to marketing.

2. Safety of FOS for its Proposed Use

a) Traditional Approach for Evaluating the Safety of Food Additives

The regulatory criterion by which the safety of a food additive is judged is that "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" (21 CFR § 170.3(i)). This regulation specifies that three factors be considered in determining safety. These are:

- The probable consumption of the substance and of any substance formed in or on food because of its use;
- the cumulative effect of the substance in the diet, taking into account any chemically- or pharmacologically-related substance or substances in such diet;
- 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.

After consideration of these factors, the FDA usually establishes an acceptable daily intake (ADI) for the additive. The ADI represents the maximum amount of the additive that can be safely consumed by humans on a daily basis for a lifetime. The FDA has specified that an ADI is usually established by application of a safety factor to the highest no-observed-adverse-effect level (NOAEL) identified in the most sensitive animal species studied. Except where evidence is submitted that justifies use of a different safety factor, a safety factor in applying animal experimentation data to man of 100 to 1 is used; that is, tolerance for the use of a human food ingredient will not exceed 1/100th of the maximum amount demonstrated to be without harm to experimental animals.

b) Approach for Evaluating the Safety of Macroingredients

Macroingredients are a class of food additives that are intended to be replacements for conventional macroingredients such as fats, proteins, and carbohydrates, and thus are intended for use at relatively high levels in food

(USFDA 1993). Macroingredients have the potential to be consumed in gram quantities per day (whereas traditional food additives are generally consumed at levels below 1 gram/day) because they are intended to replace energy-dense traditional food constituents that form a substantial part of the diet (Borzelleca 1992). The current trend is toward increased consumption of macroingredients because of a public health emphasis on reducing both total caloric intake and dietary fat intake, and because of consumer demand for more and varied products with fewer calories and less fat (Rulis 1990; FDA 1991). Thus, both the food industry and the FDA have intensified their efforts to develop a more appropriate approach to assess the safety of macroingredients.

One of the key features that differentiates the safety assessment approach for macroingredients from that for traditional food additives is the potentially large human exposure to macroingredients. If macroingredients are tested in animal studies at substantial multiples of the anticipated human exposure, similar to what is done for the testing of traditional food additives, spurious results would occur. These results would not reflect toxicological effects due to the macronutrient, but rather perturbations resulting from physiological or nutritional imbalances created by the large doses of the macronutrient administered (Borzelleca 1992; IFBC 1990; USFDA 1993; Verschuren 1988; WHO 1987; Feron et al. 1990). Therefore, macroingredients require an alternative safety assessment approach that utilizes information about the physical, chemical, and biological properties of the macronutrient.

These biological properties typically include the digestibility, absorption, and metabolic fate of the macronutrient. This information allows for the prediction of potential target organs and toxic effects (Munro 1994). Initial short-term toxicity testing with rodents can be used to identify any unanticipated systemic toxicity. However, testing should then proceed to more appropriate models, including humans, to confirm safety and to assess specific endpoints such as palatability, nutritional status, and tolerance (Borzelleca 1992; Munro 1994). Exposures to the macronutrient in human trials should be at multiples of the estimated daily intake (EDI). Human tolerance to the macronutrient can then be used as the basis for identifying an ADI (Borzelleca 1992). Characteristics, such as the similarity of a macronutrient to a traditional food, metabolic conversion into normal body constituents, and lack of overt toxicity, allow the use of human studies, supported by other data establishing safety, to be used in the development of an ADI or acceptable intake level (AIL) for a macroingredient.

Therefore, the traditional ADI/safety factor approach outlined in the previous section is generally not appropriate for use with food additives that will be consumed in relatively large quantities, such as FOS. For these macroingredients an alternative approach is generally needed to establish an AIL. The FDA's Red Book II (USFDA 1993) states:

> The common characteristic of macro-additives is that they will be consumed in large quantities compared to conventional food additives and, as a consequence, they will present testing problems that require "customized" approaches. For example, it may not be feasible to calculate safety factors in the conventional way, that is, as a fraction of the highest oral dose that has no adverse effects in animals. Other means of providing margins of safety for macro-additives will have to be used; these may include information derived from metabolic, pharmacokinetics, and human clinical studies.

Therefore, a safe intake level for FOS, used as a macroingredient, will be derived by employing an AIL approach, rather than the traditional ADI/safety factor approach. This AIL approach for FOS is described in greater detail in the following section.

c) Acceptable Intake Level (AIL) for FOS

An extensive database, consisting of both animal and human exposure and safety data, is available for determination of the safety of FOS for its proposed uses as an ingredient in foods at the levels specified in this document (see Table 1). Studies on the metabolic fate of FOS indicate that it is virtually unabsorbed and undigested by endogenous enzymes. A very small amount is hydrolyzed by stomach acid and absorbed into the body as fructose and glucose. Approximately 0.12% of ingested FOS is recovered unchanged in the urine. About 89% of undigested FOS passes unchanged into the colon where it is fermented by the microflora into gases and short-chain fatty acids (SCFA). Studies have demonstrated that the effect of FOS on the colonic microflora is not a health concern; indeed, it has been shown to help produce a normal, healthy microflora composition with improved bifidobacteria and lactobacilli numbers.

The physiologic effects of FOS are similar to those of a fiber and include resistance to digestion by endogenous enzymes, fermentation by colonic

microflora, shortened gastrointestinal transit time, increased fecal weight, reduction of fecal pH, predictable reduction in caloric value, reduction of plasma cholesterol and triglycerides, and reduction in glucose absorption.

The safety of FOS was corroborated by examining its impact on mineral absorption, nitrogen balance and colonic epithelial cells. FOS does not have a detrimental effect on mineral absorption. Indeed, β 2-1 fructans have been shown to have a positive impact on calcium and magnesium absorption and balance. The effect of FOS on nitrogen utilization and excretion indicates that there are no safety concerns, and that there is potential benefit due to the increase in nitrogen excretion in the feces, enhanced urea nitrogen transfer into the large intestine, and enhanced bacterial utilization of ammonia nitrogen. Furthermore, FOS has been shown to produce a trophic effect on colonocytes. Finally, studies in rats have demonstrated that FOS can produce colon tumor inhibition.

The systemic effects produced by FOS include its modulation of both blood glucose and lipid levels. FOS ingestion does not increase plasma glucose or stimulate secretion of insulin. In addition, similar to other soluble dietary fibers, FOS has been shown to have both hypolipidemic and hypocholesterolemic effects. Therefore, there is no concern that FOS ingestion may adversely affect either glycemic control or blood lipid levels.

Animal toxicology studies of FOS include a mutagenicity battery, acute and subchronic studies, teratology studies and a carcinogenicity bioassay. The results of toxicological studies indicate that FOS is not mutagenic or teratogenic and does not produce significant adverse effects or carcinogenicity in animals following chronic administration at levels up to 15 percent (2,664 mg/kg/day) in the diet. The effects that were noted in animal studies (i.e., increased weight of the small intestines, cecum, and colon) are consistent with gastrointestinal disturbances caused by high levels of any non-digestible material.

Historically, humans have been exposed to FOS through consumption of plants. Intake of FOS may have been higher in the past due to the use of plants such as Jerusalem artichoke as sustenance crops or as a substitute for the potato. Consumption of FOS may have been as high as 9 g/day at one time. Currently, it is estimated that FOS consumption is approximately 114 mg/day in the U.S. and 579 mg/day in the EU.

Human tolerance to consumption of FOS has been established in a number of clinical trials. These data were used to determine the AIL for FOS. Study results indicate that FOS, ingested at up to 20 g/day in adults, appears to be safe and well tolerated. In addition, a study reporting the health effects of FOS

consumption by infants in Japan reveals that it is well tolerated and has no adverse effects on health at estimated mean and 90th percentile consumptions of 3.0 and 4.2 g FOS/day, respectively. A clinical study of children ages 10 to 13 found that consumption of up to 9 g/day of oligofructose is well tolerated. Therefore, safety (and tolerance) of the β 2-1 fructans has been established, not only in adults, but in infants and children as well.

In conclusion, the AIL for FOS ingestion for the general population, excluding infants less than one year of age, is determined to be 20 g/day; the AIL for infants less than one year old is 4.2 g/day.

d) Estimated Daily Intake (EDI) of FOS from its Proposed Use

FOS is proposed for use as an ingredient in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup, and yogurt at the levels specified in Table 1.

Estimates of the intake of FOS from its proposed uses are based on the 1994-96 Continuing Survey of Food Intakes by Individuals (94-96 CSFII), the most recent data available from the national food consumption survey conducted by the U.S. Department of Agriculture (USDA). The 94-96 CSFII was a three-year survey in which data were collected from a stratified area probability sample of individuals residing in households in the U.S. Households represented a cross-section of the population of the 50 states and the District of Columbia.

Using the 94-96 CSFII survey data, the EDIs of FOS for the general public, excluding infants less than one year old, associated with its proposed uses in 18 food categories range from 3.9 to 6.2 g/day (mean intake) and from 7.1 to 12.8 g/day (90th percentile intake) for consumers of these food products. The EDIs of FOS for infants less than one year old for the proposed use of FOS in the same 18 food categories are 1.6 and 3.1 g/day for the mean and 90th percentile consumers of these food products, respectively.

The EDIs for both the general public and infants assume that FOS will be used at the proposed use level in all 18 food categories to which FOS is intended to be added. As such, the EDIs derived herein are considered highly conservative estimates of potential FOS intake.

e) **Safety Evaluation Following Chronic and Acute Intake of FOS**

Evaluation of the safety of FOS is accomplished through a review of the extensive database on the safety of FOS, including history of human exposure, animal and human studies, and a comparison of the AIL for FOS to its EDI. If the EDI is less than (or approximates) the AIL, then the proposed use can be considered to be safe.

The AIL is derived from clinical studies of FOS tolerance. Results of these studies indicate that up to 20 g FOS/day in the general population, excluding infants less than one year old, and up to 4.2 g/day in infants less than one year of age, is safe and well tolerated.

The EDI for the general population, excluding infants less than one year old, is estimated to be as high as 6.2 g/day for the mean consumer, and as high as 12.8 g/day for the 90th percentile consumer. Even at the 90th percentile consumption level, the EDI is below the AIL of 20 g/day. The EDIs for infants less than one year old are estimated to be as high as 1.6 g/day for the mean consumer and 3.1 g/day for the 90th percentile consumer. Both of these EDIs are below the AIL for infants of 4.2 g/day.

In conclusion, the EDIs of FOS resulting from consumption of the food products that will contain FOS are all below the corresponding AILs for FOS of 20 g/day (for the general population) and 4.2 g/day (for infants less than one year old). It can thus be concluded, based on scientific procedures, and supplemented with evidence of safety from common use in food prior to January 1, 1958, that the proposed use of FOS, as a bulking or bifidogenic agent for the food types and at the use levels specified herein, is safe.

3. General Recognition of the Safety of FOS

This document presents the information used to determine the GRAS status of FOS for use as an ingredient in acidophilus milk, nutritional bars, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored milk, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt. This determination of safety is based upon a critical review of both toxicological and clinical safety studies.

A review of the following information was completed in the evaluation of the GRAS status of FOS: production process, background information (i.e., composition and uses), consumer exposure data, and toxicological studies in both animals and humans. The sources of the reviewed information included pertinent studies and published literature provided by GTC Nutrition Company, or identified in literature searches

conducted through online bibliographic retrieval systems, including Medline® and Dialog®.

In the previous section, the proposed use of FOS as an ingredient in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt was determined to be safe through the use of scientific procedures, and supplemented with evidence of safety from common use in food prior to January 1, 1958. Under 21 CFR §170.30, general recognition of this safety requires that the scientific data and information upon which the determination of safety rests must ordinarily be published, but may be corroborated by unpublished studies and other data and information. The scientific data and information on which the safety determination of FOS is based are available in the published literature or are otherwise publicly available to experts qualified by training and experience to evaluate the safety of food and food additives. Thus, the data reviewed meet the common knowledge element required for all GRAS determinations.

Determination of the GRAS status of FOS as a food ingredient has been made through the deliberation of experts, Dr. Vasilios H. Frankos and Dr. Claire L. Kruger, scientists qualified by training and experience to evaluate the safety of food ingredients. These experts have carefully reviewed all the available data on the metabolism, acute and chronic toxicity of FOS in animals, as well as consideration of the human exposure and tolerance to FOS along with human studies evaluating the safety of administered intakes of FOS, and have concluded that:

> There is no evidence in the available information on FOS that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when it is used at levels that are now current or that might reasonably be expected from the proposed application. Therefore, FOS is GRAS for the uses and at the levels proposed by GTC Nutrition Company.

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, FOS, to be used as an ingredient in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt is GRAS, at an intake level up to and including 20 g/day for the general population and 4.2 g/day for infants less than one year old. Because FOS

is GRAS for its intended use, it is excluded from the definition of a food additive, and thus may be marketed for this use without the need to promulgate a specific food additive regulation under 21 CFR.

E. Availability of Information

The data and information that serve as the basis for this GRAS notification 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 Claire Kruger, Ph.D., Senior Science Manager, ENVIRON Corporation, 4350 North Fairfax Drive, Suite 300, Arlington, VA 22203, telephone: (703) 516-2309, facsimile: (703) 516-2393.

II. DESCRIPTION OF SUBSTANCE

A. Identity

1. Chemical Composition

Short-chain fructooligosaccharides (scFOS or FOS) are a mixture of 1^F-(1-β-fructofuranosyl)$_{n-1}$ sucrose oligomers; n may vary from 2 to 4. FOS is composed of sucrose molecules (glucose-fructose disaccharides, GF) to which one, two, or three additional fructose units have been added by β 2-1 glycosidic linkages to the fructose unit of sucrose. GF_2 (α-D-glucopyranoside-(1↔2)-β-D-fructofuranosyl-(1←2)-β-D-fructofuranosyl or 1-*kestose*), GF_3 (α-D-glucopyranoside-(1↔2)-β-D-fructofuranosyl-(1←2)-β-D-fructofuranosyl-(1←2)-β-D-fructofuranosyl or *nystose*), and GF_4 (α-D-glucopyranoside-(1↔2)-β-D-fructofuranosyl-(1←2)-β-D-fructofuranosyl-(1←2)-β-D-fructofuranosyl-(1←2)-β-D-fructofuranosyl or *1^F-β-fructofuranosyl nystose*) are the abbreviations for the components comprising FOS (Kamerling et al. 1972).

2. Common and Trade Names

Common or trade names for the substance that is the subject of this GRAS determination include the following: Fructooligosaccharide, Short-chain Fructooligosaccharide, FOS, Neosugar, NutraFlora®, Meioligo®, and Actilight®.

3. Structure

The chemical structure of FOS is shown in Figure 1.

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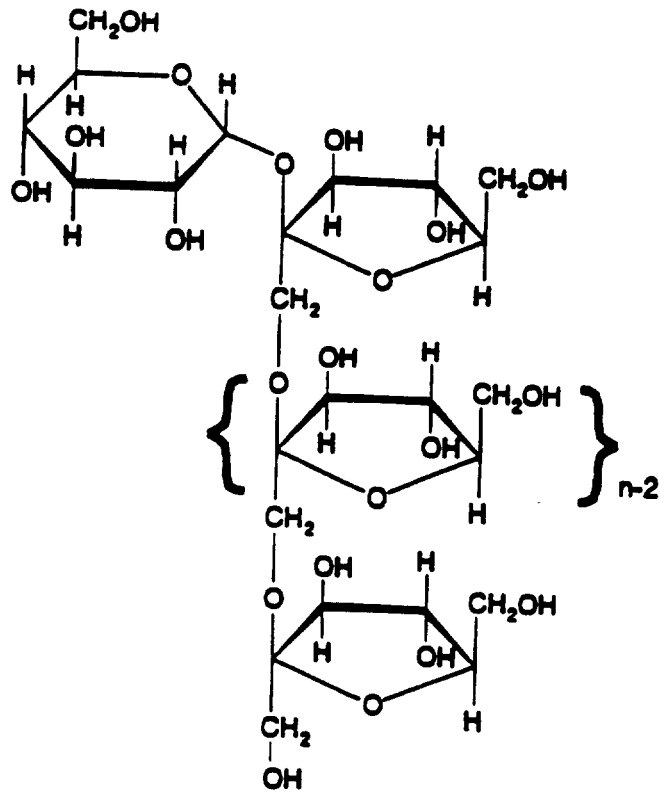
[redacted]

FIGURE 1
Chemical Structure of scFOS



n = 2-4

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B. Production Process

1. Synthesis of FOS

a) Microbial Source and Production of Active Enzyme

FOS is produced by the action of β-fructofuranosidase on a sucrose syrup solution. β-Fructofuranosidase is an endocellular enzyme preparation made by a natural, genetically unmodified strain of the fungus, *Aspergillus japonicus*, which is registered under the American Type Culture Collection (ATCC) number 20611.

Aspergillus species have common use in food production. *Aspergillus niger* (*A. niger*) is considered non-pathogenic and non-toxic by FDA (USFDA 1998). *A. niger* is used in the production of numerous GRAS ingredients including chymosin (21 CFR §184.1685), glucono delta-lactone (21 CFR §184.1318), carbohydrase and cellulase for use in clam and shrimp processing (21 CFR §173.120) and food-grade citric acid (21 CFR &173.280). *A. oryzae* is used in the production of α-amylase, which is a GRAS ingredient that can be used in the production of cereal flours (21 CFR §137.103, 21 CFR §137.200). In the 1960's, FDA authored an advisory opinion determining that carbohydrase and protease enzymes produced by *A. oryzae* are considered GRAS ingredients (USFDA 1998).

A. japonicus ATCC 20611 is a specific species of Aspergillus that is commonly called *A. japonicus Saito*. It was originally classified as *A. niger* until November 4, 1997 when ATCC renamed it *A. japonicus* based on its morphology. Hidaka et al. (1988) and Hirayma et al. (1989) both describe the use of Aspergillus spp. ATCC 20611 to produce the β-fructofuranosidase used in the production of FOS.

A mycotoxin screen conducted on a sample of the furanosidase enzyme revealed no detectable levels of aflatoxin B_1, B_2, G_1, or G_2 (at a limit of detection of 5 ppb) or sterigmatocystin (at a limit of detection of 50 ppb). The Certificate of Analysis for this mycotoxin screen is contained in Appendix A.

b) Process Description

The process by which FOS is produced is shown schematically in Figure 2. FOS is produced from sucrose by the action of the enzyme β-fructofuranosidase, derived from *Aspergillus japonicus* ATCC 20611 (previously named *Aspergillus niger* ATCC 20611). The enzyme acts as both an invertase on sucrose molecules and a fructosyltransferase between sucrose molecules and

fructofuranosyl-sucrose molecules (i.e., comprised of fructose chains with a terminal glucose). The invertase activity cleaves sucrose into its glucose (G) and fructose (F) subunits. The fructofuranosidase activity transfers the fructose subunit from sucrose to a growing fructose chain to yield 1-kestose (GF_2), nystose (GF_3), and fructosyl-nystose (GF_4) molecules. This enzyme reaction is followed by decoloration, filtration, desalting and concentration of the reaction product.

Production of FOS includes numerous in-process quality controls. Initially, granular sucrose, water, enzyme (Aspergillus whole cells), and HCl or NaOH (to adjust the pH) are combined, and the resulting reaction is monitored by an in-process test for concentration (measured as °Bx) and pH. After the enzyme reaction is complete (approximately 20 hours at 60±0.5° C), another in-process test is performed for sugar composition and pH. Then, an inactivation procedure is done by heating the reaction mixture at 80° C for 30 minutes and the first decolorization is performed by adding 0.5% activated carbon. This is followed by the first filtration, a second decolorization, a second filtration, and a desalting procedure. At this point, another in-process test is performed for electric conductivity. This is followed by the final decolorization, the final filtration, and concentration of FOS. At this point, in-process test is performed for sugar composition and pH. The three filtrations remove the *Aspergillus* cells so that residues do not remain in the final product. This is confirmed by microbiological quality control tests conducted on the final product.

Treated well water (0.3 ppm $NaClO_4$ is added) is used as the process water and this water is evaluated using the following criteria (acceptable values are listed in parentheses): taste (negative), odor (negative), color (pass), turbidity (pass), NH_3-N (not detected), NO_2-N and NO_3-N (less than 10 mg/l), Cl^- (less than 200 mg/l), organic matter (less than 10 mg/l), pH (5.8 - 8.6), standard plate count (less than 100/ml), enteric coliforms (not detected), iron (less than 0.3 mg/l), and residual chlorine (not more than 0.1 ppm) (Meiji Seika Kaisha 1988).

With regard to the enzyme, the amount added is 150 U/g sucrose or 150 x 10^6 U of enzyme per metric ton of sucrose, according to the manufacturing specifications (Meiji Seika Kaisha 1988). Because a whole cell has an activity of 1 x 10^6 U/g. Thus, 6.67 metric tons of sucrose can be reacted by about 1 kg of whole cells. One kg of whole cells can produce 8.44 metric tons of FOS.

Enzyme reaction time is variable depending on the particular lot; 20 ± 2 hours are usually required to complete the reaction (Meiji Seika Kaisha 1988). Completion of the enzyme reaction is judged by the sugar composition of the reaction mixture. The temperature must be maintained at 60 ± 0.5°C during the

enzyme reaction. To avoid any problems with enzyme stability, the enzyme is stored in a cool, dark place, typically in a refrigerator at temperatures below 5°C.

FIGURE 2
Production Process

Production process of NutraFlora Powder, NutraFlora Syrup and NutraFlora G Syrup

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[redacted]

2. Finished Product Specifications

The manufacturing specifications for FOS are listed in Table 2 below.

TABLE 2
Product Specifications

Item	Nutraflora® Powder	Nutraflora® G Syrup	Nutraflora® Syrup
Moisture % (w/w)	< 5.0	< 25.0	< 25.0
Carbohydrate Composition (% dry basis)			
Glucose + Fructose + Sucrose	<5.0	<35.0* and 10±2**	< 5.0
Fructooligosaccharides	>95.0	>55.0	>95.0
GF_2	35.0 ± 6.0	25.0 ± 3.0	42.0 ± 5.0
GF_3	50.0 +6.0	25.0 ± 3.0	45.0 ± 5.0
GF_4	10.0 + 4.0	5.0 + 3.0	8.0 ± 3.0
		*glucose + fructose	
		**sucrose	
Appearance	White powder	Clear syrup	Clear syrup
Granular Size	40 mesh pass	Not applicable	Not applicable
Color (nanometers)	Not Applicable	<0.15	<0.15
Ash (%w/w)	< 0.1	< 0.1	< 0.1
pH (10% solution)	5.0 –7.0	6.0 ± 1.0	6.0 ± 1.0
Total heavy metal (as Pb)	< 1 ppm	< 1 ppm	< 1 ppm
Arsenic (as As_2O_3)	not detected at 1 ppm	not detected at 1 ppm	not detected at 1 ppm
Foreign taste and odor	free	free	free
Foreign substance	free	free	free
Microbiological results (counts/g)			
Mesophilic bacteria	0 counts/gm	<300 ml	<300 ml
Mold and Yeast	0 counts/gm	<20 ml	<20 ml
Coliforms	Negative	Negative	Negative

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III. USE AND CONSUMER EXPOSURE

A. Historical Exposure

1. Historical Exposure from Plant Sources

FOS accumulates in the vegetative tissues of many fruits and plants world-wide (Albrecht et al. 1993; Raffinerie Tirlemontoise 1993; Gibson et al. 1994). These FOS-containing plants are consumed by humans as vegetables and include asparagus, garlic, leek, onion, artichoke, endive, Jerusalem artichokes, chicory, dahlia, murnong, and yacon (Fuchs 1991). Of these vegetables, Jerusalem artichokes and chicory contain substantial concentrations of FOS (Campbell et al. 1997a; Van Loo et al. 1995). In addition, some of these vegetables, such as Jerusalem artichoke, have been used as dietary staples or sustenance crops in times of hardship. Others, such as garlic, onion, asparagus, leek, artichoke and endive, have long been a part of the Western diet.

Yacon, a plant from the Asteraceae family, is produced in the Andes highlands of South America (Asami et al. 1989). Its roots serve as a dietary source of FOS, and were introduced into Japan in 1985. It is eaten raw, boiled or fried and has been reported to taste like Japanese pears (Asami et al. 1989; Wei et al. 1991).

Onion originates from western Asia and has been cultivated as a vegetable since "antiquity" in the European region (Van Loo et al. 1995). The carbohydrate content of onions is comprised of glucose, fructose, and sucrose in addition to FOS.

Dahlia and the Jerusalem artichoke, in particular, have historically been consumed by Native populations in North, Central, and South America. Because these foods are able to be stored for long periods, and are readily cultivated, these plants commonly served as staple foods during both the winter months and in times of drought (Shoemaker 1927; Wyse and Wilfahrt 1982; Whitley 1985; and Raffinerie Tirlemontoise 1993).

The Jerusalem artichoke tuber is considered one of the oldest cultivated crops in North America (Van Loo et al. 1995). There is evidence that North American Indians and early settlers of Canada consumed the Jerusalem artichoke in the 1600s. The Jerusalem artichoke was introduced into parts of Europe in 1612 and was known in Spain and the Mediterranean region by 1650 (Van Loo et al. 1995) before it was replaced by the potato in the middle of the 18th century (Kosaric et al. 1985). In the early 1900s, the Jerusalem artichoke was still commonly consumed in France and the

Netherlands (Van Loo et al. 1995), and in 1927, the USDA published a recipe for the preparation of Jerusalem artichokes to be baked and consumed much like a potato (Wyse and Wilfahrt 1982). Immediately following World War II, Jerusalem artichokes were again cultivated as a staple crop by the French and Germans because of the scarcity of the potato at that time.

The Jerusalem artichoke has been referred to as wild potato, horse potato, and diabetic potato because of its historical use in the diet as an adequate potato substitute. Using contemporary 90th percentile consumption values for white potatoes as a substitute for the historical consumption of Jerusalem artichokes, it can be estimated that 158 grams per day of Jerusalem artichokes were consumed (Pao et al. 1982). Assuming an FOS content of 58.4 mg/g of Jerusalem artichoke (Campbell et al. 1997a), approximately 9 grams of FOS could have been consumed per day by these populations.

Chicory is indigenous to Europe and has been used as a vegetable since Greek and Roman antiquity (Van Loo et al. 1995). It was discovered in 1919 to contain β 2-1 fructans in its root, although both the roots and greens (known as "Belgian endive") have been historically consumed. Post-World War II populations in England and Germany used the roasted chicory plant root as either an additive or a substitute for coffee beans, and chicory is still used in several brands of European and American coffee to impart additional color, body, and bitterness. In addition, chicory heads and crowns, forced in the dark from the tap roots and better known as "chicons" or the delicacy "witloof," are a major export crop for Belgium (Raffinerie Tirlemontoise 1993; Meijer et al. 1993). During the roasting process, more β 2-1 fructan is converted to fructose; however, even after roasting, approximately half to three-quarters of the inulin remains (Van Loo et al. 1995). [Inulin belongs to a class of carbohydrates known as fructans. Inulin and FOS are similar chemical entities, sharing the same basic structure of β (2-1) linked fructosyl units. See also Table 6.]

In the 16th century, dahlia tubers were noted for their medicinal capabilities, and were used as a diuretic, diaphoretic, and as a flatulence treatment (Whitley 1985). The tuber of the dahlia plant was first considered for food use in France in 1800 (Harrison 1953), and was intended to be eaten as a potato substitute. It was not found to be palatable to either humans or animals, however, and was therefore only used as an ornamental plant. In the 1900s, it was again considered for food use (Harrison 1953). Dahlia tubers are still a popular food in some parts of Mexico, and wild tubers are dug up, cooked, and sold by sidewalk vendors in large cities in south and central Mexico (Whitley 1985).

Murnong, a flowering plant that is part of the Compositae family, has a long history of use as a food of the Australian aborigines. Gott (1984) reported that murnong

roots may have been consumed daily in amounts estimated to be greater than two kilograms. Although the FOS content is not known with certainty, the inulin content of the roots is estimated to range from 8 to 13 percent. Even if FOS was only 1 percent of tuber, the intake of FOS from murnong consumption may have been as high as 20 grams per day.

2. Current Consumption from Fruits, Vegetables and Feedstuffs

a) Concentration of FOS in Plants

FOS is produced naturally in the roots, tubers, and fruits of plants in the Compositae, Amaryllidadeae, Liliaceae, and Gramineae families (Fuchs 1991; Campbell et al. 1997a). These plant families include such common edible foods as rye, wheat, barley, oats, onions, leeks, asparagus, garlic, lettuce, and artichokes. Additional food sources of FOS include banana and other fruits, brown sugar, and tomato (Spiegel et al. 1994).

The FOS content that occurs naturally in a variety of plants has been characterized most recently and extensively by Campbell et al. (1997a). These authors obtained samples of fruits, vegetables, and feedstuffs (grains, field crops and other grasses) many of which have been reported to contain FOS, and analyzed each for their content of GF_2, GF_3, and GF_4. Table 3 presents the total FOS content (i.e., the sum of GF_2, GF_3, and GF_4) of the foods included in their analysis.

Twenty-five fruit samples were analyzed for FOS. Twenty samples contained detectable levels of FOS; the FOS content ranged from 0.1-0.2 mg/g (on an as-is or wet weight basis) for most (12/20) of the fruits. The highest FOS content was found in ripe bananas, which contained 2.0 mg/g FOS. Of the 40 vegetable samples analyzed, 16 did not contain FOS. An additional 6 vegetables contained 0.1 or 0.2 mg/g FOS, while the remaining 16 vegetables contained from 0.3 to 58.4 mg/g FOS. The highest content was found in Jerusalem artichokes, an indigenous American tuber known to have a high inulin content (Labell 1992). Little (trace) or no FOS was found in almost half of the feedstuffs (15/34), and low levels (0.1 mg/g) were found in another five. The FOS content of the remaining 14 feedstuffs ranged from 0.2 to 4.6 mg/g. The highest FOS content was found in wheat middlings (a wheat milling by-product used in animal feeds).

TABLE 3

FOS Composition of Foods

Fruits	FOS Concentration (mg/g[1] as is)	Vegetables	FOS Concentration (mg/g as is)	Feedstuffs	FOS Concentration (mg/g as is)
Apple, Red Delicious	0.1	Acorn squash	0.4	**Grains**	
Apple, Golden Delicious	0.0	Artichoke, globe	2.4	barley	1.7
Apple, Granny Smith	0.1	Asparagus	0.0	corn	0.0
Apple, Jonagold	0.1	Bean, green	0.0	hominy	trace
Apple, Rome	0.0	Bean, kidney	0.1	milo	0.0
Banana	1.4	Beet, red	0.0	oats	0.3
Banana, green	0.7	Carrot, Bunny Luv	0.3	rice, brown	0.0
Banana, red	0.5	Carrot, Dole	0.2	rice, white	0.0
Banana, ripe	2.0	Celery	0.0	rye	3.8
Blackberry	0.2	Chicory root, raw	3.9	soybean	trace
Cantaloupe	0.0	Chicory root, roasted	4.2	wheat	1.3
Gooseberry	0.1	Chinese chive	0.0	**Forages**	
Grapes, black	0.2	Daikon	0.0	alfalfa	0.0
Grapes, Thompson	0.0	Eggplant	0.0	bromegrass	trace
Muskmelon	0.1	Endive	0.0	clover hay	0.4
Orange, Navel	0.3	Garlic	3.9	oat straw	0.7
Peach	0.4	Garlic powder	1.6	orchardgras	0.2
Pear, bosc	0.1	Ginger root	0.0	timothy hay	1.0
Pear, d'Anjou	0.2	Jerusalem artichoke	58.4	wheat straw	0.0
Plantain	0.4	Kiwi	0.0	**Other**	
Plum, red	0.2	Leek	0.9	alfalfa meal	2.1
Raspberry, red	0.2	Lettuce	0.5	beet pulp	0.1
Rhubarb	0.0	Onion, red	1.4	brewer's rice	0.0
Strawberry	trace	Onion, Welch	1.1	canola meal	0.0
Watermelon	0.2	Onion, white	3.1	corn distillers sol	trace
		Onion, yellow	2.6	corn gluten feed	0.1
		Onion, powder	45.0	corn gluten meal	0.3
		Peas	0.1	oat groats	0.1
		Peas, snap	1.1	peanut hulls	2.2
		Peas, snow	0.6	rice bran	0.1
		Potato, Idaho	0.0	rice hulls	0.0
		Potato, sweet	0.2	seaweed	0.0
		Radish, red	0.1	soybean hulls	0.1
		Shallot	8.5	soybean meal	0.0
		Taro root	0.0	wheat bran	3.5
		Tomato	0.0	wheat germ	4.2
		Tomato, cherry	0.0	wheat middlings	4.6
		Tomato, Roma	0.0		
		Yam	0.2		
		Zucchini	0.0		

[1] Source: Campbell et al. (1997a) All concentrations are reported on a wet weight basis.

Campbell and colleagues (1997a) reported that their FOS content analysis was in good agreement with the FOS content of specific foods reported by Spiegel et al. (1994) and Tashiro et al. (1992). Although Campbell et al. (1997a) did not find FOS in raw tomatoes, which was reported by both Spiegel et al. (1994) and Tashiro et al. (1992), they did find quantifiable amounts in tomato paste.

It is not unexpected that the content of FOS in specific foods may vary across studies. Variations in the distribution of inulin chain lengths in specific foods is known to vary depending on specific plant characteristics, growing conditions, time of harvest, and storage time (Darbyshire and Henry 1978; Suzuki and Cutcliffe 1989; Wei et al. 1991; Albrecht et al. 1993; Campbell et al. 1997a). In particular, long storage times increase FOS content of foods, as high DP fructans are hydrolyzed to lower DP fructans (Suzuki and Cutcliffe 1989).

b) FOS Intake from Plant Consumption

Humans consume FOS on a daily basis when consuming plants that naturally contain FOS. The FOS content of commonly-eaten plants was presented in Table 3, based on a recent analysis of foods by Campbell et al. (1997). To estimate the average daily intake of FOS from these foods, FOS concentration was combined with food intake data available for the U.S. population. The food intake data were obtained from the 1994-96 United States Department of Agriculture's (USDA) Continuing Survey of Food Intakes by Individuals (CSFII). CSFII design and methodology have been described in detail elsewhere (USDA 1998). Briefly, in the CSFII 1994-1996, two nonconsecutive days of dietary data for individuals of all ages were collected between January 1994 and January 1997 through in-person interviews using 24-hour recalls. The CSFII 1994-1996 sample was a stratified, multistage area probability sample. Demographic information and limited anthropometric data including region, race and national origin, household income, height and weight were collected for each respondent.

Survey respondents reported consumption of nearly 6,000 foods in CSFII 1994-1996. Recipes containing household ingredients were developed by USDA for many foods reported in the surveys; ENVIRON developed commodity-mapping files for ingredients that are themselves mixtures of more fundamental ingredients (e.g., the amount of wheat germ in whole wheat bread or the amount of soybean oil in vegetable shortening). The ENVIRON recipe database contains a recipe for each food code reported in the CSFII 1994-1996. Recipes in these

files are composed of commodity ingredients and their proportionate amounts by weight.

The primary reference tool for ENVIRON commodity-mapping files was *USDA's Composition of Foods: Raw, Processed, Prepared, Agricultural Handbook No. 8*. For some ingredients, e.g., meats and cheeses, direct translation of ingredients to commodities was possible using Handbook 8 (e.g., to determine the proportions of fat and lean meat in a chicken breast with skin for translation into the raw commodities, "chicken [boneless]-fat" and "chicken [boneless]-lean/fat free"). For other foods, e.g., breakfast cereals, manufacturers' ingredient lists were obtained, and *Handbook 8* was used to estimate the proportions of ingredients in the product based on nutrient composition.

A database of the FOS concentration per 100 grams of each of the commodity ingredients was obtained from the analysis published by Campbell et al. (1997) and reported in Table 3. The FOS concentration data were linked to the food consumption data. The resulting intake estimates for the general U.S. population, by age group, are presented in Table 4. These results include the mean and 90th percentile of total daily intake. All results are based on weighted data and represent two-day average intakes.

The mean FOS intake for adults in the U.S., based on the foods included in the analysis reported by Campbell et al. (1997), is estimated to be 114 mg/day. The FOS intakes for U.S. teenagers and children 2 years and older were somewhat lower, with mean intakes of 82 and 65 mg/day, respectively. For adults, an upper bound estimate of daily FOS intake, based on the 90th percentile food intake, is 248 mg/day. For children and teens, the upper bound intakes are estimated to be 145 mg/day and 182 mg/day, respectively. The food types that contributed the most to FOS consumption were onions, bananas, lettuce, and wheat (in rough and bran forms).

TABLE 4 Estimated Intake of Fructooligosaccharides (FOS) in the U.S. based on FOS Concentrations in the Current Food Supply (Campbell et al. 1997): Employing Mean Total Two-Day Average Intake per User[a]			
Population	**Number of Subjects**	**Mean Intake per User (mg/day)**	**90th Percentile Intake per User (mg/day)**
Children (2 to 12 y)	3701	65.3	145.3
Teenagers (13 to 19 y)	1217	82.0	181.7
Adults (20 y and older)	9208	114.2	247.7

[a] Food intake data source: 1994-96 Continuing Survey of Food Intakes by Individuals; intake estimates based on FOS concentrations in food commodities; WesVar Complex Samples 3.0 (SPSS Inc., 1998) and CSFII sampling weights were used to calculate estimates.

In addition to estimates of the daily FOS intake for the U.S. population, average FOS intakes for Western Europeans can be estimated using per capita consumption of certain foods reported by Van Loo et al. (1995). Table 5 presents the daily intake of five FOS-containing foods for the Benelux countries, Spain, and Europe, using the FOS content of those foods reported by Campbell et al. (1997).

Estimated daily FOS intakes are significantly higher in Western Europe than for the U.S. population. The estimated average FOS intake for Western Europeans ranges from 319 to 579 mg/day. These estimates are three to five times higher than the estimated intake of FOS for adults in the U.S., and are based only on five foods for Europeans provided by Van Loo et al. (1995), compared to numerous food items available for the U.S. population.

[redacted]

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TABLE 5 Estimated Average Daily Intake of FOS for Selected European Populations							
Foodstuff	FOS Content (mg/g)[1]	Benelux		Spain		Europe	
		Average Food Intake (g/day)[2]	Average FOS Intake (mg/day)	Average Food Intake (g/day)[2]	Average FOS Intake (mg/day)	Average Food Intake (g/day)[2]	Average FOS Intake (mg/day)
Artichoke	2.4	0.5	1.20	18.8	45.1	7.9	19.0
Garlic	3.9	0.4	1.56	19.2	74.9	3.6	14.0
Leek	0.9	16.4	14.8	4.0	3.60	4.9	4.41
Onion	3.1	18.5	57.4	63.2	196	26.8	83.1
Wheat	1.3	187.7	244	199.7	260	195	254
TOTALS			319		579		374

[1]Campbell et al. (1997)
[2]Van Loo et al. (1995)

In summary, average background consumption of FOS from naturally occurring foods of plant origin is 114 mg/day for adults in the U.S. Europeans consume more FOS per day because of higher intakes of the foods that contain relatively high levels of FOS. Average daily intakes in Europe are estimated to be as high as 579 mg.

3. Current Commercial Products

FOS represents only a fraction of the inulin class of carbohydrates known as fructans. In this class are different chain length species, including inulin, oligofructose and FOS. They are chemically similar entities; they share the same basic structure of β (2-1) linked fructosyl units, sometimes ending with a glucosyl unit. Since all these fractions are mixtures of molecules that differ only in chain length, they can be described by their range and average degree of polymerization (DP). Various terms describing fructons have been used interchangeably in the published literature; however, the nomenclature used in this report will be consistent with that given in Table 6.

Currently, there are several commercial sources of FOS, inulin, and oligofructose. These products are sold and consumed as fat replacements and sugar substitutes for use in

a variety of foods such dairy products, candies and chocolates, spreads, baked goods and breakfast cereals, meat products, ice cream and frozen yogurt.

In the U.S., FOS is sold as a nutritional supplement at recommended doses of up to 4 to 8 g/day to promote the growth of bifidobacteria, and as an ingredient in Ensure® Fiber With FOS (lg/8 oz can) (Abbott Laboratories, Ross Product Division), a source of dietary fiber.

In Japan, FOS has been used by adults and as an ingredient in infant formula since 1989 (Meiji brand). The FOS concentration in Meiji powder is 2.3 g/100 g and in prepared infant formula is 0.32 g/100 ml, resulting in estimated mean and 90th percentile consumptions of 3.0 and 4.2 grams FOS/day, respectively. In addition, Meiji also sells a milk-free, soy isolate infant formula containing 1.0 g FOS/100 g powder. Other products from Meiji sold for infant use in Japan include: Petit-Ghurt, a yogurt drink with 1 g FOS/100 ml; Baby Wafer, a wafer containing calcium, tea extract and 1.1 g FOS/50 g wafer; High Calcuit, a biscuit kneaded with milk that contains 4 g FOS/40 g of bicuit; and Calcuit BORO, which is calcium supplemented and contains 1.4 g FOS/100 g product.

The following two tables, Tables 7 and 8, present a list of commercial products available in Japan and Europe that contain FOS.

TABLE 6

Definitions and Descriptions for Fructans, FOS, Inulin and Oligofructose

Name	Source, DP Average, and Range
Fructans (Fructosans)	Linear chains of fructose with β 2-1 or 2-6 linkages, with or without terminal glucose. Includes inulins, levans or phleins, and branched fractions.
Inulin	A fructan with linear chains of β 2-1 linked fructose, with or without terminal glucose, and a minor amount of branched β 2-6 linked fructose. DPs range from 3 to 60. Average DP varies depending upon the plant source, growing conditions, time of harvest, and storage conditions.
Oligofructose	A fructan with linear chains of fructose with β 2-1 linkages, with or without terminal glucose, and a minor amount of branched β 2-6 linked fructose. DPs range from 3-20. Average DP varies depending upon the plant source, growing conditions, time of harvest, and storage conditions.
FOS of scFOS	A fructan with linear β 2-1 linked chains of fructose with a terminal glucose (a mixture of GF_2, GF_3, and GF_4 sugars). DPs range from 3 to 5. Commercially available FOS is synthetically produced.

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TABLE 7 Commercial Products Available in Japan Containing FOS			
Name of Product	**Manufacturer**	**Type of Food**	**FOS Content**
Meioligo, syrup	Meiji Seika	Tabletop sugar	37.5 g/100 g
Meioligo, syrup	Meiji Seika	Tabletop sugar	5.6 g/15 g
Meioligo, granule	Meiji Seika	Tabletop sugar	2.3 g/2.5 g
Oligo's effect	Meiji Seika	Tabletop sugar	31.5 g/100 g
Oligosaccharides containing honey	Coop	Tabletop sugar	4.5 g/15 g
Yoglet	Meiji Seika	Confectionery	3.0 g/29 g
Hi lemon	Meiji Seika	Confectionery	3.0 g/29 g
Tokuno Milk	Meiji Seika	Confectionery	3.0 g/29 g
Orange Up	Meiji Seika	Confectionery	3.0 g/29 g
Yoglet candy	Meiji Seika	Confectionery	3.0 g/75 g
Hi lemon candy	Meiji Seika	Confectionery	3.0 g/75 g
Propolis candy	Van Life	Confectionery	5.2 g/100 g
Denroku mame	Denroku	Confectionery	Not indicated
Black karinto	Ito Yoka Do	Confectionery	Not indicated
Popcorn (sweet)	Daiei	Confectionery	2.0 g/100 g
Calcuit	Meiji Seika	Confectionery	2.1 g/90 g
Calcuit bolo	Meiji Seika	Confectionery	1.3 g/100 g
Calcuit for teeth	Meiji Seika	Confectionery	1.8 g/8 pieces
Hi Calcuit	Meiji Seika	Confectionery	4 g/40 g
Calciut wafers	Meiji Seika	Confectionery	0.4 g/12 sheets
Calcium wafers	Ikeda Mohan Do	Confectionery	Not indicated
Yogut raisin	Toyo Nuts	Confectionery	590 mg/100 g
Soft rice cracker	Kuriyama Beika	Confectionery	200 mg/100 g
MILO, brick back	Nestle Japan	Drink	1.4 - 2.4 g/200 ml
Bifidus drink yogurt	Zennoh (JA)	Drink	0.4 g/150 g
Furano drink yogurt	Zennoh (JA)	Drink	0.5 g/200 ml
Furano plain yogurt drink	Zennoh (JA)	Drink	0.2 g/100 g
Lactic acid bacteria fermented milk	Zennoh (JA)	Drink	0.5 g/200 ml
Ice cream packed in a wafer cake	Sakaeya Milk	Ice cream	0.5 g/pack
Hohoemi	Meiji Milk Product	Infant formula	2.3 g/100 g
LOLA's Bifidus + Oligo	Meiji Seika	Health food	250 mg/1.5 g
Seaweed gentle for body	Nikoniko Nori	Food	580 mg/27 g

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TABLE 8 Examples of Food Products Launched in the EU containing Actilight® Brand FOS		
Product	**Category of Food**	**FOS Content**
Jour apres Jour de Lactel	Milk and Dairy Product	0.95%
L'equilibre et moi de Gervais-Nactalia	Milk and Dairy Product	1.2%
Mimosa de Lactogal	Milk and Dairy Product	1.0%
Forte de Danone	Milk and Dairy Product	1.0%
Lait Lagassa	Milk and Dairy Product	1.0%
Bio de Danone aux cereales	Milk and Dairy Product	0.5%
BioFibra de Asturiana	Milk and Dairy Product	2.0%
Calcio de Asturiana	Milk and Dairy Product	1.0%
Farine Blevit Plus de Ordesa	Baby Food	1.0%
Sorbets hypoglucidiques de Thiriet	Frozen Products	22.6%
Actilife de Midor pour Migros	Frozen Products	5.6%
Flocons de froment Actilife de Zwicky pour Migros	Breakfast Preparations	5.0%
Flocons de froment Z-Plus de Zwicky	Breakfast Preparations	5.0%
Biscuits aux fibres Diet Fibra de Gullon	Biscuits	9.0%
Biscuits Naranja de Santiveri	Biscuits	8.0%
Galettes de riz complet Actilife de Migros	Biscuits	2.0%
Confiture El Loro de Helios	Jam	2.0%
Gamme ligne Bifide de Vivis/Distriborg	Meal Substitutes	4.0 – 16.0%
Gamme FOS equilibre de la flore de Vitagermine	Meal Substitutes	3.0 – 20.0%
Gamme Pleniday de Distriborg	Meal Substitutes	5.8 – 13.0%
Substituts de repas minceur Gaylord Hauser/Danone	Meal Substitutes	1.0 – 10.0%
Repas pour le controle du oids Figure Control de Sponser pour Migros	Meal Substitutes	3%
Substitut de repas BioManan de Merck	Meal Substitutes	4.3 – 4.7%
Substitut de repas minceur Nutricare	Meal Substitutes	1.5 g/portion
Sucaflore des Laboratoires EFA Sante	Dietary Supplements	87.86%
Purete totale du Laboratoire Oenobiol	Dietary Supplements	25.0%
Serenite du Laboratoire Oenobiol	Dietary Supplements	21.0%
Anti-age du laboratoire Oenobiol	Dietary Supplements	20.0%
Bioprotus 5000 des Laboratoires Carrare	Dietary Supplements	74%
Sveltonic des Laboratoires Carrare	Dietary Supplements	74%
Evanea Ventre Plat de Brugier-Sillon	Dietary Supplements	27%
Juvamine Ventre Plat Purete Interieure de Juvamine	Dietary Supplements	63.2%
Complement alimentaire de Beaute Cosmence du Club des Createurs de Beaute	Dietary Supplements	87.5%
Complement nutritionnel ventre plat Bionalab des	Dietary Supplements	87.5%

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TABLE 8 Examples of Food Products Launched in the EU containing Actilight® Brand FOS		
Product	**Category of Food**	**FOS Content**
Laboratoires R.D.C.I. fabrique par PharmEurop-Italie	Dietary Supplements	87.5%
Complement nutritionnel ventre plat de PharmEurop	Dietary Supplements	87.5%
Oligocaps – CAR – Ventre Plat B+	Dietary Supplements	6%
HygiaFlore de Super Diet	Dietary Supplements	3 g/dose
Trifit de Bionic	Dietary Supplements	not listed
E'lifexir de Phergal	Dietary Supplements	33.3%
BioFibra de Pfizer	Dietary Supplements	77%
Bien-etre de Delifruits (orange and pink grapefruit)	Refrigerated Drinks	1.5%
Bio Nature de Miau (mussels, tuna, white tuna)	Canned fish	3.1 – 3.6%

B. Estimated Daily Intake (EDI) of FOS from Proposed Use

FOS is proposed for use as an ingredient in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt at the levels specified in Table 1.

Estimates of the intake of FOS for its proposed uses were derived using the 1994-96 Continuing Survey of Food Intakes by Individuals (94-96 CSFII), the most recent complete dataset from the national food consumption survey conducted by the U.S. Department of Agriculture (USDA). The 94-96 CSFII was a three-year survey in which data were collected from a stratified area probability sample of individuals residing in households in the U.S. Households represented a cross-section of the population of the 50 states and the District of Columbia.

Using the 94-96 CSFII survey data, the EDIs of FOS for its proposed use in 18 food categories were derived for infants 5 to 11 months old, toddlers 1 year old, children 2 to 12 years old, teenagers 13 to 19 years old, and adults (20 years and older). The EDIs for the mean and 90th percentile consumers of the food products to which FOS is intended to be added, by age group and food category, are presented in Tables 9 to 13. Table 14 presents mean and 90th percentile total daily intakes of FOS by age group. The total EDIs presented in Table 14 are based on the assumption that FOS is present in all foods for which its use is proposed at the use levels provided in Table 1. Estimated mean and 90th percentile intakes of FOS for the various age groups are as follows:

- Infants: 1.6 g/day (mean) and 3.1 g/day (90th percentile);
- Toddlers (1 year): 3.9 g/day (mean) and 7.1 g/day (90th percentile);
- Children (ages 2 to 12 years): 5.4 g/day (mean) and 10.0 g/day (90th percentile);

- Teenagers (ages 13 to 19 years): 6.2 g/day (mean) and 12.8 g/day (90th percentile); and
- Adults: 4.4 g/day (mean) and 9.1 g/day (90th percentile).

The total EDIs derived for all age groups assume that FOS will be used at the proposed use level in all 18 food categories to which FOS is intended to be added. As such, the EDIs derived herein are considered to overestimate potential FOS intake.

TABLE 9

Estimated Intake of Fructooligosaccharides (FOS) in the U.S. based upon Proposed Use Levels in 18 Categories of Foods: Intake per User per Food Category per Day

Infants – 5 through 11 Months Old [a]

Food Category	Number of User Days	Mean Daily Intake per User [b]		90th Percentile Daily Intake per User [b]	
		(mg)	(mg per kg BW)	(mg)	(mg per kg BW)
Acidophilus milk	0	.	.	.	.
Baby foods	306	1065	126	1982	233
Bars	0	.	.	.	.
Beverages	6	2767	351	3096	409
Biscuits	17	795	82	1273	128
Cakes	12	766	85	1073	124
Confectionery	1	325	31	325	31
Cookies	20	452	50	793	84
Crackers	59	226	24	362	40
Flavored/unflavored milk	71	2118	225	4200	498
Hard candy	2	292	35	350	42
Ice cream	7	350	40	443	63
Jams and Jellies	8	141	16	275	33
Muffins	2	1656	187	1871	214
Ready-to-eat cereals (RTEs)	47	455	51	974	126
Sorbet and Sherbet	0	.	.	.	.
Soup	26	528	61	839	97
Yogurt	6	1102	126	2086	242

[a] Food intake data source: USDA 1994-96 Continuing Survey of Food Intakes by Individuals; intake estimates based on proposed use levels in 18 categories of food. Analyses include pregnant and/or lactating females and breast-feeding infants; individuals with missing bodyweight data were excluded from all analyses. WesVar Complex Samples 3.0 and CSFII sampling weights were used to calculate estimates.

[b] Mean intake estimates based on a sample size smaller than 48, and 90th percentile intake estimates based on a sample size smaller than 128 are potentially statistically unreliable based on an insufficient sample size criterion established by FASEB (1995) in its statistical reporting standards.

TABLE 10

Estimated Intake of Fructooligosaccharides (FOS) in the U.S. based upon Proposed Use Levels in 18 Categories of Foods: Intake per User per Food Category per Day

Toddlers - 1 year old [a]

Food Category	Number of User Days	Mean Daily Intake per User [b]		90th Percentile Daily Intake per User [b]	
		(mg)	(mg per kg BW)	(mg)	(mg per kg BW)
Acidophilus milk	0	.	.	.	.
Baby foods	254	797	73	1727	157
Bars	26	3318	284	5234	513
Beverages	40	3722	307	8382	724
Biscuits	21	790	69	1616	139
Cakes	152	1397	115	2407	202
Confectionery	94	932	80	1646	138
Cookies	283	830	72	1597	141
Crackers	446	347	31	699	61
Flavored/unflavored milk	1161	2176	189	3970	365
Hard candy	26	1093	91	1606	126
Ice cream	124	892	77	1918	163
Jams and Jellies	229	105	9	176	17
Muffins	19	1231	108	1866	190
Ready-to-eat cereals (RTEs)	592	1474	124	2768	223
Sorbet and Sherbet	11	734	66	972	100
Soup	205	755	67	1490	133
Yogurt	108	1856	162	3099	289

[a] Food intake data source: USDA 1994-96 Continuing Survey of Food Intakes by Individuals; intake estimates based on proposed use levels in 18 categories of food. Analyses include pregnant and/or lactating females and breast-feeding infants; individuals with missing bodyweight data were excluded from all analyses. WesVar Complex Samples 3.0 and CSFII sampling weights were used to calculate estimates.

[b] Mean intake estimates based on a sample size smaller than 48, and 90th percentile intake estimates based on a sample size smaller than 128 are potentially statistically unreliable based on an insufficient sample size criterion established by FASEB (1995) in its statistical reporting standards.

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TABLE 11

Estimated Intake of Fructooligosaccharides (FOS) in the U.S. based upon Proposed Use Levels in 18 Categories of Foods: Intake per User per Food Category per Day

Children – 2 to 12 years old [a]

Food Category	Number of User Days	Mean Daily Intake per User [b]		90th Percentile Daily Intake per User [b]	
		(mg)	(mg per kg BW)	(mg)	(mg per kg BW)
Acidophilus milk	7	8019	482	9975	666
Baby foods	52	372	25	684	46
Bars	217	3852	151	5872	265
Beverages	144	4568	178	8193	358
Biscuits	0	.	.	.	.
Cakes	1304	2455	97	4354	171
Confectionery	1336	1988	77	4238	161
Cookies	1791	1394	58	2600	110
Crackers	1372	549	25	998	49
Flavored/unflavored milk	5883	1720	74	3024	145
Hard candy	385	1540	60	3151	118
Ice cream	1304	1824	70	3046	136
Jams and Jellies	1223	160	7	328	14
Muffins	173	2334	94	3843	168
Ready-to-eat cereals (RTEs)	3608	2866	117	5171	206
Sorbet and Sherbet	94	1467	70	2601	149
Soup	713	1180	50	2033	92
Yogurt	308	1838	86	2969	149

[a] Food intake data source: USDA 1994-96 Continuing Survey of Food Intakes by Individuals; intake estimates based on proposed use levels in 18 categories of food. Analyses include pregnant and/or lactating females and breast-feeding infants; individuals with missing bodyweight data were excluded from all analyses. WesVar Complex Samples 3.0 and CSFII sampling weights were used to calculate estimates.

[b] Mean intake estimates based on a sample size smaller than 48, and 90th percentile intake estimates based on a sample size smaller than 128 are potentially statistically unreliable based on an insufficient sample size criterion established by FASEB (1995) in its statistical reporting standards.

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TABLE 12

Estimated Intake of Fructooligosaccharides (FOS) in the U.S. based upon Proposed Use Levels in 18 Categories of Foods: Intake per User per Food Category per Day

Teenagers – 13 to 19 years old [a]

Food Category	Number of User Days	Mean Daily Intake per User [b]		90th Percentile Daily Intake per User [b]	
		(mg)	(mg per kg BW/)	(mg)	(mg per kg BW)
Acidophilus milk	0	.	.	.	.
Baby foods	0	.	.	.	.
Bars	74	4020	71	5774	103
Beverages	83	8390	123	12002	205
Biscuits	0	.	.	.	.
Cakes	440	3567	57	6518	110
Confectionery	374	2840	47	5665	94
Cookies	404	1907	33	3325	63
Crackers	230	828	14	1578	28
Flavored/unflavored milk	1342	1935	32	3759	63
Hard candy	93	2189	39	4118	80
Ice cream	321	2891	47	5286	85
Jams and Jellies	214	272	4	519	8
Muffins	71	2923	45	5084	75
Ready-to-eat cereals (RTEs)	699	4136	69	7195	114
Sorbet and Sherbet	14	3130	50	5148	82
Soup	212	1804	29	2963	53
Yogurt	43	2695	43	3235	74

[a] Food intake data source: USDA 1994-96 Continuing Survey of Food Intakes by Individuals; intake estimates based on proposed use levels in 18 categories of food. Analyses include pregnant and/or lactating females and breast-feeding infants; individuals with missing bodyweight data were excluded from all analyses. WesVar Complex Samples 3.0 and CSFII sampling weights were used to calculate estimates.

[b] Mean intake estimates based on a sample size smaller than 48, and 90th percentile intake estimates based on a sample size smaller than 128 are potentially statistically unreliable based on an insufficient sample size criterion established by FASEB (1995) in its statistical reporting standards.

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TABLE 13

Estimated Intake of Fructooligosaccharides (FOS) in the U.S. based upon Proposed Use Levels in 18 Categories of Foods: Intake per User per Food Category per Day

Adults – 20 years and older [a]

Food Category	Number of User Days	Mean Daily Intake per User [b]		90th Percentile Daily Intake per User [b]	
		(mg)	(mg per kg BW)	(mg)	(mg per kg BW)
Acidophilus milk	8	3540	58	6906	98
Baby foods	7	441	7	669	11
Bars	306	4634	64	7250	103
Beverages	438	6528	89	12044	172
Biscuits	4	522	6	580	8
Cakes	3022	2959	40	5453	75
Confectionery	1782	2621	36	5166	70
Cookies	2691	1606	22	2994	43
Crackers	2646	696	10	1303	18
Flavored/unflavored milk	9562	1242	17	2498	36
Hard candy	252	1192	17	2329	38
Ice cream	2786	2350	31	4043	58
Jams and Jellies	2345	176	2	334	5
Muffins	611	2607	36	4142	63
Ready-to-eat cereals (RTEs)	4349	3214	45	5383	76
Sorbet and Sherbet	145	2140	29	3904	56
Soup	2084	1561	23	2659	40
Yogurt	679	2445	35	3228	55

[a] Food intake data source: USDA 1994-96 Continuing Survey of Food Intakes by Individuals; intake estimates based on proposed use levels in 18 categories of food. Analyses include pregnant and/or lactating females and breast-feeding infants; individuals with missing bodyweight data were excluded from all analyses. WesVar Complex Samples 3.0 and CSFII sampling weights were used to calculate estimates.

[b] Mean intake estimates based on a sample size smaller than 48, and 90th percentile intake estimates based on a sample size smaller than 128 are potentially statistically unreliable based on an insufficient sample size criterion established by FASEB (1995) in its statistical reporting standards.

TABLE 14

Estimated Intake of Fructooligosaccharides (FOS) in the U.S. based upon Proposed Use Levels in 18 Categories of Foods: Total Intake per User per Day [a]

Population	Number of User Days	Mean Daily Intake per User		90th Percentile Daily Intake per User	
		(mg)	(mg per kg BW)	(mg)	(mg per kg BW)
Infants (5 through 11 m)	350	1624	186	3085	337
Toddlers (1 y)	1294	3896	336	7054	614
Children (2 to12 y)	6726	5407	222	10023	426
Teenagers (13 to19 y)	1959	6216	102	12795	211
Adults (20 y and older)	14787	4370	60	9085	127

[a] Food intake data source: USDA 1994-96 Continuing Survey of Food Intakes by Individuals; intake estimates based on proposed use levels in 18 categories of food. Analyses include pregnant and/or lactating females and breast-feeding infants; individuals with missing bodyweight data were excluded from all analyses. WesVar Complex Samples 3.0 and CSFII sampling weights were used to calculate estimates.

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IV. INTENDED TECHNICAL EFFECTS

A. Bulking Agent

The main objective for the use of carbohydrate replacements is to obtain caloric reduction without the loss of taste quality (sweetness). This can be achieved by replacing the usual sweetening carbohydrate with an intense sweetener plus a bulking agent. Bulking agents can be any reduced-calorie or non-calorie carbohydrate.

One of the intended uses of FOS is as a bulking agent in foods. FOS is undigested and unabsorbed from the human intestinal tract. It passes unchanged into the colon where it is fermented, producing short-chain fatty acids (SCFA) that are bioavailable to the host. FOS can therefore be used as a bulking agent, because it confers a predictable reduction in caloric content.

The caloric contribution of FOS (including supporting data from inulin and oligofructose), has been determined in seven studies, using various approaches. The results indicate that the calorie value of FOS is between 1.0 to 2.8 kcal/g. Table 15 summarizes these studies.

TABLE 15
Summary of Studies Used to Determine the Caloric Value of FOS

Reference	Substrate	Method of Determination	Results
Oku (1991)	FOS	Biochemical balance based on metabolic pathways, and published fermentation pathways and combustion energies of fatty acids	1.7 kcal/g
Drevon and Bornet (1992)	FOS	Factorial method accepted by French and Dutch authorities	2 kcal/g
Hosoya et al. (1988)	FOS	Radiorespirometric method in man	1.5 kcal/g
Molis et al. (1996)	FOS	Calculation based on measurement in humans of fraction of FOS absorbed in the small intestine, fraction excreted in urine and stools, energy content of FOS absorbed from small intestine and estimated energy available from products of FOS digestion in large intestine.	2.3 kcal/g
Castiglia-Delavaud et al. (1998)	Inulin	Energy balance calculated using whole-body calorimetry.	2.8 kcal/g
Ranhotra et al. (1993)	Oligofructose	Conversion of gross food energy to net energy (carcass energy) in rats	1.5 kcal/g
Roberfroid et al. (1993)	Oligofructose	Biochemical balance charts for carbon atoms, metabolic pathways, energy yield to host	1 - 1.5 kcal/g

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B. Prebiotic Properties

The composition of the bacteria that comprise the gastrointestinal microflora has important ramifications for human health (Mitsuoka 1982; Drasar and Roberts 1989). Bifidobacteria are considered to be one of the most important genera of beneficial bacteria (Modler 1994; Gibson and Roberfroid 1995). The genus Bifidobacterium consists of at least 25 distinct species, 10 of whose main area of colonization is the human large intestine. In infants, bifidobacteria are one of the first bacterial groups to establish themselves in the intestinal tract and, within one week, become the predominant group (Hoover 1993; Mitsuoka 1982, 1984; Poupard et al. 1973).

There are many purported beneficial effects of bifidobacteria on human health. Bifidobacteria may provide a defense against pathogenic bacteria. The predominance of the bifidobacteria in breast-fed infants is believed by many to afford some of the protection against enteral as well as systemic disorders caused by bacterial pathogens. It is thought that the predominance of bifidobacteria produces the lower morbidity and mortality seen among breast-fed infants (Yoshioka et al. 1983; Roberts 1986; Ogawa et al. 1992). In elderly persons, or persons suffering from an illness such as chronic renal failure, bifidobacteria often decrease or diminish, with *Clostridium perfringens* increasing. Antibiotic therapy in both adults and children can result in a disruption of the normal gut microflora, producing an environment where potentially harmful species predominate over nonpathogenic strains (Gibson and Roberfroid 1995; Gibson et al. 1995; Wang and Gibson 1993; Ballongue 1993; Miller-Catchpole 1989). Bifidobacteria have been used therapeutically to restore the normal intestinal flora during antibiotic therapy (Gibson and Roberfroid 1995; Gibson et al. 1995; Wang and Gibson 1993; Ballongue 1993; Miller-Catchpole 1989).

A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson and Roberfroid 1995). FOS can be used as a prebiotic. FOS does not produce an adverse effect on the balance of colonic microflora and indeed has been shown in numerous *in vitro* and *in vivo* studies to produce a beneficial manipulation of the microflora that can counteract the disruptions that are caused by such factors as age, disease, or a less than optimal diet.

In vitro studies have measured bacterial growth using FOS, inulin or oligofructose and have found that FOS is a preferred growth substrate for bifidobacteria (Yamazaki and Matsumoto 1994; Yamazaki and Dilawri 1990; Gibson and Wang 1994a,b; Newton et al. 1996; Muramatsu et al. 1992; May et al. 1994; Sghir et al. 1998; Hidaka et al. 1986; Hartemink et al. 1997; McKellar and Modler 1989; McKellar et al. 1993; Tashiro et al. 1997). End products of fermentation are SCFA. A pH reduction is also noted. Study summaries are found in Table 16.

[redacted]

Many *in vivo* studies in animals have demonstrated the selective utilization of FOS, inulin and oligofructose by bifidobacteria as well as lactobacilli (Bunce et al. 1995a,b; Oli et al. 1995; Campbell et al. 1997b; Howard et al. 1995a,b; Gallaher et al. 1996; Hill and Rao 1988; Hirayama et al. 1994). In addition, animal studies suggest that FOS can have a modulating effect on tissue damage produced by the pathogenic organism, *C. difficile,* by suppressing growth of this organism, indirectly affecting toxin production and protecting intestinal epithelial tissue, in part, through growth promotion of a normal gut (Gaskins et al. 1996; May et al. 1995; Wolf et al. 1997). Study summaries are found in Table 17.

FOS has been shown to act as a selective substrate for bifidobacteria in several human clinical trials (Anchour et al. 1997; Bouhnik et al. 1994, 1996; Buddington et al. 1996; Hidaka et al. 1986, 1991; Mitsuoka et al. 1986, 1987; Kawaguchi et al. 1993; Macfarlane and Gibson 1994; Rochat et al. 1994; Sano 1986; Takahashi 1986; Williams et al. 1994) (Table 18). Both inulin and oligofructose act similarly in human subjects (Macfarlane and Gibson 1994; Gibson et al. 1995; Bouhnik et al. 1996; Kleesen et al. 1997).

The selective increase in bifidobacteria populations resulting from FOS consumption appears to be more effective in subjects whose microflora has been compromised due to disease or old age. Using several studies reported in the literature, Roberfroid et al. (1998) correlated daily dose of β(2-1) fructan with increase in bifidobacteria concentration. He found that the log increase of bifidobacteria concentration is not dose related, but correlates with the initial bifidobacteria level. The lower the starting concentration of bifidobacteria the greater the response to β(2-1) fructan consumption within a dose range of 4 to 20 or more grams per day.

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TABLE 16

***In Vitro* Studies on the Effects of FOS on Colonic Microflora**

Reference	Substrate	Study Design	Effect
Gibson and Wang 1994a	FOS, oligofructose	Batch culture growth of eight species of bifidobacteria utilizing FOS compared to glucose	*B. infantis, B. pseudolongum,* and *B. angulatum* growth was significantly enhanced with FOS compared to glucose. In general, the preferred growth substrate for bifidobacteria was short chain (DP 4) linear FOS.
Gibson and Wang 1994b	FOS, inulin	Chemostat cultures of mixed fecal bacteria from healthy human subjects used to determine the bifidogenic effect of oligofructose	In single-stage continuous culture systems, FOS was the preferred growth substrate for bifidobacteria. Bifidobacteria cell numbers had an increase by 2 log values with FOS compared to glucose and inulin. A decrease in bacteroides was also observed with FOS compared to the other substrates. Growth of bifidobacteria was enhanced at parameters thought to approximate those in the proximal colon: a high dilution rate (0.3 h^{-1}), high substrate concentration (1% w/v FOS), and low pH (5.5). These conditions suppressed the growth of bacteroides, clostridia, and coliforms. FOS increased bifidobacteria numbers, suppressed the numbers of clostridia, and increased total short-chain fatty acids (SCFA) production compared to sucrose in multiple-chamber systems.
Hartemink et al. 1997	FOS, inulin	The growth of enterobacteria isolated from human or swine feces, and other species) on 1/2 PY broth supplemented with FOS or inulin was measured by high performance anion exchange chromatography	FOS added to the growth medium resulted in increased growth rates. Most enterobacteria were able to ferment FOS to some extent. Fermentation occurred rapidly, especially for smaller oligosaccharides. No significant differences in fermentation were observed between FOS and inulin.
Hidaka et al. 1986	FOS, inulin, glucose, lactose, lactulose	Utilization of the sugars by numerous intestinal bacteria species (including bifidobacterium, lactobacillus, eubacterium, propionibacterium, bacteroides, clostridium, enterococcus, etc.) was measured	FOS was utilized by all bifidobacterium species except *B. bifidum*. FOS was not utilized by *Lactobacillus fermentum, Escherichia coli,* or *Clostridium perfringens* compared to other sugars tested.

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TABLE 16

***In Vitro* Studies on the Effects of FOS on Colonic Microflora**

Reference	Substrate	Study Design	Effect
May et al. 1994	FOS, cellulose, modified pectin, XOS, gum arabic	Pig fecal inoculum donors were used to investigate the effect of various fiber sources on the growth and toxin production of *C. difficile* and acidogenic bacteria	Growth of acidogenic bacteria (bifidobacteria and lactobacilli) was greatest for FOS. Cellulose, modified pectin, and FOS resulted in the lowest growth of total clostridia compared to XOS and gum arabic, which allowed the greatest growth. No culturable counts of *C. difficile* were observed for any treatment. Toxin A was not detected with FOS. FOS resulted in the lowest pH (4.17; initial pH was 6.7), due possibly to production of lactic acid, and intermediate concentrations of SCFA.
McKellar and Modler 1989	FOS, Jerusalem artichoke flour, inulin	Several strains of bifidobacteria were measured in culture for the ability to use Jerusalem artichoke flour, FOS, and inulin as carbohydrate sources	For *B. adolescentis, B. longum,* and *B. thermophilum,* FOS was the best substrate, followed by sucrose and inulin. Maximum activity was obtained with short chain FOS (DP 3-5). All 3 strains utilized Jerusalem artichoke flour as a carbohydrate source. All bifidobacteria strains produced acid with FOS, as well as inulinase. The synthesis of inulase was proportional to the molecular weight of the carbohydrate utilized.
McKellar et al. 1993	FOS, inulin	19 species of bifidobacterium were evaluated for the ability to grow on various carbohydrate sources	All strains tested grew on FOS (DP $\leq$ 5), with the best growth observed with *B. minimum, B. cuniculi,* and *B. thermophilum* (all of animal origin). These 3 strains also had the best growth on inulin (DP $\geq$ 15). Strains with the best growth on FOS also had the best growth on inulin, suggesting that the same enzyme is responsible for metabolism of the two substrates. FOS gave a significantly greater cell yield for all strains combined and was thus the best overall carbohydrate source.
Muramatsu et al. 1992	FOS, inulin, sucrose	The production of β-fructofuranosidase was measured from 6 strains of bifidobacteria grown in modified BL broth containing a mixture of FOS, inulin, or sucrose as the only carbon source	All bifidobacteria strains hydrolyzed FOS and inulin. Peak growth occurred at 10, 12, or 24 hours after inoculation, followed by a gradual decrease in the absorbances of the cultures. pH decreased according to the growth of bifidobacteria, and acid production stopped with growth. The pH of the broths was 4.4-4.8 after 36 hours of cultivation. Enzyme activity increased with growth; peak enzyme activity occurred at 10, 12, and 24 hours.

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TABLE 16 *In Vitro* Studies on the Effects of FOS on Colonic Microflora			
Reference	**Substrate**	**Study Design**	**Effect**
Newton et al. 1996 (abstract)	FOS, pectin, fructose, polydextrose, starch, inulin, oligofructose, palatinose, XOS, soya-oligosaccharides, malto-oligosaccharides	Batch culture fermentation systems inoculated with fecal bacteria were used to compare the bifidogenic effect of various dietary carbohydrates	All substrates were fermented to some extent; all resulted in an increase in SCFA. FOS demonstrated the highest bifidogenic potential. Other bacteria (bacteroides, clostridia, fusobacteria, gram-positive cocci, lactobacilli, and coliforms) remained relatively unchanged.
Sghir et al. 1998	FOS	Lactic acid-producing bacteria in continuous culture fermentors using FOS as the selective substrate were followed over a 21-day period after inoculation with mixed human feces from healthy adults.	Lactobacilli and bifidobacteria increased from 11.2% of the total cultural count on day 1 to 98.7% on day 21, with bifidobacteria representing a greater proportion than lactobacilli. About 97% of FOS was utilized by bifidobacteria between day 2-3. The bifidobacterium population reached its maximum on day 2. FOS utilization was 99.75% between days 13-15, correlating with the peak lactobacilli time. Bifidobacteria isolates used FOS more rapidly than lactobacilli isolates.
Tashiro et al. 1997 (abstract)	FOS, starch, guar gum, pectin, inulin, inulooligosaccharides, other polysaccharides	Fermentation tests with healthy human stool were conducted to delineate differences in fermentability between the various substrates	FOS was the most fermentable substrate; inulin was the least fermentable. FOS had a significantly higher fermentation rate compared to guar gum or pectin. FOS produced SCFA similar to starch and other polysaccharides. No significant differences were evident in the rates of substrate disappearance and SCFA production between FOS and inulooligosaccharides. FOS had slightly higher molar proportion of butyrate after 6 hours compared to inulooligosaccharides. Molar proportion of butyrate was lower in fructans with higher DP. (Lactic acid production decreased at 1-6 hours in fructans with higher DP suggesting that fructans with higher DP are less fermented by bifidobacterium.)
Yamazaki and Dilawri 1990	Enzymatically-prepared FOS, Jerusalem artichoke oligofructose, crude Jerusalem artichoke flour, inulin from dahlia tubers	Continuous measurement of 3 bifidobacteria strains in air evacuated culture tubes	*B. longum, B. infantis,* and *B. adolescentis* had good growth on Jerusalem artichoke oligofructose and FOS. Jerusalem artichoke flour and inulin had slower growth.

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TABLE 16 *In Vitro* Studies on the Effects of FOS on Colonic Microflora			
Reference	**Substrate**	**Study Design**	**Effect**
Yamazaki and Matsumoto 1994	Fructans from Jerusalem artichoke tubers, enzymatically-prepared FOS, inulin	Measurement of various carbon sources on the growth of 3 bifidobacteria species	Jerusalem artichoke fructans and enzymatically-prepared FOS were good substrates for bifidobacteria. Much slower growth occurred with inulin.

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000045.001

TABLE 17 Animal Studies on the Effects of FOS on Colonic Microflora		
Reference	**Study Design**	**Effect**
Bunce et al. 1995a (abstract)	Neonatal pigs not vaccinated against *E. coli* were administered a non-medicated milk replacer diet for 6 days. The animals then received milk replacer diet alone or milk replacer diet plus 3 g/day FOS for another 6 days. On day 7, the animals were challenged with an oral administration of *E. coli*.	The milk replacer diet plus FOS resulted in a nonsignificant decrease in *E. coli* counts and increased bifidobacteria counts compared to animals who received the milk replacer diet alone. Clostridium counts were not significantly different between groups. The authors concluded that FOS can provide protection from infectious *E. coli* in the large intestines of pigs.
Bunce et al. 1995b (abstract)	Holstein calves were fed a non-medicated milk replacer diet alone, the milk-replacer diet plus 4 g/day FOS, or the milk replacer diet plus 7 g/day FOS.	The milk replacer diet plus FOS caused nonsignificant decreases in *E. coli* and clostridia counts and significant increases in bifidobacteria counts compared to animals fed the milk replacer diet alone. Ingestion of FOS also produced a decrease in total anaerobic flora and total bacterial population comprised of *E. coli*, and increased the total bacterial population comprised of bifidobacteria. The authors concluded that FOS can prevent intestinal *E. coli* infection in calves.
Campbell et al. 1997b	Male Spraque-Dawley rats were randomly assigned to receive (1) a control diet, (2) control diet plus 5% microcrystalline cellulose, (3) control diet plus 5% microcrystalline cellulose plus 6% FOS, (4) control diet plus 5% microcrystalline cellulose plus 6% oligofructose, or (5) control diet plus 5% microcrystalline cellulose plus 6% xylooligosaccharide (XOS) for 14 days.	Cecal bifidobacteria concentrations were significantly higher in animals fed the oligosaccharide diets compared to controls. The oligosaccharide diets resulted in significantly higher total anaerobes and reduced total aerobes. Lactobaccili concentrations did not significantly change with any diet. Consumption of the FOS and oligofructose diets produced lower cecal pH and higher total cecal SCFA pools, cecal butyrate concentrations, cecal total weight, and cecal wall weight compared to the XOS, cellulose and control diets.

000045.008

TABLE 17

Animal Studies on the Effects of FOS on Colonic Microflora

Reference	Study Design	Effect
Gallaher et al. 1996	Male Wistar rats were gavaged with 15 mg/kg/wk 1,2-dimethylhydrazine for 2 weeks. After one week, animals received various diets consisting of skim milk, saline, skim milk w/bifidobacteria (10^8), and skim milk w/*Lactobacillus* (via gavage) or 2% FOS (in feed). The diets and durations were: *Experiment 1*: skim/basal diet or skim milk/bifido/FOS diets for 24-25 days. *Experiment 2*: saline/basal, skim/basal, bifido-basal, bifidio/FOS, or skim/FOS diets for 29-35 days. *Experiment 3:* skim/basal, bifido/basal, bifido/FOS, or skim/FOS diets for 28-31 days. *Experiment 4:* skin/basal, skim/FOS, bifido/FOS, lacto/FOS, or bifido/lacto/FOS for 44-66 days.	The effects of dietary FOS and/or bifidobacteria on colonic microflora were inconsistent between experiments: *Experiment 1:* There were no significant differences in bifidobacteria or *C. perfringens* populations between the two groups. *Experiment 2:* The skim/FOS diet produced significantly higher bifidobacteria counts and lower *C. perfringens* counts compared to the basal diets. Cecal pH was decreased in all the diets with FOS. *Experiment 3: C. perfringens* numbers were reduced and bifidobacteria counts increased with the skim/FOS diet compared to the basal diets. Cecal pH was lower with both FOS diets. *Experiment 4:* Bifidobacteria and *Lactobacillus* counts were not significantly affected by the diets. *C. perfringens* counts were lower in the bifido/FOS, lacto/FOS, and bifido/lacto/ FOS groups. No significant effect on cecal pH was noted.
Gaskins et al. 1996	Male C57BL/6NHsd mice were fed a basal diet for 5 days. On day 5, the animals were given 100 µg/g cefoxitin or vehicle orally and then randomly assigned on day 6 to receive the basal diet alone or the basal diet plus 30 g/L FOS in drinking water for 5 days. On day 11, the animals were challenged with an inoculation of *Clostridium difficile* (2.5 x 10^8 CFU/ml) and continued on the diets for an additional 5 days.	Levels of *C. difficile* were higher for antibiotic-treated animals receiving FOS supplementation and the basal diet only compared to non-antibiotic-treated animals. FOS-treated animals challenged with *C. difficile* had less diarrhea and lower detectable titres of toxin A compared to animals not supplemented with FOS. Three days after antibiotic challenge, animals fed the basal diet only and treated with antibiotic had lower fecal concentrations of total anaerobes and total clostridia compared to non-antibiotic-treated controls. Animals supplemented with FOS and treated with antibiotic had higher fecal concentrations of total anaerobes and total clostridia compared to non-antibiotic-treated controls.
Hill and Rao 1988 (abstract)	Male Fisher F344 rats were fed 0.5%, 1.0%, or 5.0% FOS or administered equivalent daily doses by gavage for 2 weeks.	FOS administration resulted in a significant increase in bifidobacteria counts and a decrease in cecal pH. A greater magnitude of decrease in cecal pH was observed in animals administered FOS by gavage compared to animals who ingested FOS. No significant changes in streptococcus counts were observed.

000045.009

TABLE 17 Animal Studies on the Effects of FOS on Colonic Microflora		
Reference	**Study Design**	**Effect**
Hirayama et al. 1994	Germ-free BALB/c mice who had been cage-mated for 1 month to female germ-free BALB/c mice that were inoculated with human feces (to produce human-flora associated mice), were administered 0.1% FOS in drinking water for 4 months and 0.5% FOS for 1 week after an interval of 3 weeks.	Administration of FOS resulted in nonsignificant changes in the fecal flora of human-flora-associated mice. The rate of bifidobacteria to total bacteria increased during ingestion of 0.1% FOS for 4 months, followed by a decrease to the initial level when FOS ingestion ceased, and an increase when ingestion of 0.5% FOS was resumed. The number of bacteroidaceae and enterobacteriaceae tended to decrease during the 0.1% and 0.5% FOS ingestion periods and increased during the non-FOS period.
Howard et al. 1995a	Male BALB/c mice randomly fed 4 soluble fiber diets: no supplemental fiber (control), or 30 g/l FOS, XOS, or gum arabic in drinking water for 14 days.	Consumption of FOS resulted in a significantly higher concentration of viable bifidobacteria, significantly higher concentration of bifidobacteria expressed as a percentage of total anaerobic flora, and a nonsignificant increase in total anaerobic microflora compared to the other diets. The authors concluded that dietary supplementation with FOS can enhance the population of bifidobacteria in the large intestines.
Howard et al. 1995b	Male neonatal pigs randomly fed formula alone or formula plus 3 g/l FOS for 6 days.	Fecal bifidobacteria counts were similar in all animals on days 1 and 3; however, on day 6, there was a tendency for animals ingesting FOS to have higher numbers of bifidobacteria compared to controls. The authors conclude that dietary supplementation with FOS can enhance bifidobacteria populations and prevent colonic epithelial mucosa atrophy in neonates fed an elemental diet.

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TABLE 17 Animal Studies on the Effects of FOS on Colonic Microflora		
Reference	**Study Design**	**Effect**
May et al. 1995	Male BALB/c mice were fed a basal diet for 3 days and given 100 µg/g cefoxitin via gavage on day 4. The mice were then fed ad libitum for 5 days, either (1) a control diet with no fiber supplementation, (2) the control diet plus 44 mg gum arabic/ g diet, (3) the control diet plus 44 mg FOS/ g diet, or (4) the control diet plus 30 g XOS/ l drinking water. The mice were then challenged with an inoculum of *C. difficile* (2.8 x 10^8 CFU/ml) and sacrificed 6 days after the inoculation.	3-days after *C. difficile* inoculation, all animals became infected with the pathogen; however, 6 days post-inoculation, animals fed the FOS and XOS diets had lower *C. difficile* counts compared to animals fed the control and gum arabic diets. Animals fed the FOS and XOS diets had a less than 50% incidence of toxin A; animals fed the control or gum arabic diets had a greater than 50% incidence of toxin A. The FOS and XOS diets appeared to ameliorate lesions induced by *C. difficile* in the colon and cecum that occurred in animals receiving the control and gum arabic diets. Only animals fed the control or gum arabic diets had severe diarrhea or died. Animals consuming FOS had the highest concentrations of fecal total SCFA. The FOS and XOS diets resulted in a significantly greater molar proportion of butyrate compared to the other diets. The authors concluded that FOS and XOS appear to provide protection to the intestinal epithelial tissue, due in part, to the promotion of growth of the normal gut microbiota.
Oli et al. 1995	Weanling pigs with cholera toxin-induced diarrhea were treated with oral electrolyte solutions with and without FOS. Dose of FOS and treatment duration not reported.	Diarrhea caused a reduction in total bacterial densities in luminal contents (from 10^{10} to 10^6 CFU/mg). The decline was especially noted for lactobacilli. A lesser effect was observed for bacteria associated with the mucosa. After 24 hours, animals receiving FOS had total bacterial counts similar to counts before diarrhea. FOS produced a 10-fold increase in lactobacilli counts. Authors concluded that the addition of FOS to oral electrolyte solutions accelerates the recovery of beneficial bacteria groups.

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TABLE 17

Animal Studies on the Effects of FOS on Colonic Microflora

Reference	Study Design	Effect
Wolf et al. 1997	Female golden Syrian hamsters were orally gavaged with 250 mg/kg/day ciprofloxain for 6 days. On days 3 and 7, the animals were inoculated with *C. difficile* (10^9 CFU/ml). Experiment 1: Hamsters were randomly assigned to receive for 24 days: 0 or 30 g/l FOS in drinking water and 0, 0.5 x 10^9, or 2.0 x 10^9 CFU *C. difficile* inoculum. Experiment 2: Hamsters were randomly assigned to receive for 24 days: 0 or 30 g/l FOS in drinking water, 0 or 2.0 x 10^9 CFU *C. difficile* inoculum, and 0 or 50 mg/kg/day vancomycin via gavage on days 7-13. Experiment 3: A ten fold dilution (10^0 to 10^{-5}) of *C. difficile* toxin-laden supernatant was added to saline or 60 g/l FOS. 1 ml of this inoculum was injected intraperitoneally into female CF-1 mice.	Experiment 1: FOS significantly increased median survival time in hamsters challenged with *C. difficile* and improved (but not significantly) the median survival time in hamsters not inoculated. In experiment 2, FOS had an overall effect of significantly improving median survival time. In experiment 3, all mice injected with 10^0, 10^{-1}, or 10^{-2} dilutions died of apparent toxemia within 24 hours. All animals receiving higher injections (10^{-3} to 10^{-5}) lived. According to the authors, the results indicate that FOS does not directly bind *C. difficile* toxins. The authors concluded that FOS increases median survival time in a hamster model of *C. difficile*-colitis, and may be used to prevent outbreaks of *C. difficile* in long-term care treatment institutions and hospitals.

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TABLE 18 Clinical Studies on the Effects of FOS on Colonic Microflora		
Reference	**Study Design**	**Effect**
Anchour et al. 1997 (abstract)	12 healthy subjects studied for 3 consecutive periods: 2-week basal period; 4-week FOS period (8 g/day FOS), and 4-week control period (8 g/day sucrose).	There was no change in total anaerobe counts, oro-fecal transit time, fecal weight, or % of fecal water during the study periods. Fecal bifidobacteria counts were significantly increased during the FOS period but returned to their initial values during the control period. Fecal pH tended to decrease during both the FOS and control periods.
Bouhnik et al. 1994 (abstract)	20 healthy subjects randomly assigned to 2 groups and studied for 12-day periods consisting of a basal period, ingestion period, and post-ingestion period. Subjects ingested 12.5 g/day FOS or placebo in 3 divided doses during the ingestion period.	Bifidobacteria counts as well as β-galactosidase, β-glucosidase, and fructo-oligohydrolase activity significantly increased with FOS ingestion. The increases were transient and disappeared a few days after cessation of FOS ingestion. FOS ingestion had no effect on fecal total anaerobe counts, nitroreductase, azoreductase, and β-glucuronidase activities. No changes in fecal bacteria counts and enzymes were observed during any period in the placebo group.
Bouhnik et al. 1996	20 healthy male and female subjects randomly assigned to 2 groups and studied for consecutive periods consisting of a 12-day basal period, 12-day ingestion period, and a 12-day post-ingestion period. Subjects ingested 12.5 g/day FOS or placebo (saccharose) in 3 divided doses during the ingestion period. Subjects were allowed to consume their usual diet, minus fermented dairy products, food containing FOS, and food known to promote abdominal symptoms.	Bifidobacteria counts and β-fructosidase activity significantly increased with FOS ingestion; there was a significant correlation between fecal β-fructosidase activity and bifidobacteria counts in subjects consuming FOS. The increases returned to basal values within the 12-day post-ingestion period. FOS ingestion had no effect on total fecal anaerobe counts or fecal pH. No changes occurred in fecal bacterial counts, fecal pH, or fructosidase activity in subjects given placebo. There were no changes in nitroreductase, azoreductase, and β-glucuronidase in either group through out the study periods.
Buddington et al. 1996	12 healthy subjects fed a controlled diet (lower in fat and protein, with a higher fiber content) for 42 days and given 4 g/day FOS on days 7-32.	Both the controlled diet alone and the diet plus FOS resulted in increased densities of total anaerobe and bifidobacteria counts; however, significantly greater increases were observed with FOS. Total aerobe and enterobacteria counts were less affected by the controlled diet and FOS. The increases in anaerobic microbiota correlated with decreased activity of β-glucoronidase and glycocholic acid hydroxylase, which have been implicated in carcinogenesis. FOS ingestion had no effect on nitroreductase activity.

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TABLE 18

Clinical Studies on the Effects of FOS on Colonic Microflora

Reference	Study Design	Effect
Hidaka et al. 1986, Mitsuoka et al. 1987	23 senile inpatients (50 to 90 yrs of age) were given 8 g/day FOS for 2 weeks.	Bifidobacteria and total bacteria counts were significantly increased 4-7 days after ingestion of FOS. The average number of bifidobacteria per gram of stool increased about 10-fold after 14 days of FOS ingestion. The increases were observed only during FOS administration; bifidobacteria counts decreased after cessation of FOS ingestion. FOS was more rapidly utilized by bifidobacteria compared to *Bacteroides fragilis,* which are the dominant bacteria in the human intestine, and other species. Lactobacilli numbers had a tendency to increase and *Clostridium perfringens* showed a tendency to decrease following FOS ingestion. Persons with a relatively large number of bifidobacteria per gram of stool did not show marked changes but *Clostridium perfringens* was not detected during FOS ingestion, suggesting that a large number of bacteria may suppress the growth of *Clostridium perfringens* in the human intestines. Persons initially with diarrhea or moderate constipation had normalized stool conditions during FOS ingestion. Mean fecal pH decreased by 0.3 during FOS ingestion.
Hidaka et al. 1991, Mitsuoka et al. 1986	8 hypercholesterolemic subjects with type IIa hyperlipoproteinemia given 8 g/day FOS for 1 month. 10 chronic renal failure patients and 6 healthy subjects given 8 g/day FOS for 1 year.	Bifidobacteria counts (analyzed in 6/8 pts) were increased by 5 times in subjects with a 10% reduction in total cholesterol. There was a slight increase in bifidobacteria counts in subjects with < 10% reduction in total cholesterol. In both healthy subjects and chronic renal failure patients, total intestinal microflora slightly increased after 1 month and remained at that level for 12 months thereafter. In chronic renal patients, bifidobacteria counts increased from $10^{8.7}$/g stool (2.5%) to $10^{9.8}$/g stool (23%) after 1 month; counts remained increased for 12 months thereafter and varied between $10^{9.6}$ to 10^{10} (12% to 27%). Enterobacteria counts tended to gradually decrease. In healthy subjects, bifidobacteria counts were slightly increased, and there were no changes in other intestinal bacteria. 2 renal patients with abnormal flora caused by administration of antimicrobial agents prior to the study had normalized flora after 3 months of FOS ingestion.

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TABLE 18 Clinical Studies on the Effects of FOS on Colonic Microflora		
Reference	**Study Design**	**Effect**
Kawaguchi et al. 1993	1 male subject, 68 yrs of age received a low dose of FOS for 2 months prior to start of study. During the study, the subject received (1) a low dose of FOS of 6.2 g/day, (2) a high dose of FOS of 18.6-24.8 g/day, (3) a low dose of lactulose of 6.3 g/day, or (4) a high dose of lactulose of 18.9-25.2 g/day.	The subject had increased bifidobacteria counts after ingestion of FOS, the increase was more pronounced after ingestion of the high dose of FOS. Ingestion of lactulose produced similar results.
MacFarlane and Gibson 1994 (abstract)	8 healthy volunteers fed a controlled diet for 45 days. The diet was supplemented with 15 g/day sucrose on days 1-15, 15 g/day FOS on days 16-30, and 15 g/day sucrose on days 31-45.	Bifidobacteria counts were significantly increased during the FOS supplementation period compared to the sucrose supplementation periods.
Mitsuoka et al. 1986	30 hyperlipidemia patients (mean age 70.2 years) randomly received 1, 2, or 4 g/day FOS for 4-10 weeks.	There was a tendency for bifidobacteria counts to increase with increasing dose. The increase in bifidobacteria counts reached statistical significance at the highest dose level.
Rochat et al. 1994 (abstract)	38 healthy male subjects 8 g/day FOS, placebo, or 10^9 CFU/day Lactobacillus acidophilus in a dairy product for 2 weeks each in a crossover design consisting of five 2-week periods.	Bifidobacteria counts increased during the FOS ingestion period, but gradually returned to baseline values after cessation of FOS ingestion. Ingestion of FOS resulted in reduced *Clostridium perfringens* counts and slightly decreased fecal pH. Enterobacteriaceae and bacteriodaceae counts were not affected.
Sano 1986	13 subjects with non-insulin dependent diabetes and complications of diabetic neurosis and symptoms of bowel movement abnormalities were administered 8-10 g/day FOS for 4-16 weeks.	5/13 patients were analyzed for intestinal microflora. In these patients, there was a tendency for increased total bacteria counts. Bifidobacteria counts were significantly increased and clostridium counts reduced after 4 weeks of FOS ingestion. 9/13 subjects (69%) had improvements in bowel movement after 4 weeks of FOS ingestion, which were maintained after 16 weeks.
Takahashi 1986 (abstract)	9 chronic renal failure patients administered 6 g/day FOS for 12 months. 9 patients served as the control group.	Prior to FOS ingestion, bifidobacteria counts in the renal failure patients had been low. Ingestion of FOS improved (increased) bifidobacteria counts in 8/9 patients (89%).

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TABLE 18 Clinical Studies on the Effects of FOS on Colonic Microflora		
Reference	**Study Design**	**Effect**
Williams et al. 1994	10 subjects administered 4 g/day FOS for 14 days	Total bacterial counts increased in 9/10 subjects (90%). FOS ingestion resulted in 3.7-300-fold increases in bifidobacteria counts (the average was about a 40-fold increase). Bifidobacteria increased from 1.3% to 6.8% of the total microbiota. No bifidobacteria was detected in fecal samples from 2 subjects. Enterics (as a % of the total microbiota) decreased in 8/10 subjects from an average of 2.3% to 0.2%.

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V. ANALYTICAL METHODOLOGY

An ion chromatographic method (ion-exchange high-performance liquid chromatography (HPLC) with pulsed electrochemical detection) has been developed to measure FOS in food and feed ingredients. This method is described in Campbell et al. (1997a).

VI. REVIEW OF SAFETY DATA

A. Fate of FOS in the Gastrointestinal Tract

FOS, as well as inulin and oligofructose, are polymers of D-fructose characterized by β (2-1) bonds. This linkage is responsible for many of the physical and chemical properties of these fructans. Therefore, studies of gastrointestinal fate for inulin and oligofructose can be used to corroborate findings from studies of FOS. *In vitro* and *in vivo* studies in animals and humans have demonstrated that FOS is virtually undigested and subject to fermentation by the colonic microflora.

1. *In Vitro* Studies

The indigestibility of FOS by rat and human digestive enzymes has been demonstrated *in vitro* using intestinal and pancreatic homogenates and the rat everted small intestinal sac (Oku et al. 1984; Hidaka et al. 1986; Tsuji et al. 1986; Ziesenitz and Siebert 1987; Molis et al. 1996). The fermentation of undigested FOS to CO_2 and SCFA by the colonic microflora has been also demonstrated *in vitro* (Hosoya et al. 1988; Tokunaga et al. 1989; Ziesenitz and Siebert 1987). These studies are described in Table 19.

Digestion and fermentation of inulin and oligofructose have also been examined *in vitro*. Acid hydrolysis of the fructofuranosidic linkages of β 2-1 fructans has been studied. Since FOS, inulin and oligofructose are acid-labile, it is possible that these β 2-1 linked fructans may be hydrolyzed in the stomach. Nilsson et al. (1988) studied hydrolysis of fructans (trisaccharides, tetrasaccharides, pentasaccharides and two β 2-1 linked fructans with DP of 9 and 16) *in vitro* using human gastric juice and homogenate of rat intestinal mucosa. Both *in vitro* experiments showed very slow hydrolysis by the rat intestinal mucosal homogenate and by the human gastric juice. Hydrolysis by human gastric juice at pH 1.05 was 10-15% but only 1% at pH 2.25. The hydrolysis by the rat intestinal mucosal homogenate was less than 1%. In an *in vitro* study by Nilsson and Bjorck (1988), about 8% of inulin was hydrolyzed and converted to fructose by 0.10 M HCl. Okey et al. (1919) showed that inulin was hydrolyzed by HCl at 37°C at concentrations known to be present in the stomach. However, the amount of hydrolysis that would occur in the 2-3 hours that inulin may be expected to remain in the stomach was comparatively small.

TABLE 19 *In Vitro* Studies Showing Non-digestibility and Subsequent Fermentation of FOS		
Study Design	**Effect**	**Reference**
FOS was incubated with pancreatic and intestinal mucosal homogenate from rats.	GF_2 and GF_3 were essentially undigested by the pancreatic and small intestinal digestive enzymes.	Hidaka et al. (1986)
Radiolabeled FOS was incubated with human fecal homogenate.	More than 90% of FOS was converted to $^{14}CO_2$ within 4 hours.	Hosoya et al. (1988)
Pancreatic and intestinal mucosal homogenate from rats and purified sucrase-isomaltase complex were used to determine the digestibility of GF_2, GF_3, sucrose and maltose.	In presence of pancreatic homogenate, starch was readily digested, but hydrolysis of GF_2 and GF_3 was negligible. In presence of sucrase-isomaltase complex GF_2 and GF_3 was not digested.	Oku et al. (1984)
Cecal contents from male Wistar rats fed diet with or without FOS (10%) for 2 weeks was incubated anaerobically with [U-^{14}C] for 6 hours.	During the 6-hour incubation period more than 10% of the radioactivity was converted to $^{14}CO_2$ in rats on FOS diet and 66% was converted to ^{14}C-volatile fatty acids in rats on FOS-free diet.	Tokunaga et al. (1989)
FOS hydrolysis and digestibility was determined by measuring the transmural potential difference evoked by Na^+ dependent active transport of glucose (produced by hydrolysis of sugar substitute) using everted small intestinal sac.	Hydrolysis of FOS was negligible.	Tsuji et al. (1986)
Human jejunal homogenate was obtained from 10 patients and incubated with enzyme-saturating concentrations of maltose, sucrose, palatinose and 30 mM of FOS. FOS and sucrose (20 mM) were fermented anaerobically using the cecal contents from 3 male rats fasted for 18 hours.	FOS was not cleaved to any appreciable extent by the human intestinal carbohydrases. The percent cleavage of FOS was <0.1%. The cecal microflora ferments FOS anaerobically into SCFA at a rate that is about 60% of the fermentation rate for sucrose.	Ziesenitz and Siebert (1987)
FOS was incubated with duodenal mucosal homogenate from 15 patients to investigate enzymatic hydrolysis by the intestinal brush border.	FOS was not hydrolyzed by the intestinal brush border enzymes.	Molis et al. (1996)

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2. *In Vivo* Studies in Animals

In vivo studies in animals demonstrate that FOS is not digested by the digestive enzymes of the alimentary canal. It passes unchanged into the colon where it is subsequently hydrolyzed and fermented by the microflora. A minor amount of acid hydrolysis of FOS may take place in the stomach resulting in release of fructose and glucose.

Animal studies that investigated the digestibility and fermentability of FOS are summarized in Table 20. Oku et al. (1984) demonstrated that digestion of FOS was not stimulated even after long-term (6 weeks) intake of FOS (20%) in male rats. Further, they also demonstrated that long-term intake of FOS does not affect the activities of other enzymes such as sucrase and maltase. This study also included intravenous dosing of FOS in order to determine what would happen to trace levels of this fructan if some of it were to be absorbed from the intestine without digestion. The radioactivity excreted by expiration, over 24 hours, as $^{14}CO_2$ was less than 1% of the total radioactivity injected. About 80% of the radioactivity was excreted in the urine within 6 hours and more than 95% was excreted over 24 hours. The radioactivity in the gastrointestinal (GI) tract and feces during the 24-hour period was only 1.24% of the injected radioactivity. This study strongly suggests that there is no hydrolysis of FOS in the internal organs of the rat.

Fermentation of FOS to SCFA (highest concentration in the feces is of acetic acid followed by propionic acid and butyric acid) and CO_2 by the colonic microflora was demonstrated in several animal studies (Tokunaga et al. 1986, 1989; Campbell et al. 1997b).

Several investigators (Bjorck and Nilsson 1991; Nilsson and Bjorck 1988; Graham and Aman 1986) have demonstrated, *in vivo*, partial hydrolysis of inulin by stomach acid. In a study by Bjorck and Nilsson (1991), a group of rats was given omeprazol to block HCl in the stomach. The inulin recovered in feces of these rats was not significantly hydrolyzed. This was in contrast to a second group of rats which were treated with the antibiotic nebacitin. Gel chromatography of the inulin recovered in the feces from this group showed that there was some acid hydrolysis of the inulin. In another study, rats were fed inulin or fructan, and 1 group of rats on each diet was treated with nebacitin (Graham and Aman 1986). Results from this study also demonstrated an increase in low molecular weight sugars following feeding of fructans due to hydrolysis in the gastrointestinal tract of pigs. Nilsson et al. (1998) demonstrated very low disappearance and poor absorption of cereal fructans in the small intestine. Although acid hydrolysis of inulin, oligosaccharides and FOS can occur in the stomach, the extent

of hydrolysis may depend on factors such as the buffering capacity of food components and the rate of gastric emptying (Bjorck and Nilsson 1991).

TABLE 20

Animal Studies Showing Non-digestibility and Subsequent Fermentation of FOS

Study Design	Effect	Reference
Male rats were fed diets supplemented with 5% microcrystalline cellulose or 6% FOS for 14 days.	Cecal SCFA content was higher in rats on FOS diet	Campbell et al. (1997b)
Male Wistar rats were fed diets with and without FOS (20%) for 6 weeks to study the effects of long-term ingestion of FOS on hydrolyzing enzymes. [U – ^{14}C]FOS was intravenously administered to rats to determine the digestibility of FOS by internal organs.	The digestion of GF_2 and GF_3 was not stimulated even after long-term ingestion of FOS. Long-term ingestion of FOS does not affect the activities of sucrase and maltase. After the intravenous dose, less than 1% of radioactivity was expired as $^{14}CO_2$ and 80% of the radioactivity was excreted in the urine within 6 hours and more than 95% was excreted over 24 hours. Radioactivity in the contents of the gastrointestinal tract and feces during the 24-hour period was only 1.24% of the injected radioactivity.	Oku et al. (1984)
Male Wistar rats (6 rats per group) were fed diets with either 10% FOS, 20% FOS, 20% glucomannan or control diet for 6 – 8 weeks. The concentration of SCFA was measured in feces.	The concentration of SCFA in feces increased in rats fed FOS compared to the control. The greatest increase was in acetic acid concentration in feces followed by propionic acid and butyric acid in rats fed FOS.	Tokunaga et al. (1986)
Male Wistar rats were fed diets with or without FOS (10%) for 2-3 weeks. [U-^{14}C]FOS was administered with a gastric tube to conventional, germfree and antibiotic-treated male rats.	In conventional rats, about 55% of [U-^{14}C]FOS was expired as $^{14}CO_2$ within 24 hours. The radioactivity excreted in urine and feces was 3.2% and 3.0%, respectively. In antibiotic-treated rats the $^{14}CO_2$ expired was lower and the radioactivity excreted in feces was higher than conventional rats. In germ-free rats, the $^{14}CO_2$ expired was 40% and the radioactivity excreted in the feces was much higher than the other groups. Radioactivity excreted in urine was not different between the three groups. More than 50% of FOS given orally was fermented to CO_2 within 24 hours. The results suggest that FOS is fermented mainly to SCFA by intestinal microorganisms, and the SCFA produced are absorbed and further converted to CO_2 in the body.	Tokunaga et al. (1989)

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3. Studies in Humans

Studies examining the fate of ingested FOS in humans are summarized in Table 21. These studies corroborate the findings from *in vitro* and animal studies that show that FOS is not absorbed or digested. A small amount of FOS may be hydrolyzed by the acid in the stomach. The unabsorbed FOS passes to the large intestine and is fermented by the microflora resulting in the production of SCFA and H_2. The H_2 produced is absorbed and expired in the breath.

In a multiple crossover study by Alles et al. (1996), 24 young healthy subjects were supplemented with 0, 5 or 15 g/d of FOS for 7 days with two 7-day washout periods. Breath H_2 excretion was significantly increased at 15 g of FOS. Breath H_2 excretion was higher than the control at 5 g FOS, bud did not reach statistical significance. FOS was not recovered in the urine suggesting that FOS was not absorbed. No FOS was recovered in fecal samples. The breath H_2 excretion and lack of FOS in the feces suggest complete fermentation in the large intestine by microflora.

Stone-Dorshow and Levitt (1987) supplemented 10 subjects with 10 g/d of FOS and 5 subjects with 5 g/d sucrose for 12 days. Breath H_2 excretion increased with FOS supplementation and was similar to H_2 excretion after lactulose ingestion, suggesting malabsorption of FOS by the small intestine.

In another crossover study, Molis et al. (1996) supplemented six subjects with placebo or 20.1 g/d FOS for 11 days, after an initial equilibrium phase. Eighty-nine percent of FOS was delivered to the colon, and FOS appeared in the ileum within 0.5–1 hour after ingestion. Most of the unabsorbed FOS was in an intact unhydrolyzed form. The FOS recovery in urine after 24 hours was 0.12% of the ingested amount. Approximately 1% of the FOS that disappeared from the small intestine was recovered in the urine; the remaining portion of the FOS that disappeared from the small intestine was hydrolyzed during passage through the GI tract and absorbed as glucose and fructose. FOS was not recovered in the feces. These results suggest that only about 0.12% of ingested FOS is absorbed by the small intestine and that only a small amount is hydrolyzed in the upper GI tract. The remaining FOS is fermented in the colon.

Other investigators (Knudsen and Hessov 1995; Rumessen et al. 1990) have reported similar results on absorption and digestion of inulin. Rumessen et al. (1990) observed increased breath H_2 in subjects supplemented with 5, 10 or 20 g/d fructans purified from Jerusalem artichokes. Traces of fructans were recovered in urine of only one subject receiving a 20-g dose. Knudsen and Hessov (1995) also demonstrated that inulin was not digested in the small intestine. Seven ileostomy subjects were fed 10 or 30 g/d inulin purified from Jerusalem artichokes. The recovery of inulin in the ileal effluent

was about 87% for both levels of dosing. The concentration of acetic acid was higher in the ileal effluent followed by propionic and butyric acid. The loss of inulin during the passage through the small intestine may be due to the hydrolysis by either the acid or enzymes, or microbial degradation in the distal intestine.

4. Conclusions

Both *in vitro* and animal studies corroborate the findings in humans that FOS is virtually indigestible in the upper GI tract, although some acid hydrolysis into absorbable fructose and glucose can occur. Approximately 0.12% of ingested FOS is absorbed unchanged and recovered in the urine. In humans, approximately 89% of ingested FOS is delivered to the colon where it is completely fermented by the microflora. None is recovered in the feces. The fermentation of FOS generates H_2 and SCFA. The SCFA is predominantly acetic acid, while propionic and butyric acids are generated in smaller amounts.

TABLE 21

Human Studies Showing Non-digestibility and Subsequent Fermentation of FOS

Study Design	Effect	Reference
In a multiple crossover trial, 24 healthy young men were supplemented with 0, 5 or 15 g of FOS for 7 days (for each dose) with two 7-day washout periods. The control group received glucose. Breath H_2 excretion was measured. Fecal and urine samples were collected to measure the concentrations of FOS.	Breath H_2 excretion increased with 5 and 15 g of FOS, but the increase was not significant at the 5 g dose. FOS was not recovered in the urine or in fecal samples. The concentrations of SCFA in feces did not increase with FOS supplementation. No changes in the fecal water pH or fecal weight was observed with FOS supplementation. The results suggest that FOS was not absorbed by the small intestine and it was metabolized completely in the large intestine.	Alles et al. (1996)
Six healthy individuals were supplemented with 6.1 g/d of FOS for 7 days and 6.1 g/d of radiolabeled FOS on day 8. The $^{14}CO_2$ expiration in breath was measured.	Forty-nine and 55% of $^{14}CO_2$ was detected in the breath after 24 and 48 hours, respectively. About 10% of radioactivity was recovered in feces and only 1.9% was detected in urine.	Hosoya et al. (1988)
In a crossover trial, six subjects (3 men and 3 women) were supplemented, after an equilibrium phase, for 11 days with placebo or 20.1 g /d of FOS. Output of FOS and its constituent in the distal ileum were determined using intestinal aspiration after a single meal. Fecal and urine samples were analyzed to determine the amount of FOS.	In the ileal samples, the percentage of constituent oligosaccharides were similar to the ingested FOS, and fructose was not detected. Eighty-nine percent of FOS was recovered in the ileum and FOS appeared in the ileum within 0.5–1 hour after ingestion. FOS recovery in urine after 24 hours was 0.12% of the ingested amount. Approximately 1% of the FOS that disappeared from the small intestine was recovered in the urine; the remaining portion of the FOS which disappeared from the small intestine was hydrolyzed during the passage through the gastrointestinal tract and absorbed as glucose or fructose. FOS was not recovered from the feces. The results suggest that FOS was partly digested in the upper gastrointestinal tract and then fermented in the colon.	Molis et al. (1996)
In a blinded study, 10 subjects were supplemented with 10 g/d FOS for 12 days. Five subjects received 5 g/d sucrose for 12 days. Breath H_2 excretion after supplementation with FOS was compared to H_2 excretion after ingestion of lactulose.	Breath H_2 excretion increased with FOS supplementation and was 50% higher after 12 days of supplementation. The breath H_2 excretion after FOS supplementation was similar to H_2 excretion after lactulose ingestion suggesting no absorption of FOS by the small intestine.	Stone-Dorshow and Levitt (1987)

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B. Physiological Effects of FOS in the Gastrointestinal Tract

1. Fiber-Like Properties

Dietary fibers can be classified as soluble-fermentable (e.g., pectins, gums, inulin) and insoluble-unfermentable (e.g., lignin, cellulose) (FASEB 1987). The physiological properties of dietary fibers are attributed to their undigestibility and physical properties such as viscosity (Asp 1995). Dietary soluble fibers are a class of carbohydrates that are resistant to digestion by the human digestive enzymes, but are hydrolyzed by the gut microflora.

The physiological effects of soluble-fermentable fiber include: a) resistance to hydrolysis by digestive enzymes; b) fermentation by the gut microflora; c) shortening of gastrointestinal transit time; d) increased fecal weight; e) reduced fecal pH; f) predictable reduction in caloric value; g) reduced plasma cholesterol; and h) delay or reduction in glucose absorption. Insoluble dietary fiber may increase fecal weight and shorten gastrointestinal transit time, but is ineffective in lowering cholesterol or blood glucose (FASEB 1987).

Investigators have looked at these physiologic effects with respect to FOS, as well as inulin and oligofructose, and results of their work support the view that the β 2-1 fructans produce a soluble fiber-like effect. The experimental evidence to corroborate this view is presented in Table 22.

TABLE 22 Soluble Dietary Fiber-Like Properties of FOS (and Inulin and Oligofructose)	
Predictable Effect	**Citations**
Resistance to digestion by endogenous enzymes	Roberfroid (1993); Oku et al. (1984); Tsuji et al. (1986); Ziesenitz and Siebert (1987); Nilsson and Bjorck (1988); Nilsson et al. (1988); Graham and Aman (1986); Bjorck and Nilsson (1991); Andrieux et al. (1991); Tokunaga et al. (1986; 1989)
Fermentation by colonic microflora to produce SCFA	Roberfroid (1993); McKellar and Modler (1989); Yazawa et al. (1978); Wang and Gibson (1993); Gibson and Wang (1994a,b); Yamazaki and Dilawri (1990); Gibson et al. (1994); Hidaka et al. (1986); Williams et al. (1994); Hidaka et al. (1990a,b); Macfarlane and Gibson (1994); Mitsuoka et al. (1987); Rumessen et al. (1990); Stone-Dorshow and Levitt (1987); Tokunaga et al. (1986, 1989); Gibson et al. (1995); Rowland et al. (1998); Fontaine et al. (1996); Garleb et al. (1996); Kawaguchi et al. (1993)
Shortened gastrointestinal transit time	Roberfroid (1993); Tokunaga et al. (1986); Rumessen et al. (1990)
Increased fecal weight	Roberfroid (1993); Tokunaga et al. (1986); Oku et al. (1984)
Reduction of fecal pH	Roberfroid (1993); Modler et al. (1990); Mitsuoka et al. (1987); Ohta et al. (1994, 1995); Morisse et al. (1993); Berggren et al. (1993); Fontaine et al. (1996)
Predictable reduction in caloric value	Oku (1991); Hosoya et al. (1988); Ranhotra et al. (1993); Roberfroid et al. (1993); Drevon and Bornet (1992)
Reduction of plasma cholesterol and triglycerides	Roberfroid (1993); Tokunaga et al. (1986); Tomomatsu (1994); Bhattathiry (1971); Yamashita et al. (1984); Hidaka et al. (1986, 1990a,b, 1991); Delzenne et al. (1993); Vanhoof and DeSchrijver (1995)
Reduction in glucose absorption	Roberfroid (1993); Oku et al. (1984); Rumessen et al. (1990); Yamashita et al. (1984); Luo et al. (1996)

2. Effect on Mineral Absorption

Nondigestible carbohydrates (dietary fiber) have been thought to impair mineral absorption in the small intestine because of their binding/sequestering effects. The proposed mechanisms for this effect are: a) decreased mineral solubility due to ionic or coordination/chelation interactions with fiber; b) increased luminal viscosities which reduce the rate of migration of nutrients from bulk phase of the lumen to the mucosal surface; c) reduced transit time through the gut, reducing the timespan available for nutrient absorption; d) entrapment of minerals in the fiber matrix; and e) possible changes in morphology of the gut surface (as cited in Ink 1988; Roberfroid and Delzenne 1998). Experimental evidence has shown, however, that dietary fiber *per se* does not affect mineral absorption or mineral balance.

Many studies (Ohta et al. 1993, 1994a,b, 1995a,b, 1996, 1997, 1998; Baba et al. 1996; Delzenne et al. 1995) have investigated the effects of FOS on mineral absorption and balance in animals (Table 23). Results from these studies indicate that FOS does not produce a detrimental effect on mineral absorption. Indeed, β 2-1 fructans have been shown to have a positive impact on calcium and magnesium absorption and balance.

Investigators have reported that FOS ingestion decreases fecal excretion, increases apparent absorption, increases retention and increases urinary Ca excretion (Ohta et al. 1993, 1994a, 1995a,b, 1996,1997, 1998; Delzenne et al. 1995). Similarly, studies have demonstrated increased absorption, increased or no change in retention, increased urinary excretion and decreased fecal excretion of Mg with FOS ingestion (Ohta et al. 1993, 1994a,b, 1995a,b, 1996, 1997, 1998; Baba et al. 1996; Delzenne et al. 1995). Ohta et al. (1993, 1998) showed that the effect of FOS on apparent absorption of Ca and Mg was dose dependent.

Similar effects on mineral absorption and balance have been observed with inulin and oligosaccharides in animals. Delzenne et al. (1995) reported that fecal excretion of Fe, Zn, and Cu decreased and the apparent absorption of Fe and Zn increased in rats fed 10% oligofructose in the diet compared to rats fed a control diet. Ohta et al. (1995b) reported a non-significant increase in apparent absorption of Fe, an increase in hematocrit ratio, an increase in hemoglobin concentration, and an increase in hemoglobin regeneration efficiency in anemic rats fed an FOS diet compared to rats fed a control diet. Serum iron, total iron binding capacity (TIBC), and unsaturated iron binding capacity (UIBC) were not affected by FOS in the diet. Several investigators (Delzenne et al. 1995; Levrat et al. 1991; Remesy et al. 1993; Brommage et al. 1993) have reported an increase in Ca and Mg absorption with inulin or oligofructose ingestion

in rats. An increase in absorption and a decrease in fecal excretion of Fe, Zn and Cu in rats fed inulin was reported by Delzenne et al. (1995).

Three crossover trials investigated the effects of inulin and oligofructose on mineral absorption and balance in humans (Coudray et al. 1997; van den Heuvel et al. 1998; Ellegard et al. 1997). In a crossover study by Coudray et al. (1997), nine male students consuming 40 g/d of inulin for 28 days showed an increase in apparent Ca absorption and balance compared to controls. No effect of inulin on absorption, urinary excretion and balance of Mg, Fe and Zn was observed in these individuals compared to controls. In a study by van den Heuvel et al. (1998), no effect of inulin (15 g/d), oligofructose (15 g/d) or galactooligosaccharide consumption on Ca and Fe absorption was observed in 12 young healthy men compared to controls. Ellegard et al. (1997) reported no effect of inulin (17 g/d) or oligofructose (17 g/d) on excretion of Ca, Mg, Zn and Fe in 10 subjects with conventional ileostomy.

Several mechanisms that have been proposed to explain the positive effect of FOS and other β 2-1 fructans on mineral absorption are: 1) osmotic effect; 2) lowering of colonic content pH due to fermentation and production of SCFA; 3) hypertrophy of the colon wall, thereby increasing the surface area for absorption; and 4) formation of salts with these SCFA (Roberfroid and Delzenne 1998). FOS and other β 2-1 fructans have been shown to produce osmotic effects, reduce colonic pH and produce hypertrophy of the colon wall.

A decrease in colonic content pH was associated with an increase in mineral absorption in rats fed inulin, oligofructose or FOS diets compared to control rats (Ohta et al. 1994a, 1995b, 1996, 1997; Baba et al. 1996; Levrat et al. 1991; Remesy et al. 1993). The acidification of the colonic contents increases the percentage of soluble Ca and Mg to insoluble Ca and Mg, thereby increasing the absorption of Ca and Mg (Remesy et al. 1993; Baba et al. 1996; Ohta et al. 1995b).

Mineral absorption and balance may improve due to the osmotic effect of nondigestible polysaccharides that transfer water into the large bowel, thus allowing the minerals to become more soluble (Roberfroid and Delzenne 1998). This is demonstrated by animal studies that have reported either an increase in the ratio of cecal contents in the liquid phase in rats fed an FOS diet (Baba et al. 1996), an increase in the wet weight of the cecal contents in rats consuming FOS (Ohta et al 1995a,b, 1997), or an increase in the cecal water content in rats consuming FOS (Levrat et al. 1991).

Consumption of FOS, inulin or oligofructose has been shown to produce proliferation of the cecal tissue, an increase in cecal weight, and an increase in blood flow to the cecum (Levrat et al. 1991; Remesy et al. 1993; Delzenne et al. 1995). This could potentially result in a greater surface area for mineral absorption.

In conclusion, data from animal and human studies show that FOS and other β 2-1 fructans do not negatively affect mineral absorption or balance. On the contrary, FOS enhances the absorption and retention of Ca and Mg and improves Ca balance. Studies also demonstrate that FOS, inulin and oligofructose produce either an increase or no change in the absorption of Fe, Zn and Cu and have no negative effect on the balance of these minerals.

TABLE 23 Effect of FOS on Mineral Absorption		
Study Design	**Effect**	**Reference**
The purpose of the study was to examine the role of the large intestine in the absorption of Ca and Mg using cecectomized rats in a mineral balance study. Sham-operated and cecectomized male Sprague-Dawley rats were fed a diet containing sucrose at 100 g/kg of diet or 50 g/kg sucrose + 50 g/kg FOS for 28 days (4 groups). Urine and feces samples were collected to determine apparent absorption and retention of minerals (balance study). The samples were collected for 5 days at 4 time points each (days 4-8, 10-14, 17-21 and 24-28).	The fecal excretion of Mg in both sham-operated (FSO diet 0.91 ± 0.29 mg/d; FOS free diet 2.96 ± 0.34 mg/d) and cecectomized rats (FOS diet 2.45 ± 0.24 mg/d; FOS free diet 3.86 ± 0.71 mg/d) decreased significantly ($p<0.05$). Apparent Mg absorption ratio increased significantly ($p<0.05$) in rats fed FOS diet In sham-operated rats, the fecal excretion of Ca was lower in rats fed an FOS diet (19.1 ± 4.2 mg/d) compared to rats fed a sucrose diet (35.5 ± 5.1 mg/d). The apparent Ca absorption ratio increased significantly ($p<0.05$) during all but the final 5-day period in sham-operated rats fed an FOS diet, but not in cecectomized rats fed an FOS diet. Fecal dry weight was 30% heavier in cecectomized rats fed an FOS diet compared to the rats on a sucrose diet. In sham-operated rats fed an FOS diet, the pH of the fecal contents was lower (5.36, $p<0.05$) than in rats fed a sucrose diet (6.47). In both sham-operated (5.7) and in cecectomized rats (5.43) fed an FOS diet, the pH of colon contents was lower ($p<0.05$) compared to the rats fed a sucrose diet (sham-operated 6.89, cecectomized 6.56).	Ohta et al. (1994a)

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TABLE 23 Effect of FOS on Mineral Absorption		
Study Design	**Effect**	**Reference**
The purpose of the study was to investigate *in vivo* the effects of an FOS diet on the net absorption of Mg from the hindgut by a cecal cannulation method. Male Sprague-Dawley rats were fed a pelleted diet for one week and then operated for cecum cannulation. The animals were divided into 2 groups where one group received Mg in the diet and the other group received Mg via cecum infusion. Both groups were subdivided (4 groups, 7 rats per group) where one subgroup in each group received FOS-containing diet and the other received FOS-free diet for 15 days. Fecal and urine samples were collected from days 3-7 and 11-15 for Mg balance study.	The apparent Mg absorption diet (amount administered – fecal excretion) increased significantly in rats receiving Mg orally or via cecum infusion and fed FOS diet. Mean (± SEM) Mg absorption (μmol/d) values on days 3-7 were 81.8 ± 10 (oral Mg administration without FOS), 126 ± 11 (oral Mg administration + FOS), 70.3 ± 7.4 (cecum Mg infusion without FOS) and 125 ± 11 (cecum Mg infusion + FOS). The increase in Mg absorption in the cecal and oral groups was similar. On days 11-15, the mean Mg absorption values were 97.9 ± 12.7, 133 ± 22, 93.8 ± 9.6 and 130 ± 10 mol/d respectively, for the above-mentioned groups. The mean (± SEM) Mg retention (amount administered – fecal excretion - urinary excretion) increased significantly in rats receiving Mg through diet or cecum infusion and fed FOS diet. Mean (± SEM) Mg retention (μmol/d) values on days 3-7 were 65.7 ± 8.0 (oral Mg administration without FOS), 96.8 ± 11.9 (oral Mg administration + FOS), 61.4 ± 10.1 (cecum Mg infusion without FOS) and 103 ± 11 (cecum Mg infusion + FOS). On days 11-15, the mean Mg retention values were 85.1 ± 12.5, 102 ± 15, 84.4 ± 6.9 and 97.3 ± 7.4 respectively, for the above-mentioned groups. The pH of the cecal contents was significantly lower and the cecal weight was significantly higher in rats fed an FOS-containing diet compared to rats fed an FOS free-diet. The distribution of Mg in the liquid phase of the cecal contents and the proportion of soluble Mg in the cecum was significantly higher in rats fed an FOS-containing diet compared to those fed an FOS-free diet.	Baba et al. (1996)
The purpose of the study was to assess the apparent retention of energy, nitrogen, and Ca, Mg, Fe, Zn and Cu in rats supplemented with fructooligosaccharides with high and low DP. 30 male Wistar rats were equally divided into 3 groups and fed a diet containing sucrose (control), 10% Oligofructose (DP=4.8), or 10% Inulin (DP=10) for 24 days. On day 18 of the study, animals were placed in metabolic cages for 8 days and feces and urine samples were collected.	The fecal excretion of Ca, Mg, Fe, Zn and Cu was decreased in rats fed diets with Oligofructose or Inulin compared to rats fed sucrose. Dietary intake of Ca, Mg, Fe, Zn and Cu decreased in rats fed Oligofructose, but only Fe and Cu intake decreased in rats fed Inulin. The apparent absorption of Ca, Mg, Fe and Zn increased in rats fed Oligofructose or Inulin diets. The apparent retention of Mg was doubled and the retention of other minerals was modestly increased in rats fed Oligofructose or Inulin diets. Fecal weight was higher in rats fed Oligofructose or Inulin diets compared to rats fed sucrose. Cecal tissue weight was increased in rats fed Oligofructose or Inulin diets compared to rats fed sucrose.	Delzenne et al. (1995)

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TABLE 23 Effect of FOS on Mineral Absorption		
Study Design	**Effect**	**Reference**
The purpose of the study was to determine the contribution of the large intestine in the absorption of Ca and Mg from their low solubility salts, and to determine the effects of FOS on Ca and Mg absorption in the large intestine *in vivo*. Twenty-eight male Sprague-Dawley rats were fed a stock diet for one week and then implanted with a tube in the cecum. The rats were divided into four groups (7 rats per group), two groups were fed an FOS-containing diet and two groups were fed an FOS-free diet, and all groups were fed a Ca- and Mg-free diet. Half of the rats in each group were administered $CaCO_3$ and MgO via cecal infusion and the other half were administered the same suspension via stomach intubation for 10 days. Fecal and urine samples were collected for the last seven days for the balance study. Coprophagy was prevented by using wire-mesh anal cups.	The apparent Ca and Mg absorption increased in rats fed Ca via cecal and stomach infusion and fed FOS. The mean (± SD) apparent absorption values (mmol/d) for Ca were 639 ± 99 (stomach infusion without FOS), 872 ± 163 (stomach infusion with FOS), 558 ± 84 (cecum infusion without FOS) and 637 ± 131 (cecum infusion with FOS). The mean absorption values for Mg were 161 ± 15, 209 ± 18, 155 ± 9 and 199 ± 9, respectively, for the above-mentioned groups. The apparent absorption of P decreased with FOS diet (stomach infusion without FOS = 2.31 ± 0.21; (stomach infusion with FOS = 2.06 ± 0.30; cecum infusion without FOS = 2.66 ± 0.12 and cecum infusion with FOS = 2.35 ± 0.28). The mean (± SD) Ca retention values (μmol/d) were 634 ± 99 (stomach infusion without FOS), 865 ± 159 (stomach infusion with FOS), 552 ± 84 (cecum infusion without FOS) and 630 ± 131 (cecum infusion with FOS). The Ca retention increased with FOS, but did not differ significantly from Mg. FOS decreased the pH of the cecal contents.	Ohta et al. (1997)

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TABLE 23 Effect of FOS on Mineral Absorption		
Study Design	**Effect**	**Reference**
The purpose of the study was to investigate the effects of FOS on the apparent absorption of Mg and on the incidence of skin inflammation. Male Sprague-Dawley rats were fed constant concentrations of Ca and P plus two concentrations of Mg and three concentrations of FOS (0, 1 or 5%, w/w) for 25 days. Feces and urine samples were collected on days 2-6, 7-11 and 21-25 for balance study.	FOS diet decreased fecal excretion of Mg significantly ($p<0.05$) and increased absorption and retention of Mg. Mean fecal excretion values (mg/d) for Mg were 2.72 ± 0.36 (Ca + P + low Mg), 5.41 ± 0.43 (Ca + P + high Mg), 1.98 ± 0.34 (Ca + P + Mg + FOS 1%), 1.81 ± 0.32 (Ca + P + Mg + FOS 5%).	Ohta et al. (1994b)
The purpose of the study was to investigate the effects of FOS on absorption of Ca, Mg and water from the colon and rectum of rats fed diets with and without FOS. Twenty-eight male Sprague-Dawley rats were fed a pelleted diet for one week. The rats were divided into two groups (14 per group) and fed either an FOS diet of 50 g/kg (FOS group) or a sucrose diet (control group) for 8 days.	The apparent absorption of Ca and Mg and the absolute amount of Ca and Mg absorbed was higher in rats fed an FOS diet compared to rats fed a sucrose diet. Mean (± SD) apparent Ca absorption (μmol/d) in rats fed a control diet was 924 ± 142 and 1185 ± 157 in rats fed an FOS diet. Mean (± SD) apparent Mg absorption (μmol/d) in rats fed a control diet was 197 ± 32 and 278 ± 24 in rats fed an FOS diet. The wet weight of cecal contents was three times higher in rats in the FOS group (6.13 ± 2.71 g) compared to the rats in the control group (1.78 ± 0.38 g). Concentrations of both Ca and Mg were lower in the cecal content and feces of rats in the FOS group compared to the rats in the control group. The amount of water in the cecal contents was not significantly different between the two groups (control group = 70.5 ± 1.3 g/100g; FOS group = 73 ± 5.8 g/100g).	Ohta et al. (1995a)

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TABLE 23 Effect of FOS on Mineral Absorption		
Study Design	**Effect**	**Reference**
The purpose of the study was to investigate the effects of FOS on absorption of Fe, Ca and Mg and on biochemical parameters in anemic rats. Twenty-eight male Sprague-Dawley rats were fed an Fe-deficient diet for three weeks. The rats were divided into four groups and were fed the following experimental diets for 2 weeks: 1) 15 mg/kg Fe (group 1); 2) 15 mg/kg Fe + 50 g/kg FOS (group 2); 3) 30 mg/kg Fe (group 3); and 4) 30 mg/kg Fe + 50 g/kg FOS (group 4). Feces samples were collected on days 3-6 and 10-13.	Apparent Fe absorption increased, but not significantly, with FOS diet. The apparent absorption of Ca ($p<0.05$) and Mg ($p<0.001$) increased significantly in rats fed an FOS diet during the two time periods, compared to rats fed diets without FOS. Mean (± SEM) Ca apparent absorption (mg/d) values on days 3-6 were 64.6 ± 1.7 (group 1), 67.0 ± 1.7 (group 2), 62.7 ± 2.5 (group 3) and 68.8 ± 1.3 (group 4) and on days 10-13 were 64.8 ± 5.3 (group 1), 65.0 ± 3.4 (group 2), 65.3 ± 2.1 (group 3) and 79.5 ± 3.8 (group 4). Mean (± SEM) Mg apparent absorption (mg/d) values on days 3-6 were 4.80 ± 0.19 (group 1), 5.62 ± 0.13 (group 2), 4.87 ± 0.25 (group 3) and 6.64 ± 0.13 (group 4) and on days 10-13 were 4.11 ± 0.30 (group 1), 6.21 ± 0.31 (group 2), 4.84 ± 0.33 (Group 3) and 7.28 ± 0.40 (group 4). The weight of the cecal contents increased and the pH decreased in rats with an FOS diet. The distribution of Fe, Ca and Mg increased in the liquid phase and decreased in the solid phase of the cecal contents with FOS feeding. FOS increased the hematocrit ratio on days 3 and 10, increased the hemoglobin concentration on days 3, 10 and 14 and increased hemoglobin regeneration efficiency. FOS did not affect serum iron, UIBC and TIBC.	Ohta et al. (1995b)

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TABLE 23 Effect of FOS on Mineral Absorption		
Study Design	**Effect**	**Reference**
The purpose of the study was to determine if the absorption of Ca and Mg and the stimulatory effects of FOS on absorption of Ca and Mg were altered by prevention of coprophagy (re-ingestion of feces) in rats. Male Sprague-Dawley rats (7-11 rats per group) were divided into two groups and received either 100 g/kg of sucrose diet (control diet) or 50 g/kg of sucrose + 50 g/kg of FOS diet. Each group was subdivided into two groups and the four groups were given the following diets: 1) control diet, without coprophagy; 2) control diet, with coprophagy; 3) FOS diet, without coprophagy; and 4) FOS diet, with coprophagy, for 15 days. Feces and urine samples were collected on days 3-7 and 10-14 for balance study.	The absorption of Ca and Mg increased in rats fed an FOS diet with or without coprophagy compared to the control rats. The apparent absorptive ratio of Ca (days 3-7 $p<0.001$; days 10-14 $p=0.008$) and Mg (days 3-7 and 10-14 $p<0.001$) were significantly increased with FOS diet regardless of coprophagy prevention during both the time periods. The mean (± SE) apparent absorptive ratios (%) for Ca for days 3-7 were 44.0 ± 1.6 (control diet, without coprophagy), 45.4 ± 1.3 (control diet, with coprophagy), 66.8 ± 1.8 (FOS diet, without coprophagy) and 60.9 ± 2.9 (FOS diet, with coprophagy). The mean (± SE) apparent absorptive ratios (%) for Ca for days 10-14 were 47.7 ± 2.1 (control diet, without coprophagy), 47.7 ± 2.7 (control diet, with coprophagy), 59.6 ± 2.8 (FOS diet, without coprophagy) and 52.9 ± 4.2 (FOS diet, with coprophagy). The Ca retention ratio was higher in rats fed FOS diets compared to the control rats, and the ratio tended to be higher in rats with coprophagy compared to the rats without coprophagy. The mean (± SE) apparent absorptive ratios (%) for Mg for days 3-7 were 55.9 ± 1.9 (control diet, without coprophagy), 58.8 ± 2.3 (control diet, with coprophagy), 87.9 ± 0.5 (FOS diet, without coprophagy) and 76.3 ± 2.3 (FOS diet, with coprophagy). The mean (± SE) apparent absorptive ratios (%) for Mg for days 10-14 were 52.0 ± 2.2 (control diet, without coprophagy), 52.0 ± 3.7 (control diet, with coprophagy), 83.6 ± 1.4 (FOS diet, without coprophagy) and 68.1 ± 3.6 (FOS diet, with coprophagy). The Mg retention ratio was higher in rats fed FOS diets compared to the control rats. The cecal content pH was significantly reduced ($p<0.001$) with FOS diet in rats both with or without coprophagy, compared to the control rats with and without coprophagy. The mean (± SE) cecal pH values were 7.49 ± 0.15 (control diet, without coprophagy), 7.27 ± 0.19 (control diet, with coprophagy), 6.0 ± 0.15 (FOS diet, without coprophagy) and 5.88 ± 0.21 (FOS diet, with coprophagy).	Ohta et al. (1996)

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TABLE 23

Effect of FOS on Mineral Absorption

Study Design	Effect	Reference
The purpose of the study was to determine if there were differences among FOS with respect to their effects on Ca, Mg and nitrogen balance. Forty-nine male Sprague-Dawley (7 rats per group, 7 groups) were provided with the following experimental diets for 4 weeks: 1) control diet containing sucrose; 2) FOS 5%; 3) FOS 10%; 4) GF_2 5%; 5) GF_2 10%; 6) GF_3 5%; and 7) GF_3 10%. Feces and urine samples were collected on days 10-14 and 24-28 for mineral and nitrogen balance studies.	Fructooligosaccharides (10%) significantly decreased fecal excretion and significantly increased urinary excretion of Ca during both the time periods (days 10-14 and days 24-28). The effect of fructo-oligosaccharides was dose-dependent and the effect was similar regardless of the type of fructo-oligosaccharides studied. The apparent absorption ratio for Ca was higher with 10% fructo-oligosaccharides diets compared to the control diet. The mean (± SD) fecal Ca excretion (mmol/d) values for days 10-14 were 0.7 ± 0.2 (control diet), 0.5 ± 0.1 (FOS 5%), 0.2 ± 0.1 (FOS 10%), 0.5 ± 0.2 (GF_2 5%), 0.2 ± 0.1 (GF_2 10%), 0.6 ± 0.3 (GF_3 5%) and 0.3 ± 0.2 (GF_3 10%), and for days 24-28 were 1.0 ± 0.2, 0.9 ± 0.1, 0.7 ± 0.2, 1.0 ± 0.2, 0.6 ± 0.2, 0.9 ± 0.2 and 0.7 ± 0.2 for the above-mentioned diets, respectively. The mean (± SD) urinary Ca excretion (mmol/d) values for days 10-14 were 13.3 ± 2.0, 51.1 ± 25.2, 98.8 ± 31.8, 67.7 ± 21.1, 117.4 ± 37.3, 48.9 ± 19.5 and 128.6 ± 43.8, and for days 24-28 were 15.4 ± 3.5, 33.6 ± 19.1, 112.5 ± 4.9, 516 ± 12.7, 113.4 ± 34.8, 67.4 ± 31.5 and 151.8 ± 58.1 for the above-mentioned diets, respectively. The mean (± SD) apparent absorption of Ca (%) for days 10-14 was 73.1 ± 4.4, 79.4 ± 4.8, 90.6 ± 2.8, 81.1 ± 5.8, 90.6 ± 3.6, 78.9 ± 8.0 and 88.3 ± 7.3, and for days 24-28 were 66.9 ± 3.0, 66.8 ± 4.8, 76.2 ± 4.4, 68.4 ± 3.4, 78.1 ± 5.0, 71.2 ± 4.5 and 74.3 ± 7.3 for the above mentioned diets, respectively. Fructo-oligosaccharides (10%) significantly decreased fecal excretion (both periods) and significantly increased urinary excretion (first period) of Mg. The effect of fructo-oligosaccharides was dose-dependent and the effect was similar regardless of the type of fructo-oligosaccharide studied. The apparent absorption ratio for Mg was higher with 10% fructo-oligosaccharides diets compared to the control diet. The mean (± SD) fecal Mg excretion (mol/d) values for days 10-14 were 66.1 ± 25.5, 49.8 ± 14.0, 29.3 ± 5.9, 44.3 ± 14.7, 28.3 ± 7.5, 54.5 ± 21.0 and 35.4 ± 9.9 and for days 24-28 were 110.3 ± 24.7, 75.7 ± 17.8, 51.0 ± 13.5, 83.0 ± 20.0, 47.9 ± 10.4, 67.0 ± 10.1 and 53.6 ± 14.9 for the above-mentioned diets, respectively. The mean (± SD) urinary Mg excretion (μmol/d) values for days 10-14 were 134.3 ± 16.7, 166.5 ± 20.7, 168.0 ± 15.2, 151.1 ± 21.0, 162.7 ± 18.6, 155.8 ± 17.1 and 170.5 ± 19.7, and for days 24-28 were 157.7 ± 14.9, 189.2 ± 27.2, 217.3 ± 24.2, 209.5 ± 38.1, 221.1 ± 19.4, 142.4 ± 15.8 and 161.8 ± 39.8 for the above-mentioned diets, respectively. The mean (± SD) apparent absorption of Mg (%) for days 10-14 was 80.0 ± 7.0, 84.7 ± 4.1, 90.7 ± 1.9, 86.0 ± 4.3, 90.7 ± 2.6, 83.4 ± 5.4 and 89.3 ± 2.7 and for days 24-28 were 70.0 ± 5.2, 78.8 ± 5.1, 85.9 ± 3.1, 78.2 ± 5.1, 86.7 ± 2.2, 82.0 ± 2.4 and 85.0 ± 4.0 for the above-mentioned diets, respectively.	Ohta et al. (1998)

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TABLE 23 Effect of FOS on Mineral Absorption		
Study Design	**Effect**	**Reference**
The purpose of the study was to investigate the effects of lactose, FOS and oligosaccharides on absorption of Ca, Mg and P in rats. Experiment 1: Male Sprague-Dawley rats (7 rats per group) were administered either a control diet, lactose (LA) 5%, LA 15%, FOS 1%, FOS 3%, FOS 5% or FOS 15% diet for 10 days. Feces samples were collected from days 7-10 for the mineral balance study. Experiment 2: Male Sprague-Dawley rats (7 rats per group) were administered either a control diet or FOS 5% diet for 31 days. Feces samples were collected from days 3-7, 15-19 and 27-31 for the mineral balance study. Experiment 3: Male Sprague-Dawley rats (7 rats per group) were administered either a control diet, isomaltooligosaccharide 5%, galactooligisaccharide 5%, raffinose 5%, or FOS 5% diet for 10 days. Feces samples were collected from days 7-10 for the mineral balance study.	Experiment 1: The absorption of Ca, Mg and P was significantly higher ($p<0.05$) in rats fed FOS in a dose-dependent manner compared to the rats fed the control diet. The mean (± SD) absorption values expressed as percent for Ca were 56.5 ± 3.0 (control diet), 60.4 ± 3.7 (LA 5 %), 64.2 ± 3.4 (LA 15 %), 61.0 ± 5.4 (FOS 1 %), 61.1 ± 2.8 (FOS 3 %), 65.2 ± 3.4 (FOS 5 %) and 82.0 ± 3.4 (FOS 15 %). The mean (± SD) absorption values expressed as percent for Mg were 69.2 ± 1.7, 71.3 ± 2.3, 80.8 ± 3.6, 65.9 ± 1.2, 75.2 ± 3.2, 83.4 ± 4.0 and 88.8 ± 2.0 for the above-mentioned diets, respectively. The mean (± SD) absorption values (expressed as percent) for P were 77.2 ± 1.8, 77.5 ± 2.2, 79.7 ± 3.0, 78.5 ± 2.3, 77.7 ± 1.4, 78.3 ± 1.1 and 87.6 ± 2.7 for the above-mentioned diets, respectively. Experiment 2: The absorption of Ca ($p<0.001$), Mg ($p<0.001$) and P ($p<0.05$) was significantly higher in rats fed FOS compared to the rats fed the control diet during all time periods. The mean (± SD) absorption values (expressed as percent) for Ca for days 3-7 were 58.6 ± 3.9 (control diet) and 75.0 ± 4.8 (FOS 5 %); for days 14-18 were 55.3 ± 3.0 (control diet) and 64.0 ± 4.0 (FOS 5 %); for days 27-31 were 38.3 ± 3.2 (control diet) and 51.4 ± 3.0 (FOS 5 %). The mean (± SD) absorption values (expressed as percent) for Mg for days 3-7 were 61.0 ± 3.2 and 83.1 ± 3.0; for days 14-18 were 55.3 ± 3.7 and 78.3 ± 5.2; for days 27-31 were 49.8 ± 5.3 and 73.3 ± 4.9 for the above-mentioned diets, respectively. The mean (± SD) absorption values (expressed as percent) for P for days 3-7 were 71.1 ± 3.5 and 80.7 ± 3.4; for days 14-18 were 68.3 ± 1.9 and 72.5 ± 3.1; for days 27-31 were 60.6 ± 2.7 and 63.7 ± 1.3 for the above-mentioned diets, respectively. Calcium retention was significantly higher ($p<0.001$) in rats fed an FOS diet compared to the control diet during all time periods. Magnesium retention was not significantly different in rats fed an FOS diet compared to the control diet and P retention was significantly higher ($p<0.001$) only for the first time period (days 3-7), but not for the other time periods in rats fed an FOS diet. The mean (± SD) Ca retention values (expressed as percent) for days 3-7 were 58.2 ± 3.8 (control diet) and 73.9 ± 4.9 (FOS 5 %); for days 14-18 were 54.9 ± 3.0 (control diet) and 62.9 ± 3.9 (FOS 5 %); for days 27-31 were 37.7 ± 3.0 (control diet) and 50.2 ± 3.2 (FOS 5 %). Experiment 3: Isomaltooligosaccharide had no effect, galactooligisaccharide and raffinose had variable effect and FOS increased the absorption of Ca, Mg and P in rats (no data for available from this experiment). FOS reduced the cecal content pH from 6.88 ± 0.14 (control) to 5.24 ± 0.16 (FOS 5%).	Ohta et al. (1993)

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3. Effect on Nitrogen Balance

FOS has been shown to increase the amount of nitrogen excreted in the feces, enhance urea nitrogen transfer into the large intestine, and enhance bacterial utilization of ammonia nitrogen. FOS ingestion, however, has no statistically significant effect on nitrogen balance. The effect of FOS on nitrogen utilization and excretion indicates that there are no safety concerns, and potentially a beneficial effect of FOS consumption due to the reported potential adverse health consequences of excessive resorption of toxic ammonia from the large intestine, as well as the potential neoplastic influence of ammonia on intestinal mucosal cells (Thorton 1981; Bingham 1988; Lupton and Marchand 1989; Bode and Schäfer 1985; Macfarlane and Cummings 1991).

The main product of nitrogen metabolism is ammonia, which enhances cell proliferation, alters DNA synthesis and has been implicated in large bowel carcinogenesis. The potential health consequences are due to the reabsorption of toxic ammonia from the large intestine (as cited in Levrat et al. 1993; Bingham 1988; Lupton and Marchant 1989). Several studies (Delzenne et al. 1995; Younes et al. 1995, 1996; Ohta et al. 1998) have shown an increase in fecal excretion and decrease in urinary excretion of nitrogen in rats fed an FOS diet or a blend of FOS with other fiber (Table 24). Despite these changes in excretion of nitrogen, total nitrogen balance was not affected by these dietary changes (Delzenne et al. 1995; Younes et al. 1996). Similarly, studies of inulin in rats have shown a shift in nitrogen excretion from urine to the feces with no effect on nitrogen balance (Levrat et al. 1993; Goodlad and Mathers 1990; Younes et al. 1997).

TABLE 24 Effect of FOS on Nitrogen Balance		
Study Design	**Effect**	**Reference**
The purpose of the study was to assess the apparent retention of energy, nitrogen, Ca, Mg, Fe, Zn, and Cu in rats supplemented with fructo-oligosaccharides with high and low DP. Thirty male Wistar rats were equally divided into 3 groups and fed a diet containing sucrose (control group), 10% Oligofructose (DP=4.8) or 10% Inulin (DP=10) for 50 days. On day 42 of the study, the animals were placed in metabolic cages for 8 days and feces and urine samples were collected. Plasma urea concentration was also determined.	Fecal nitrogen excretion increased significantly and urinary nitrogen excretion decreased significantly in rats fed Oligofructose or Inulin diets compared to the rats fed a sucrose diet. The mean (± SEM) fecal nitrogen excretion values (in mg/4 days) are 143 ± 11 (control), 197 ± 17 (10 % Oligofructose) and 207 ± 12 (10 % Inulin). The mean (± SEM) urinary nitrogen excretion values (in mg/4 days) are 453 ± 26 (control), 330 ± 11 (10 % Oligofructose) and 306 ± 25 (10 % Inulin). Oligofructose or Inulin did not affect the total nitrogen balance. Serum urea levels (mg/dl) were significantly lower in the Oligofructose (17.0 ± 0.6, $p<0.05$) and Inulin (13.5 ± 0.2, $p<0.01$) groups compared to the control group (20.0 ± 0.1).	Delzenne et al. (1995)
The purpose of the study was to determine if there were differences among FOS with respect to their effects on Ca, Mg and nitrogen balance. Forty-nine male Sprague-Dawley (7 rats per group, 7 groups) were provided with the following experimental diets for 4 weeks: 1) control diet containing sucrose; 2) FOS 5 %; 3) FOS 10 %; 4) GF_2 5 %; 5) GF_2 10 %; 6) GF_3 5 %; and 7) GF_3 10 %. Feces and urine samples were collected on days 10-14 and 24-28 for mineral and nitrogen balance studies.	Fructo-oligosaccharides increased fecal nitrogen excretion during both the time periods. The effect of fructo-oligosaccharides was dose-dependent for the first time period, but not for the second, and the effect was similar regardless of the type of fructo-oligosaccharides studied. The mean (± SD) fecal nitrogen excretion (mmol/d) values for days 10-14 were 1.55 ± 0.25 (control diet), 2.66 ± 0.36 (FOS 5 %), 3.10 ± 0.64 (FOS 10 %), 2.67 ± 0.41 (GF_2 5 %), 2.93 ± 0.40 (GF_2 10 %), 2.83 ± 0.54 (GF_3 5 %), and 3.65 ± 0.59 (GF_3 10 %), and for days 24-28 were 1.97 ± 0.22, 3.59 ± 0.48, 3.59 ± 0.85, 3.92 ± 0.39, 3.65 ± 0.67, 3.69 ± 0.41 and 3.83 ± 0.70 for the above-mentioned diets, respectively. The urinary nitrogen excretion and the nitrogen retention ratios did not differ significantly among the groups.	Ohta et al. (1998)

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TABLE 24 Effect of FOS on Nitrogen Balance		
Study Design	**Effect**	**Reference**
The purpose of the study was to compare the ability of poorly fermentable oat fiber with soluble fermentable fiber (gum arabic and two oligosaccharides) to stimulate the transfer of urea into the cecum and shift urinary nitrogen excretion to the feces. Fifty male Wistar rats were fed either a fiber-free, 7.5 % oat fiber, 7.5 % gum arabic, 7.5 % fructooligosaccharide, or 7.5 % xylooligosaccharide diet for 17 days. After an initial 10-day adaptation period, the animals were kept in metabolic cages for the last 7 days, and feces and urine samples were collected during the last five days. Plasma urea concentration was also determined.	Cecal nitrogen levels increased by over 100% in animals fed FOS compared to the control and oat-fiber-fed animals. The fecal nitrogen excretion increased (10 % in control group vs. 27-29 % in gum arabic or FOS group) and the urinary nitrogen excretion decreased ($p<0.05$) in rats on an FOS diet compared to the rats on the control diet. Plasma urea concentrations decreased significantly (20 %, $p<0.05$) in rats fed anFOS diet compared to the rats fed the control or oat fiber diet. Total cecal weight, cecal wall weight, and cecal blood flow were significantly greater in rats fed an FOS diet compared to the rats fed the control diet. The cecal content pH was significantly lower in rats fed an FOS diet compared to the rats on a control or oat fiber diet. The cecal SCFA pool increased 2.6-fold in rats fed an FOS diet relative to those animals given the control diet.	Younes et al. (1995)

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TABLE 24 Effect of FOS on Nitrogen Balance		
Study Design	**Effect**	**Reference**
The purpose of the study was to determine if balance fiber blend at either 4 or 8% would support useful bacterial fermentations in the cecum even at lower levels of protein intake (8 %) thereby optimizing the digestive disposal of urea. Sixty male Wistar rats were fed either a low protein (8% casein) (control), low protein + low fiber (4%), low protein + high fiber (8%), high protein (14%) casein) (control), high protein + low fiber (4%) or high protein + high fiber (8%) diet for 18 days. Oligosaccharide/fiber blend was used in the experimental diet which contained 41.2% FOS, 26.4% oat fiber, 17.7% soy polysaccharide, 10.3% gum arabic and 4.4% carboxymethyl cellulose. After 10 days of adaptation, the animals were placed in metabolic cages for 8 days. Feces and urine samples were collected during the last 5 days of the study for determination of nitrogen excretion. Plasma urea concentration was also determined.	Total cecal nitrogen levels increased by over 50% with low oligosaccharide/fiber blend diet, and by over 100 % with oligosaccharide/fiber blend diet regardless of the protein level. Fecal nitrogen excretion increased with a high fiber diet in both high and low protein groups. Fecal nitrogen excretion in rats adapted to a compared low protein and high protein diet increased by 50% and 26%, respectively, compared with the rats on a high fiber diet. Urinary nitrogen excretion decreased significantly with increased fecal nitrogen excretion. Nitrogen balance was not influenced by oligosaccharide/fiber blend diet. Plasma urea levels decreased by over 30 % ($p<0.0001$) at both levels of protein supplementation with the oligosaccharide/fiber blend diet. The lowest plasma urea levels were observed in rats fed a low protein/high fiber diet. The oligosaccharide/fiber blend caused cecal enlargement and hypertrophy of the cecal wall, which was roughly proportional to the amount of fiber that was added to the diet. Cecal blood flow increased with oligosaccharide/fiber blend diet. The cecal content pH decreased significantly ($p<0.0001$) and SCFA concentrations increased significantly ($p<0.0001$) with the oligosaccharide/fiber blend diet.	Younes et al. (1996)

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4. Effect on Colonocytes

FOS is not digested by the mammalian gut enzymes, but fermented by the microflora in the colon and has physiologic effects similar to soluble dietary fibers. FOS stimulates the growth of nonpathogenic bifodobacteria, which have been associated with inhibiting tumor formation in the colon, and also suppress the growth of pathogenic organisms such as bacteriodes, clostridia and coliforms in the gut (as cited in Reddy et al. 1997). Fermentation of FOS in the gut produces SCFA, which has been shown to produce a trophic effect on the colonic epithelial cells, as well as reducing pH and ammonia concentrations in the colon. Changes in ammonia concentration may play a role in colonic tumor inhibition (cited in Reddy et al. 1997; Howard et al. 1995a). FOS has also been reported to modulate bacterial enzymes such as β-glucuronidase that can convert procarcinogens to proximate carcinogens. Furthermore, studies in rats have demonstrated that FOS can produce tumor inhibition in animal models as demonstrated by an inhibition of aberrant crypt foci (ACF).

Studies summarizing the trophic effect of FOS supplementation on colonic epithelial cells and the effect of FOS on tumor inhibition are presented in Table 25 (corroborating information from studies of inulin and oligofructose are also presented). The effect of FOS ingestion on tumor inhibition was evaluated in animal studies by measuring the number of ACF and/or by evaluating the crypt multiplicity. Aberrant crypts are considered to be precancerous lesions and the number of aberrant crypts is predictive of eventual tumor formation. Compared to normal crypts, aberrant crypts are characterized as large with a thicker lining of epithelial cells (Gallaher et al. 1996). Crypt multiplicity is assessed by the number of crypts in each focus and categorized as those containing up to three, four or more aberrant crypts/foci (Reddy et al. 1997).

Several studies (Koo and Rao 1991; Reddy et al. 1997; Gallaher et al. 1996) have demonstrated a reduction in the total number of aberrant crypts and/or ACF in animals supplemented with FOS, FOS + bifidobacteria, FOS + lactobacilli, or FOS + bifidobacteria + lactobacilli. Gallaher et al. (1996) conducted four experiments to determine the effect of bifidobacteria (experiment 1, 2 and 3) and lactobacilli, (experiment 4) with or without FOS supplementation on aberrant crypt formation in male rats. In two experiments (1 and 3), dietary FOS supplementation significantly decreased the number aberrant crypts and ACF, but in the other two experiments, FOS supplementation had no effect on the number of aberrant crypts or ACF. The authors attributed inconsistency in the results to the differences in the age of the rats at the time the carcinogen was administered, and also to the variability in the effect of bifidobacteria and lactobacilli on ACF formation in the colon.

In a study by Koo and Rao (1991), the authors demonstrated a decrease in the number of ACF, a decrease in cecal pH, an increase in cecal weight, and an increase in bifidobacteria count in mice fed FOS and bifidobacteria. The authors observed that the crypts were confined more to the distal end of the colon, and the dietary treatment appeared to suppress the process of colon cancer.

Reddy et al. (1997) observed a decrease in the total number of ACF in the distal colon, and in crypt multiplicity in F344 rats fed either an inulin or oligofructose diet. The effect of inulin was more pronounced compared to oligofructose, and this was attributed to the slower fermentation of inulin due to its higher DP. In animals fed the control or experimental diets, there was no significant change in body weight, and no adverse effects on liver, kidney, stomach, intestine or lungs. The authors concluded that inulin or oligofructose treatment inhibits the formation of preneoplastic lesions in the colon.

In a study by Pierre et al. (1997), a significant reduction in tumors, primarily in the distal colon, was observed in *Min* mice receiving 5.83 g FOS/100 g dry matter. Four of 10 animals in this group had no tumors, whereas all animals in the other treatment groups had tumors. The number of small tumors, but not large tumors, was significantly reduced in animals supplemented with FOS. Histopathological examination of the large tumors revealed an equal number of adenomas and adenocarcinomas in animals receiving FOS, but a higher proportion of adenocarcinomas in the other three treatment groups. The FOS diet was not administered until day 42 or 49 when the large tumors may have already formed, hence some large tumors may have escaped the effect of FOS and continued to grow. No effect of FOS was observed on the number of tumors in the small intestine, and the effect was limited to the colon. A significant increase in the number of detectable lymphoid nodules was observed in the small intestine in mice fed an FOS diet, suggesting a role of the immune system in inhibiting tumor formation. The authors concluded that FOS counteracts advanced stages of colon cancer, possibly due to a stimulation of immunity brought about by a modulation of the colonic ecosystem.

A study by Rowland et al. (1998) on dietary supplementation with inulin or inulin + *B. longum* showed a decrease in the number of ACF in rats. Rats were fed control, inulin, *B. longum,* or inulin + *B. longum* diets. Inulin and *B. longum* significantly decreased the number of ACF in the distal colon by 21 and 29%, respectively. The combination of inulin + *B. longum* produced a marked decrease (74%) in the number of ACF. This effect was more apparent in foci with 1-3 aberrant crypts. Inulin or *B. longum* did not affect the number of large ACF, but the total number of ACF was significantly decreased in rats fed inulin + *B. longum* diet. Consumption of diets containing *B. longum*, inulin or both were associated with decreased concentrations of ammonia (a putative tumor promoter) and decreased β-glucuronidase activity in the cecal

contents. Cecal β-glucosidase activity, on the other hand, was increased in rats fed the inulin diet, and this increase was more pronounced in rats fed both the inulin and *B. longum* diet.

In contrast, Rao et al. (1998) reported no effect of inulin on the number of ACF and crypt multiplicity in rats fed inulin, coffee, coffee fiber, pectin or piroxicam. Inulin significantly reduced the number of ACF/cm^2 in the colon. Inulin significantly increased SCFA concentration in the cecal contents. No effect of inulin was observed on β-glucuronidase activity.

The trophic effect of FOS supplementation on colonic epithelial cells was investigated by Howard et al. (1995a). BALB/c mice were fed either a control diet or diets supplemented with FOS, xylooligosaccharide, or gum arabic. Intake of food and water was comparable between the treatment groups. Dietary fiber intake was comparable among the groups supplemented with fiber. The crypt depth in the cecum was greatest with xylooligosaccharide diet, and was significantly higher in rats fed the control diet compared to the rats fed an FOS diet. The proliferation zone in the cecum was smaller with FOS and xylooligosaccharide diets, compared to the control and gum arabic diets. The cell density was reduced in animals fed FOS and gum arabic diets compared to the animals fed control and xylooligosaccharide diets. The labeling index was highest in animals fed a xylooligosaccharide diet. The crypt depth in the distal colon was greatest in animals fed a control diet followed by an FOS, gum arabic and xylooligosaccharide diet. No effect of the treatments on proliferation zone, cell density and labeling index was observed in the distal colon. The concentration of viable bifidobacteria was significantly greater with the FOS diet compared to the other diets. The results suggest that FOS and xylooligosaccharide produce a moderate effect on epithelial cell proliferation, and that this effect is limited to the cecum in mice.

Howard et al. (1995b) also studied the effects of an FOS diet on colonic epithelial cell proliferation in neonatal pigs. Supplementation with FOS increased cecal mucosal cell density, number of labeled cells, proximal colonic mucosal crypt height, leading edge, labeled cells, proliferation zone and labeling index. FOS supplementation significantly increased distal colonic mucosal crypt height, leading edge, labeled cells, proliferation zone, and labeling index. Food intake and body weight gain were not affected by the FOS diet. Viable cell count of bifidobacteria, cecal pH, and SCFA concentration in the cecal digestive contents was not affected by the FOS diet. These results suggest that FOS and xylooligosaccharide stimulate epithelial cell proliferation in the cecum and the distal colon.

In conclusion, FOS produces a favorable micro-environment in the gut by stimulating growth of beneficial bacteria, limiting growth of pathogenic bacteria,

increasing colonic SCFA concentration, decreasing pH and modulating enzyme activity. The outcome of these effects has been shown to inhibit colon cancer in animal models. FOS has also been shown to produce a trophic effect on the colonic epithelial cells.

TABLE 25

Effects of FOS Supplementation on Colonic Epithelial Cells in Animals

Species	Experimental Design	Results	Conclusion	Reference
CF_1 female mice	Female mice were fed either a normal diet or diet containing 5% neosugar during the acclimation period (2 weeks). After the acclimation period the animals were administered sc DMH or EDTA (control group, n=5) weekly for 6 weeks. One week after the last DMH injection, the mice receiving FOS were administered viable bifidobacteria suspension (10^9/mouse) via oral intubation twice a week (DMH treatment + FOS + bifidobacteria, n=20). One DMH treated group did not receive FOS or bifidobacteria (DMH treated group, n=21). After the last DMH injection the animals were sacrificed at 3 time points (day 18, 28 and 38).	Aberrant crypts were not identified in the colons of the control animals. In animals receiving the FOS diet, the mean number of aberrant crypts and foci were lower than controls. At 18 ($p<0.05$) and 38 ($p<0.01$) weeks, the mean number of aberrant crypts were significantly reduced in animals on FOS. At 28 ($p<0.05$) and 38 ($p<0.01$) weeks, the mean number of aberrant foci was significantly reduced in animals receiving FOS. The aberrant foci were confined more to the distal end of the colon in animals fed FOS. At week 38, the mean fecal bifidobacterial count increased significantly ($p<0.05$) from 8.85 ± 0.20 to 9.45 ± 0.19 in animals fed FOS. The logarithmic ratio of fecal anaerobes to aerobes increased significantly ($p<0.05$) from 0.20 ± 0.01 to 0.92 ± 0.34 in animals on FOS. The mean cecal weight increased significantly ($p<0.05$), cecal pH decreased significantly ($p<0.001$), and cecal acetic acid concentration increased (but not significantly) in animals fed FOS.	The authors concluded that, "feeding of bifidobacteria and neosugar (FOS) reduces the number of aberrant crypts in the colon thereby suppressing the process of colonic carcinogenesis".	Koo and Rao (1991)

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TABLE 25

Effects of FOS Supplementation on Colonic Epithelial Cells in Animals

Species	Experimental Design	Results	Conclusion	Reference
Experiment 1: Conventional male BALB/c weanling mice Experiment 2: Male Sprague-Dawley rats	Experiment 1: Fifty-two male BALB/c mice were fed a control diet, FOS diet, xylooligosaccharide diet, or gum arabic diet for 14 days. Experiment 2: Forty-four male Sprague-Dawley rats were fed a control diet, FOS diet, xylooligosaccharide diet, or gum arabic diet for 14 days. The cecum and distal colon tissues were examined for morphological changes of the mucosa.	Experiment 1: Intake of food and water was comparable between the treatment groups. Dietary fiber intake was comparable between the groups supplemented with fiber. The concentration of viable bifidobacteria was significantly ($p<0.05$) greater with the FOS diet compared to the other diets. The concentration of bifidobacteria expressed as a percentage of the total anaerobic flora increased with the FOS diet compared to the other diets, but the concentration of the total anaerobic flora was not altered by the FOS diet. Experiment 2: Intake of food and water was comparable between the treatment groups. Dietary fiber intake was comparable between the groups supplemented with fiber. Crypt depth in the cecum was greatest with the xylooligosaccharide diet and intermediate (between the control and FOS diet) with the gum arabic diet. The crypt depth was significantly higher ($p<0.05$) in rats fed the control diet compared to the rats fed the FOS diet. The proliferation zone was smaller with FOS and xylooligosaccharide diets compared to the control and gum arabic diets ($p<0.01$). The cell density was reduced in animals fed FOS and gum arabic diets compared to the animals fed the control and xylooligosaccharide diets ($p<0.01$). The labeling index was highest ($p<0.05$) in animals fed a xylooligosaccharide diet. The crypt depth in the distal colon was greatest in animals fed the control and FOS diet, intermediate in animals fed the gum arabic diet and smallest in animals fed the xylooligosaccharide diet. In the distal colon, the treatments had no effect on proliferation zone, cell density, or labeling index.	The authors concluded that, "dietary supplementation with FOS can be successfully used to enhance the population of bifidobacteria in the large intestine."	Howard et al. (1995a)

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TABLE 25

Effects of FOS Supplementation on Colonic Epithelial Cells in Animals

Species	Experimental Design	Results	Conclusion	Reference
Male pigs	Experiment 1: Twenty male pigs were randomly assigned to a liquid formula diet (n=10) or liquid formula + FOS (3 g/l) diet (n=10) for 15 days. Cecum and proximal colon content samples were collected to measure pH, SCFA and bifidobacteria count. Experiment 2: Twelve pigs were randomly assigned to a liquid formula diet or liquid formula + FOS (3 g/l) diet for 15 days. On days 1, 3 and 6, fecal samples were collected. Cecal, proximal and distal colonic mucosal tissues were collected for morphological measurements.	Experiment 1: Food intake and body weight gain were not affected by FOS diet. Viable cell count of bifidobacteria and the total anaerobic microbiota in the cecal and proximal colonic digestive contents and the cecal pH were not affected by consumption of FOS. SCFA concentration in the cecal digestive contents was not affected by FOS. Supplementation with FOS increased cecal mucosal cell density ($p<0.01$) and number of labeled cells ($p<0.05$). Supplementation with FOS significantly increased ($p<0.01$) the proximal colonic mucosal crypt height, leading edge, labeled cells, proliferation zone and labeling index. The cell density in the proximal colon tended to increase ($p=0.08$) with FOS supplementation. FOS supplementation significantly increased ($p<0.01$) distal colonic mucosal crypt height, leading edge, labeled cells, proliferation zone and labeling index. The proliferation zone in the distal colon tended to increase ($p<0.06$) with FOS supplementation. Experiment 2: Viable bifidobacteria cell count was similar on days 1 and 3 regardless of the treatments ($p>0.05$), but it increased on day 6 in pigs consuming the FOS diet ($p=0.08$).	The authors concluded that, "dietary consumption of FOS will enhance bifidobacteria populations and prevent colonic epithelial mucosa atrophy in neonates fed an elemental diet."	Howard et al. (1995b)

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TABLE 25

Effects of FOS Supplementation on Colonic Epithelial Cells in Animals

Species	Experimental Design	Results	Conclusion	Reference
C57BL/6J-Min/+ Mice	Forty animals were fed *ad libitum* diets with or without 5.8% dietary fiber for 42 days. The animals were divided into four groups (8 females and 2 males per group) and fed a control diet containing 2% cellulose, a retrograded corn starch diet, a starch-free wheat bran diet, or a short-chain FOS diet. Except for the control diet, the other 3 diets provided 5.8% of the total dietary fiber.	A significant reduction in tumors primarily in the distal colon was observed in animals receiving the FOS diet. Four animals in the FOS group had no tumors. whereas all animals in the other treatment groups had tumors. The number of small tumors (diameter < 1 mm), but not large tumors (diameter > 1mm), was significantly reduced ($p=0.01$) in animals supplemented with FOS. Histopathological examination of the large tumors revealed equal numbers of adenomas and adenocarcinomas in animals receiving FOS, but higher number of adenocarcinomas in the other three treatment groups. The FOS effect was limited to the colon. Regardless of the diets, aberrant crypt foci were extremely rare in the colon, and tumors mainly occurred in the small intestine. There was no significant difference between the treatments in the number of tumors observed in the small intestine. Animal growth was not affected by the dietary treatments and no age- or sex-related effects on gut tumor were observed. In animals fed an FOS diet, a significantly higher number ($p<0.05$) of detachable lymphoid nodules were observed in the small intestine.	The authors concluded that, "short-chain FOS counteracts advanced stages of colon carcinogenesis, possibly via stimulation of antitumoral immunity by modulation of the colonic ecosystem."	Pierre et al. (1997)

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TABLE 25 Effects of FOS Supplementation on Colonic Epithelial Cells in Animals				
Species	**Experimental Design**	**Results**	**Conclusion**	**Reference**
Male F344 rats	At 5 weeks of age, male F344 weanling rats were fed a control, a 10% Raftilose P95 (oligofructose, average DP = 4.5), or a 10% Raftiline HP (inulin, average DP = 25) diet. At 7-8 weeks of age, the rats were administered by sc injection either saline (saline group) or azoxymethane (15 mg/kg) (AMO group), once a week for 2 weeks. The animals in the saline and the AMO group were fed the control or experimental diet from age 5 weeks until termination (age 16 weeks).	The aberrant crypt foci were primarily observed in the distal colon. Aberrant crypt foci formation in the colon was not observed in animals fed saline along with control or experimental diets (inulin or oligofructose). AOM treatment induced, on average, 120 aberrant crypt foci/colon in animals fed the control diet. The total number of aberrant crypt foci was significantly reduced in animals fed the inulin or oligofructose diet compared to the control diet. The suppression of aberrant crypt foci formation was more pronounced in animals receiving inulin ($p<0.0006$) compared to the animals receiving oligofructose ($p<0.02$). In animals fed inulin ($p<0.02$ – 0.0001) or oligofructose ($p<0.04$ – 0.01), the crypt multiplicity in terms of 2 or 3 aberrant crypts/focus were significantly reduced compared to the animals fed the control diet. Body weight was not affected by AOM treatment or by dietary inulin or oligosaccharide treatments. No signs of adverse effects were observed on kidney, stomach, intestine or lungs in animals treated with inulin or oligofructose.	The authors concluded that, "the administration of oligofructose and inulin inhibits the formation of preneoplastic lesions in the colon."	Reddy et al. (1997)

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TABLE 25

Effects of FOS Supplementation on Colonic Epithelial Cells in Animals

Species	Experimental Design	Results	Conclusion	Reference
Male F344 rats	At 5 weeks of age, rats were started on one of the following: a control, 1% coffee fiber, 10% coffee fiber, 10% inulin, 10% pectin, or 200 ppm piroxicam until termination (age 16 weeks) (12 weeks of supplementation). At 7 weeks of age, rats were administered sc AOM or normal saline for 2 weeks at a dose of 15 mg/kg body wt/week. During and after (10 weeks) the sc injections, the animals were fed control or experimental diets.	The size and weight of the cecum increased 2- to 3-fold and the total colonic area increased in animals receiving fiber in their diet. There was no aberrant crypt foci (ACF) formation in animals given saline and fed control or experimental diets. In rats treated with AOM and the fed control diet, animals showed an average of 120 ACF/colon and 35 foci containing multiple aberrant crypts/focus. Piroxicam, coffee fiber, and pectin significantly inhibited the total number of ACF in the colon, as well as crypt multiplicity. The inulin diet had no significant ($p>0.05$) inhibitory effect on total number of ACF in the colon and on crypt multiplicity, but it reduced ($p<0.05$) the number of ACF/cm^2. The coffee and coffee fiber diet significantly decreased cecal β-glucuronidase activity compared to the control diet. The pectin diet had a moderate inhibitory effect, but the inulin diet had no significant effect on β-glucuronidase activity. The SCFAs increased by 3- to 4-fold in the cecal contents of rats fed fiber-containing diets. Rats fed inulin or coffee fiber diets had significantly increased ($p<0.05$-0.001) levels of acetate, propionate and butyrate.	The authors concluded that, "coffee fiber significantly suppressed induction of colonic aberrant crypt foci and crypt multiplicity by possibly modulating cecal bacterial β-glucuronidase activity and SCFA levels."	Rao et al. (1998)

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TABLE 25

Effects of FOS Supplementation on Colonic Epithelial Cells in Animals

Species	Experimental Design	Results	Conclusion	Reference
Male Sprague-Dawley rats	Rats were administered two doses (one dose/week, 12.5 mg/kg body wt) of AOM sc 1 week apart. One week after the second AOM dose the rats were fed one of the following: control diet, inulin diet (5% w/w), *B. longum* diet ($7x10^8$ c.f.u./g diet), or inulin + *B. longum* diet for 12 weeks. All animals were treated with AOM, with no control group.	AOM induced ACF mainly in the distal half of the colon. Inulin and *B. longum* diets significantly decreased the total number of AOM-induced ACF by 21 and 29%, respectively. The decrease was more apparent in the foci with 1-3 aberrant crypts (41% and 26% by inulin and *B. longum* diet, respectively). Inulin or *B. longum* alone had no significant effect on the total number of large ACF. In all treatment groups, the number of large ACF were lower compared to the control, but there were no significant differences between the groups. The inulin + *B. longum* diet had the most potent inhibitory effect on total ACF numbers (74%) and ACF with 1-3 crypts/focus (80%). The inulin + *B. longum* diet significantly reduced the number of large ACF (59%, $p<0.05$). Cecal weight increased significantly (25-32%, $p<0.001$) in rats fed inulin or inulin + *B. longum* compared to the rats fed the control diet. Cecal pH ($p<0.001$) and ammonia content (25-30%, $p<0.01$) decreased significantly in rats fed inulin or inulin + *B. longum*. β-glucuronidase activity decreased significantly in all treatment groups compared to the control group, with the greatest decrease in the group fed inulin + *B. longum* (55%, $p<0.01$). β-glucosidase activity in the cecum increased in all treatment groups with the greatest increase in the group fed inulin + *B. longum* (7-fold higher than the control).	The results suggest that, consumption of *B. longum* or inulin may cause beneficial changes in cecal physiology and reduce preneoplastic lesions in the colon. Combined treatment may be more effective in reducing colonic lesions.	Rowland et al. (1998)

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TABLE 25

Effects of FOS Supplementation on Colonic Epithelial Cells in Animals

Species	Experimental Design	Results	Conclusion	Reference
Male Wistar rats	Preliminary experiment: Rats were fed one of the following: basal diet, basal diet + bifidobacteria (1 ml daily at 10^9/ml), basal diet + 2% FOS, or basal diet + 2% FOS + bifidobacteria. The animals were fed these diets for 4 weeks. Experiments 1-4: In each experiment, 2 doses/week of DMH (15 mg/kg) were administered to the rats via gavage one week apart. One week after the second dose, the dietary treatments were started. Except for FOS, which was added to the feed, all other treatments (skim milk, saline, bifidobacteria (10^8) and lactobacillus) were administered via gavage. The treatments for each experiment are listed below: Experiment 1: Rats were fed either basal diet + skim milk, or basal diet + skim milk w/bifidobacteria + 2% FOS. The animals were sacrificed on day 24 and 25. Experiment 2: Rats were fed one of the following: basal diet + saline, basal diet + skim milk, basal diet + skim milk w/bifidobacteria, basal diet + skim milk w/bifidobacteria + 2% FOS or basal diet + skim milk + 2% FOS. The animals were sacrificed on day 29 through 35.	Preliminary experiment: Bifidobacteria suppressed the *C. perfringens* population. Experiment 1: The number of aberrant crypt and aberrant crypt foci were significantly lower ($p<0.05$) in the group receiving basal diet + bifidobacteria + FOS compared to the group receiving basal diet + skim milk. No significant difference in the number of bifidobacteria or *C. perfringens* between the two groups. Hyodeoxycholic acid concentration was lower in the group fed basal diet + skim milk w/bifidobacteria + FOS compared to the group fed basal diet + skim milk. No significant differences were observed for other bile acids among the groups. No significant difference in body weight and food intake between the groups was observed. Experiment 2: No significant differences among the groups were observed for either aberrant crypt or aberrant crypt foci counts. The rats receiving basal diet + skim milk + FOS had a significantly higher ($p<0.05$) number of bifidobacteria compared to the groups receiving basal diet + saline or basal diet + skim milk. The *C. perfringens* population was lower in rats fed basal diet + skim milk + FOS compared to the rats fed basal diet + saline. Deoxycholic concentration was higher ($p<0.05$) in the group fed basal diet + skim milk + FOS compared to the groups without added FOS. No significant difference in body weight and food intake between the groups was observed. The addition of FOS to the basal diet decreased the cecal pH compared to the basal diet. Experiment 3:	The authors concluded that, bifidobacteria and *L. acidophilus* have variable effects on aberrant crypt formation in the colon and on *C. perfringens* population. They attributed the inconsistency to the differences in the age of the rats at the time of DMH injection as well as the species or strains administered.	Gallaher et al. (1996)

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TABLE 25

Effects of FOS Supplementation on Colonic Epithelial Cells in Animals

Species	Experimental Design	Results	Conclusion	Reference
	Experiment 3: Rats were fed one of the following: basal diet + skim milk, basal diet + skim milk w/bifidobacteria, basal diet + skim milk w/bifidobacteria + 2% FOS, or basal diet + skim milk + 2% FOS. The animals were sacrificed on day 28 through 31. Experiment 4: Rats were fed one of the following: basal diet + skim milk, basal diet skim milk + 2% FOS, basal diet + skim milk w/bifidobacteria + 2% FOS, basal diet + skim milk w/10^6 lactobacillus + 2% FOS, or basal diet + skim milk w/bifidobacteria and lactobacillus + 2% FOS. The animals were sacrificed from day 44 through 66.	The group fed basal diet + bifidobacteria + FOS had significantly fewer ($p<0.05$) aberrant crypts compared to the group receiving basal diet + bifidobacteria. Aberrant crypt foci counts were significantly lower ($p<0.05$) in the group receiving basal diet + bifidobacteria + FOS compared to the groups receiving basal diet + skim milk or basal diet + bifidobacteria. The bifidobacteria count was significantly higher ($p<0.05$) in rats fed basal diet + skim milk + FOS compared to the group receiving basal diet + skim milk. The *C. perfringens* population was lower in the group receiving basal diet + skim milk + FOS compared to the groups receiving basal diet + saline or basal diet + bifidobacteria. The body weight was higher ($p<0.05$) in groups fed basal diet + skim milk + FOS compared to the group fed basal diet + skim milk w/bifidobacteria + FOS. The addition of FOS to the basal diet decreased the cecal pH compared to the basal diet. Food intake decreased in the basal diet + skim milk w/bifidobacteria group compared to the other groups. Experiment 4: No significant differences among the groups were observed for either aberrant crypt or aberrant crypt foci counts. Bifidobacteria and *L. acidophilus* numbers were not affected by any treatments, but *C. perfringens* numbers decreased in groups receiving basal diet + skim milk w/bifidobacteria + FOS, basal diet + skim milk w/*L. acidophilus* + FOS, or basal diet + skim milk w/bifidobacteria and *L. acidophilus* + FOS. Food intake and final body weight were not significantly different between the groups.		

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C. Systemic Effects

Similar to other nondigestible but highly fermentable dietary fibers, FOS can exert systemic effects. FOS serves as a substrate for colonic bacteria, which produce SCFA that may influence hepatic and peripheral metabolism of glucose (Luo et al. 1996). FOS has also been shown to modulate the concentration of serum lipids.

1. Glucose Metabolism

FOS ingestion does not increase plasma glucose levels or stimulate the secretion of insulin (Gibson et al. 1994; Hidaka et al. 1990a). The mechanism by which FOS improves glucose metabolism in diabetic patients is not clear; however, the lack of glycemic response may be due to the indigestibility of FOS or the production of SCFA during fermentation.

FOS has not been reported to produce adverse effects on plasma glucose or insulin concentrations in animal studies. No increases in plasma fructose, glucose, or insulin concentrations were observed in rats exposed to 1 g of FOS via gavage (Yamada et al. 1990). Oku et al. (1984) studied the effects of FOS in the diet on the hydrolysis of sucrose and maltose in rats. Ingestion of 20 percent FOS in the diet for 6 weeks had no effect on the hydrolysis of sucrose or maltose.

Several studies have evaluated the effect of FOS consumption on glycemia and insulinemia in humans. These studies are summarized in Table 26. In non-insulin-dependent diabetic subjects, an improvement in glucose tolerance is seen with FOS ingestion. Drevon and Bornet (1992) reported that a solution load of 22.5 g FOS in 200 ml did not change postprandial plasma glucose, fructose and insulin in healthy and diabetic subjects. In subjects with diabetes, hyperlipidemia, or high blood pressure, FOS consumption (1 to 4 g/day FOS for 4 to 8 weeks) decreased fasting blood sugar levels were seen (Mitsuoka et al. 1986). Yamashita et al. (1984) reported that fasting blood glucose levels were reduced by approximately 15 mg/dl in non-insulin-dependent diabetic subjects who consumed 8 g/day FOS for 14 days. No such reduction was observed in control subjects who consumed 8 g/day sucrose. Sano (1986) studied non-insulin-dependent diabetic subjects with complications of diabetic neurosis. Subjects were administered 10 g/day FOS for 4-16 weeks. A nonsignificant decrease in fasting blood sugar levels occurred after 4 weeks, with an average decrease of 16 mg/dl. Long-term consumption of FOS (8 to 16 weeks), however, resulted in significant decreases in fasting blood sugar levels. Sano et al. (1994) administered non-insulin-dependent diabetic subjects and healthy subjects one of the following: 75 g glucose, 22.5 FOS, or 75 g glucose plus 22.5 g FOS. FOS alone had no effect on blood sugar, serum insulin or plasma glucagon levels in healthy or diabetic subjects. FOS plus glucose produced

effects similar to those of glucose alone. The authors concluded that, unlike sugar, FOS does not affect blood sugar, and thus can be given to diabetics.

No effect on plasma fructose, glucose or insulin was reported in healthy subjects administered 25 g FOS or sucrose in water (Yamada et al. 1990). In contrast, sucrose produced a rapid rise in plasma fructose, glucose, and insulin within 30 minutes of ingestion. In a randomized double-blind crossover study, healthy males were administered 20 g FOS or sucrose for 4 weeks with a 2-week washout period between treatments (Luo et al. 1996). Neither FOS nor sucrose consumption produced any significant differences in fasting plasma glucose, plasma insulin or insulin binding to erythrocytes. FOS did not significantly affect exogenous glucose infusion rate and total body glucose disposal; however, basal hepatic glucose production was significantly reduced compared to that of sucrose. As measured by hyperinsulinemic clamp, insulin suppression of hepatic glucose production and insulin stimulation of glucose uptake were not significantly different for FOS or sucrose.

Consistent with the results seen with FOS, Rumessen et al. (1990) found that other β 2-1 fructans have a similar effect on blood glucose, insulin, and C-peptide responses in healthy subjects. Subjects were administered one of the following. 5, 10, or 20 g fructans isolated from Jerusalem artichoke, or 20 g fructose in tap water, or 50 g wheat starch in bread with or without 10 g fructans. There was a much larger breath hydrogen response to fructan ingestion than fructose, indicating malabsorption with subsequent fermentation by the colonic microflora. Fructans produced a lower glycemic response and a lower measured blood insulin peak. There were no detectable differences in blood glucose, insulin or C-peptide response in subjects ingesting 10 versus 20 g of fructans.

TABLE 26

Clinical Studies on the Effects of FOS on Glucose Metabolism

Reference	Study Design	Effect
Luo et al. 1996	In a randomized double-blind crossover study, 12 healthy male subjects (19-32 yrs of age) received either 20 g FOS (Actilight 950) or sucrose for 4 weeks with a 2-week washout period. Subjects followed a low-fiber diet during the 4-week study period.	There were no significant changes in fasting plasma glucose or insulin, or in insulin binding to erythrocytes during the FOS and sucrose administration periods. FOS did not significantly affect the exogenous glucose infusion rate or total body glucose disposal, but significantly lowered the basal hepatic glucose production compared to sucrose consumption. Insulin suppression of hepatic glucose production and insulin stimulation of glucose uptake were not significantly different between FOS and sucrose dietary periods, as measured by hyperinsulinemic clamp. There were no significant differences in fasting plasma C-peptide, glucagon, or fatty acid concentrations between treatment periods as measured by hyperinsulinemic clamp.
Mitsuoka et al. 1986	30 subjects with hyperlipidemia, diabetes, high blood pressure, or peripheral arterial occulsion randomly administered 1, 2, or 4 g/day FOS for 4-8 weeks.	Declining tendency observed in fasting blood sugar levels.
Sano 1986	13 NIDDM patients (mean age of 53 yrs) with complications of diabetic neurosis were administered 10 g/day FOS for 4-16 weeks.	Fasting blood sugar levels declined an average of 16 mg/dl after 4 weeks of administration; this decline was not statistically significant. In 8 subjects who continued intake of FOS for 6 months, a significant decrease in fasting blood sugar levels was observed after weeks 8 and 16.
Sano et al. 1984	24 NIDDM subjects and 6 healthy controls subjects were administered one of the following: 75 g glucose, 22.5 g FOS, or 75 g glucose + 22.5 g FOS. The NIDDM subjects were divided into 3 groups according to fasting blood sugar: 1) ≤ 120 mg/dl and HbA_1 ≤ 8.5% (8 subjects) 2) 121-160 mg/dl and HbA_1 ≤10% (6 subjects) 3) ≥ 161 mg/dl and HbA_1 ≥ 10% (10 subjects) Blood sugar and serum insulin were measured 30, 60, 90, 120, and 180 minutes after ingestion of assigned treatment.	In healthy and diabetic subjects, FOS alone had no effect on blood sugar, serum insulin or plasma glucagon. Glucose and glucose + FOS resulted in increased blood sugar levels that peaked at 60 minutes followed by a decline toward baseline values, and in early reductions in plasma glucagon. In healthy and diabetic subjects, the effects on blood sugar, serum insulin and plasma glucagon levels were not significantly different for glucose + FOS compared to glucose alone.

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TABLE 26 Clinical Studies on the Effects of FOS on Glucose Metabolism		
Reference	**Study Design**	**Effect**
Yamada et al. 1990 (abstract)	6 human male subjects (37-58 yrs of age) were given 25 g FOS or sucrose in water after an overnight fast. Blood was collected before and 15, 30, 60, and 120 minutes after ingestion of assigned treatment.	FOS had no effect on plasma fructose, glucose, or insulin. In constrast, there was a rapid increase in plasma fructose, glucose, and insulin at 30 minutes after sucrose ingestion.
Yamashita et al. 1984	NIDDM subjects were fed 8 g/day FOS (18 subjects; mean age of 48.5 yrs) or 5 g/day sucrose (10 subjects; mean age of 50.3 yrs) in food for 14 days.	FOS reduced fasting blood glucose levels by approximately 15 mg/dl. No changes in fasting blood glucose levels were seen in the control group.

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2. Effect on Blood Lipid Levels

Similar to other soluble dietary fibers, FOS has been reported to have hypolipidemic and hypocholesterolemic effects in animals. Studies in humans have not been conclusive. The mechanisms of the effects on serum lipids has not yet been fully elucidated; however, several theories have been offered, including: (1) a decrease in the daily energy intake due to the lower caloric value of FOS compared to sugar; (2) a change in the intestinal flora, possibly influencing the absorption of lipids and their metabolism; (3) binding of FOS to lipids preventing absorption at the intestinal mucosal surface; or (4) a metabolic effect that is the result of a decrease in the concentration and hepatic release of VLDL-triacylglycerol due to a decrease in fatty acid synthase activity reducing *de novo* fatty acid synthesis in the liver (Modler et al. 1990; Hata et al. 1983, Kok et al. 1996a,b, 1998). The hypocholesterolemic effect may be due to: (1) reduced cholesterol absorption; (2) increased neutral steroid and bile acid excretion; or (3) increased synthesis of fermentation by-products which may decrease cholesterol synthesis in the liver (Davidson et al. 1998; Vanhoof and DeSchrijver 1995; Yamashita et al. 1984).

Tokunaga et al. (1986) fed male rats a diet containing 10 or 20 percent FOS for 6 to 8 weeks. Both the 10 and 20 percent FOS diets produced a significant increase in triacylglycerol levels. Animals in the 20 percent FOS diet group also had significantly decreased body weight gain compared to control animals that received no FOS supplementation.

Several investigators have examined the effects of inulin and oligofructose on blood lipid levels in animals. Inulin and oligofructose, at concentrations of 5 to 25 percent in the diet, have been reported to produce significant reductions in total cholesterol, triglycerides, and phospholipids in the serum and the liver, significantly increase the ratio of HDL-cholesterol to LDL-cholesterol, and the ratio of serum apolipoprotein B to apolipoprotein A in rats (Bhattathirty 1971; Delzenne et al. 1993, Fiordaliso et al. 1995; Kim and Shin 1998). In addition, Kok and colleagues (Kok et al. 1996a,b, 1998) reported that oligofructose decreased serum and liver triacylglycerol levels in rats. At least part of the triglyceride lowering action is due to a reduction of *de novo* fatty acid synthesis in the liver, through inhibition of fatty acid synthase activity.

Studies in human subjects have reported inconsistent findings with respect to reductions in blood cholesterol and lipids from ingestion of FOS. One of the difficulties with interpretation of results from these trials has been lack of consistency with respect to many confounding factors such as diet, hypercholesterolemic status, study duration, number of subjects and study design. These studies are summarized in Table 27.

Hata et al. (1983) randomly assigned subjects with hyperlipidemia to receive 22.5 g/day FOS or sucrose for 4 weeks. FOS significantly decreased total cholesterol and produced a nonsignificant decrease in triglycerides, HDL cholesterol, and free fatty acids. In contrast, sucrose significantly raised total cholesterol, triglycerides and free fatty acids, while lowering HDL cholesterol. Hidaka et al. (1991) studied the effect of FOS on hyperlipidemic and hyperlipoproteinemic subjects. Subjects with hyperlipidemia who received 8 g/day FOS for 5 weeks had significant reductions in total cholesterol compared to subjects administered sucrose. There was also an insignificant trend of reduced serum triglycerides and free fatty acids with FOS. FOS produced no significant changes in HDL cholesterol. In subjects with hyperlipoproteinemia, 8 g/day FOS for 1 month produced nonsignificant reductions in total cholesterol, but had no effect on HDL cholesterol or triglyceride levels.

Several studies have been conducted on the effects of FOS on blood lipid levels in non-insulin dependent diabetic subjects. Sano (1986) noted a nonsignificant reduction in serum total cholesterol in diabetics administered 10 g/day FOS for 4 weeks. After 4 weeks of FOS ingestion, total cholesterol was decreased by 14 mg/dl on average. The decline in total cholesterol reached statistical significance after long-term consumption (8-16 weeks). Serum triglyceride levels showed a nonsignificant decrease of 13 mg/dl (on average) after 4 weeks of FOS consumption. Serum HDL-cholesterol was unaffected. No significant changes in serum triglycerides or plasma free fatty acids were observed in diabetic or healthy subjects administered 22.5 g FOS (Sano et al. 1984). Yamashita et al. (1984) administered diabetic subjects 8 g/day FOS or 5/day sucrose for 14 days. Total cholesterol was reduced by 19 mg/dl (on average) in the FOS group ($p<0.01$). The reduction in total cholesterol was more pronounced in hypercholesterolemic subjects compared to normocholesterolemic subjects. LDL cholesterol was reduced on average by 17 mg/dl in the FOS group ($p<0.02$). FOS produced a nonsignificant decrease in serum triglyceride levels, but had no significant effect on HDL cholesterol or free fatty acid levels.

Several studies have evaluated the effect of other β 2-1 fructans (inulin and/or oligofructose) on blood lipid levels. Results from these studies were also not conclusive with regard to a hypocholesterolemic or hypolipidemic effect. In a randomized double-blind crossover study, no significant differences in total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerol levels were observed in 64 women who consumed 14 g inulin/day for 4 weeks (Pedersen et al. 1997).

Davidson et al. (1998) conducted a randomized, double-blind, crossover trial with two six-week treatment periods, separated by a six-week washout in 21 men and women. They also observed no significant changes (slight decreases observed) in serum lipid

levels in subjects who ingested 18 g/day inulin. However, this study was confounded by the fact that consumption of control foods was associated with a significant increase in total cholesterol and LDL-cholesterol. Although participants were instructed to substitute study products for similar foods in their diets, it appeared that participants consumed approximately 25% more fat during both of the treatment periods than during the diet lead-in period, mainly from chocolate and chocolate paste (used as the vehicle for inulin delivery).

In another clinical trial, 17g inulin or oligofructose had no effect on absorption of cholesterol or excretion of cholesterol or bile acids in ileostomy subjects (Ellegard et al. 1997). This study suggests that any hypocholesterolemic effects are not due to changes in lipid excretion from the small bowel.

TABLE 27

Clinical Studies on the Effects of FOS on Blood Lipid Levels

Reference	Study Design	Effect
Hata et al. 1983	46 subjects (20 male, 26 female) with hyperlipidemia were randomized to receive either 22.5 g/day FOS or sucrose for 4 weeks.	FOS significantly decreased total cholesterol ($p < 0.001$), while insignificantly lowering other serum lipid levels (triglycerides, HDL cholesterol, and free fatty acids). Sucrose insignificantly raised total cholesterol, triglycerides, and free fatty acids, while insignificantly lowering HDL cholesterol.
Hidaka et al. 1991	46 subjects (20 male, 26 female) with hyperlipidemia were randomized to receive either 8 g/day FOS or sucrose for 5 weeks. 8 subjects (7 male, 1 female; 37-67 years of age) with type IIa hyperlipoproteinemia were administered 8 g/day FOS for 1 month.	Hyperlipidemic subjects: Total cholesterol was significantly reduced ($p < 0.05$) with the FOS diet relative to the sucrose diet. Tendency toward an insignificant reduction of serum triglycerides and free fatty acids with the FOS diet. No significant changes in HDL cholesterol or uric acids with FOS diet. Hypercholesterolemic (type IIa hyperlipoproteinemia) subjects: Apoprotein E levels significantly increased ($p < 0.05$) with the FOS diet. Total cholesterol and apoprotein B levels were reduced (insignificantly), while the apoprotein A-I level increased (insignificantly) with the FOS diet. HDL cholesterol and triglyceride levels remained unchanged with FOS.
Luo et al. 1996	In a randomized double-blind crossover study, 12 healthy males subjects (19-32 yrs of age) received 20 g FOS (Actilight 950) or sucrose for 4 weeks with a 2-week washout period. Subjects followed a low-fiber diet during the 4-week study period.	There were no significant changes in serum triacylglycerol, total and HDL cholesterol, apolipoprotein A-1 and B, and lipoprotein(a) concentrations during the FOS and sucrose administration periods.
Mitsuoka et al. 1986	30 subjects with hyperlipidemia, diabetes, high blood pressure, or peripheral arterial occulsion were randomly administered 1, 2, or 4 g/day FOS for 4-8 weeks.	In subjects who consumed 1 g/day FOS, total serum cholesterol increased by 4.2%; on average, these subjects had initial cholesterol levels in the normal range. Total cholesterol declined by 2.5% and 3.0% for subjects consuming 2 g/day and 4 g/day FOS, respectively; on average, these subjects had slightly high to high initial cholesterol levels. Triglyceride levels decreased in all groups; levels fell by 10.4%, 9.2%, and 14% in the low-, mid-, and high-dose groups, respectively. FOS consumption, at any dose level, had no effect on HDL cholesterol.

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TABLE 27

Clinical Studies on the Effects of FOS on Blood Lipid Levels

Reference	Study Design	Effect
Sano 1986	13 NIDDM patients (mean age of 53 yrs) with complications of diabetic neurosis were administered 10 g/day FOS for 4-16 weeks.	After 4 weeks of FOS consumption, there was a nonsignificant decrease in serum total cholesterol in 7/13 subjects (54%). On average, serum total cholesterol decreased by approximately 14 mg/dl. A significant decline in serum total cholesterol occurred after 8 and 16 weeks of FOS consumption. After 4 weeks of FOS consumption, serum triglyceride count decreased on average of about 13 mg/dl before and after FOS administration. FOS had no effect on serum HDL cholesterol.
Sano et al. 1984	24 NIDDM subjects and 6 healthy controls subjects were administered one of the following: 75 g glucose, 22.5 g FOS, or 75 g glucose + 22.5 g FOS. Subjects were divided into 3 groups according to fasting blood sugar: 1) ≤ 120 mg/dl and HbA_1 ≤ 8.5% (8 subjects) 2) 121-160 mg/dl and HbA_1 ≤10% (6 subjects) 3) ≥ 161 mg/dl and HbA_1 ≥ 10% (10 subjects) Serum triglycerides and plasma free fatty acids were measured 30, 60, 90, 120, and 180 minutes after ingestion of assigned treatment.	In healthy and diabetic subjects, FOS produced no significant changes in serum triglycerides or plasma free fatty acids.
Takahashi 1986 (abstract)	9 subjects with chronic renal failure administered 6 g/day FOS (15 g/day Neosugar) for 12 months. 9 patients served as the control group.	Total cholesterol and serum triacylglycerol levels decreased in patients consuming FOS. The magnitude of improvement in triacylglycerol levels and initial triacylglycerol levels were positively correlated. The decrease in total cholesterol was due to a decrease in serum LDL cholesterol.
Yamashita et al. 1984	28 non-insulin-dependent diabetic subjects divided into two groups. First group (18 subjects; mean age of 48.5 yrs) given 8.0 g of FOS for 14 days. Second group (10 subjects; mean age of 50.3 yrs) given 5.0 g of sucrose for 14 days.	Total cholesterol significantly decreased (by 19 mg/dL; $p < 0.01$) in subjects given FOS; this reduction was even greater in hyper-cholesterolemic subjects when compared with the normocholesterolemic subjects. There were no significant changes in the control (sucrose) group. LDL cholesterol was significantly reduced (by 17 mg/dL; $p < 0.02$) in subjects given FOS, whereas there were no significant changes in the control group. Average serum triglyceride levels decreased insignificantly in subjects given FOS. There were no significant changes in HDL cholesterol or free fatty acid levels in either group.

F:\CLK\WP\GTC\Table 27 doc

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3. Conclusions

Several studies, primarily in humans, have examined the systemic effects associated with consumption of FOS, oligofructose, and inulin. FOS does not produce any adverse effects on blood glucose or blood insulin levels. Studies in non-insulin dependent diabetic subjects demonstrate that ingestion of FOS can improve glucose tolerance. The effect of FOS on glucose metabolism may be due to its indigestibility and subsequent production of SCFA that may influence hepatic and peripheral metabolism of glucose.

β 2-1 Fructans have been reported to have a hypolipidemic and hypocholesterolemic effect in animals. The mechanism by which FOS exerts an effect on blood cholesterol and blood lipid levels has not been fully elucidated, however, recent research has provided compelling data to support the hypothesis that the triglyceride-lowering action of FOS is due to a reduction of *de novo* fatty acid synthesis in the liver, through inhibition of lipogenic enzymes. Several studies in humans have been conducted to determine if FOS can produce the hypocholesterolemic and hypolipidemic effect seen in animal studies, however, results have been inconclusive. Further research in humans is needed to ascertain the effects of FOS, oligofructose, and inulin on blood lipid levels.

D. Animal Toxicology

The toxicity of FOS has been evaluated in several standard toxicology studies, including a mutagenicity battery, acute and subchronic studies, teratology studies, and carcinogenicity assays.

1. *In Vitro* Studies

The genotoxicity of FOS was tested in microbial reverse mutation assays using *Salmonella typhimurium* (Ames assay) and *E. coli* WP2 *uvr*A, the L5178Y mouse lymphoma $TK^{+/-}$ mammalian cell mutation assay, and an assay for the induction of unscheduled DNA synthesis (UDS) in human epithelioid cells (HeLa S3). At a wide range of dose levels, FOS (with or without metabolic activation) did not produce an increase in mutation frequency in the microbial or mammalian assays, or a dose-related increase in UDS. In all three assays, FOS did not possess genotoxic potential under the conditions of the tests (Clevenger et al. 1988).

2. *In Vivo* Studies

Takeda and Niizato (1982; results presented at the Proceedings of the First Neosugar Research Conference and in Carabin and Flamm 1999) conducted an acute and

two short-term studies of FOS. In the acute study, four-week-old male and female JcL-IcR mice (SPF) and 6-week-old male and 10-week-old female Sprague-Dawley rats were used in four groups of 6 (a total of 24 male and 24 female mice and 24 male and 24 female rats). FOS was administered by gavage. No deaths, gross abnormalities, or changes in body weight were observed in mice given single oral doses of 3, 6, or 9 g/kg FOS. The LD_{50} was determined to be greater than 9 g/kg.

In a 6-week gavage study in Wistar rats, FOS was given in daily doses of 1.5, 3, and 4.5 g/kg, while sucrose and glucose were the control substances. On the second, fourth and sixth weeks, blood samples were obtained from 6 animals in each group. At sacrifice, liver, pancreas, adrenal glands, kidneys, brain, cerebellum, heart, lungs, spleen, pituitary gland, and testes were removed and prepared for histopathology. Study results indicated that there were no abnormalities or deaths during the study. A slight increase in body weight was observed in the 3 and 4.5 g/kg groups compared to controls. No consistent, treatment-related changes were seen in clinical chemistries. Upon dissection, swelling of the appendix was noted in rats receiving FOS, while this was not seen in the control animals. No other gross or histopathologic abnormality was reported. It was concluded that there was no treatment-related toxicity at doses up to 4.5 g/kg for 6 weeks.

In a 6-week feeding study, groups of 18 rats were fed 5 to 10 percent FOS while sucrose, glucose and sorbitol were used as controls. On the second, fourth and sixth weeks, blood samples were obtained from sex of the rats from each group. At the same time intervals, these same animals were necropsied and the liver, pancreas and adrenal glands were removed for further histopathologic examination. After the sixth week, kidneys, brain, cerebellum, heart, lungs, spleen, pituitary gland, and testes were removed at necropsy for histopathology. Results revealed no treatment-related abnormalities or deaths. On the 10th day, rats given FOS exhibited diarrhea; on the 3rd day, the sorbitol group exhibited diarrhea. Sorbitol and FOS groups had lower body weight in the 1st to 5th week, but the growth trends near completion of the study were similar to controls. No treatment-related effects were seen on clinical chemistries with the exception of a reduction in cholesterol. At necropsy on the 2nd and 6th week, swollen appendices were identified. No other treatment-related gross or histopathologic effects were noted.

The toxicity of FOS was investigated in a 90-day feeding study in rats (Meiji Seika Kaisha 1982a). Consumption of FOS at doses up to 30 percent (20.4 g/kg/day) resulted in no significant changes in urinalysis, hematology, or clinical chemistry parameters and no gross or histopathological abnormalities. A dose-related increase in soft stool and diarrhea, increased intestinal weights, and cecal distension were observed in animals fed FOS; however, these findings can be attributed to the nondigestible nature of FOS. No other findings indicative of toxicity were observed.

Developmental and reproductive toxicity of FOS was investigated in rats exposed to dietary concentrations of up to 20 percent (Henquin 1988; Sleet and Brightwell; both cited in Carabin and Flamm 1999). Henquin (1988) reported the results of a developmental toxicity study in female rats fed a diet containing 20 percent FOS during gestational days 1-21 and throughout lactation. A separate group of 17 rats with a copulation plug was fed a control diet. FOS had no effect on the number of pregnancies. Decreased body weight gain in dams and growth delay in suckling pups were observed, but were attributed to the poor caloric content of FOS and reduced food intake and/or diarrhea observed in the dams. The study concluded that a diet containing 20 percent FOS has no significant effects on the course of pregnancy in rats and on the development of the fetuses and newborns.

In a study by Sleet and Brightwell (1990, cited in Carabin and Flamm 1999), pregnant rats were pretreated with 4.75 percent FOS in the diet on days 0 to 6 postcoitum in an attempt to avoid diarrhea observed with earlier studies. Rats were then fed 5, 10, or 20 percent FOS from day 6 to day 15 postcoitum and sacrificed on day 20. No diarrhea was observed in any of the test animals and no deaths were recorded. Body weight was reduced in all FOS groups relative to controls. Body weight change in the treatment groups was decreased in a dose-related manner. The 5 percent group exhibited a lower weight gain relative to that of controls, whereas the 10 percent and 20 percent groups lost weight. From day 11 to the end of the study, the body weight and body weight change were similar among groups, with the exception of the 20 percent group that remained below controls. Treatment with FOS had no effect on number of pups/litter, sex ratio, and viability of both the embryo and fetus. Litter and fetal weights were not reduced; fetal weight of the 20 percent dose group was statistically greater than that of the controls. Structural development of the fetuses was unremarkable. Dietary supplementation with FOS at concentrations up to 20 percent, did not negatively affect the pregnancy outcome or *in utero* development of the rat.

FOS was found to have no long-term toxicity or carcinogenic potential in rats exposed to 8,000, 20,000, or 50,000 ppm FOS in the diet for 104 weeks (Clevenger et al. 1988). Ingestion of FOS produced no significant dose-related effects on body weight, food consumption, survival, growth, hematology, blood chemistry, or organ weights. Numerous nonneoplastic lesions, common in aging rats, were observed in treated and control animals. However, it can be concluded that FOS had no effect on the incidence of these lesions because of sporadic increases and decreases in the incidences of the lesions and the lack of a significant dose-response relationship with regard to incidence and severity. The incidences of rare and spontaneous tumors were similar in all dose groups, with the exception of a dose-related increase in the incidence of pituitary

adenomas in male rats. All incidences of this spontaneous tumor, however, were within the range of historical controls, and no dose-response trend was evident. Therefore, it is unlikely that the occurrence of pituitary adenomas in male rats was treatment-related.

Following a review of the toxicology studies of FOS by the Scientific Committee for Food of the European Union, a 90-day feeding study was conducted on behalf of Beghin Meiji Industries in order to interpret the findings observed in the subchronic toxicity study (Meiji Seika Kaisha 1982a) and carcinogenicity study (Clevenger et al. 1988) of FOS. Various relevant hepatic parameters were measured to determine if FOS has any significant biological or toxicological effects at the molecular level. Because it is known that FOS is fermented by the colonic microflora and that intestinal bacteria play a role in colon carcinogenesis, a microadenoma assay was conducted as part of the 90-day study to determine if FOS has a promoting effect on colon carcinogenesis. Ingestion of FOS at doses of 7.5 and 15 percent did not result in significant increases in the number of colonic aberrant foci in the colon of rats, or significantly affect hepatic free radical scavenger levels (as measured by hepatic content of retinol, retinol palmitate, and α-tocopherol), hepatic drug metabolizing cytochromes P450 enzymes, or cytochrome P450-dependent metabolism of the steroid hormone testosterone (Meiji Seika Kaisha 1982b).

3. Conclusions

The results of toxicological studies indicate that FOS is not mutagenic or teratogenic and does not produce significant adverse effects or carcinogenicity in animals following chronic administration at up to 15 percent (2,664 mg/kg/day) in the diet. The effects that were noted in animal studies (i.e., increased weight of the small intestines, cecum, and colon) are consistent with the gastrointestinal changes caused by ingestion of high levels of any non-digestible material.

F. Human Clinical Trials

1. FOS

Gastrointestinal (GI) tolerance to FOS has been documented in a number of clinical trials. GI intolerance is the result of fermentation of FOS into SCFA and gases by the colonic microflora. In addition, as with any fiber-like substance that is undigested and passes unchanged into the colon, diarrhea may occur at high levels of intake as a result of an osmotic response (Briet et al. 1995). Clinical trails that are used to derive a level of FOS ingestion that is well tolerated by most individuals consuming the sugar are summarized in Table 28. Based on these clinical studies, FOS ingestion appears to be well tolerated at doses up to 15-20 g/day.

Yamashita et al. (1984) studied 28 non-insulin-dependent diabetic subjects (13 males, 15 females) to examine the effects of FOS on blood glucose and lipids. Subjects were divided into a test group, who received 8.0 g/day of FOS for 14 days, and a sucrose control group, who received 5.0 g/day of sucrose for 14 days. The authors reported that GI symptoms such as flatulence and diarrhea were not observed in subjects during the study period.

Hata and Nakajima (1985) studied a group of 85 healthy male and female adult subjects who participated in a number of experiments over a one- to two-week period. Subjects were given single doses of FOS, depending on their body weight, that corresponded to six intake levels for men and women (males: 12, 17, 25, 33, 41, and 50 g/day; females: 10, 14, 20, 26, 34, and 41 g/day). A wash-out period of at least one week was provided between doses. In men and women, no symptoms of diarrhea occurred at the lowest dose levels, 17 g and 14 g, respectively. In men, the first signs of diarrhea were noted at 25 g (9 percent experienced symptoms). At 33 and 41 g, 23 percent of the men experienced diarrhea, and at 50 g, 54 percent of subjects reported diarrhea. In women, diarrhea was first observed at 26 g, at which 14 percent reported symptoms, and the same incidence (14 percent) was observed at an intake of 34 g. At the next highest intake level (41 g), 43 percent of female subjects reported diarrhea. The investigators determined the FOS effective dose (i.e., that dose that produced diarrhea in 50 percent of test subjects) to be 0.78 g/kg in men and 0.84 g/kg in females. These doses correspond to an intake of approximately 47 g based on an average reported body weight for men and women of 58 kg.

Hidaka et al. (1986) conducted several clinical studies on both healthy adults and elderly senile patients to examine effects of FOS on intestinal flora, stool consistency, blood lipids, and production of SCFA. Subjects were given 25 g FOS/day as a single dose, or up to 8 g FOS/day for up to two months. Improvement in moderate constipation was observed at an intake of 8 g FOS/day in the elderly, senile patients with no reports of adverse GI effects or intolerance. In four healthy adults given 8 g FOS per day for 2 months, no GI or other intolerance was reported.

Stone-Dorshow and Levitt (1987) conducted a study to examine whether FOS produces flatulence as measured subjectively, with symptom reports and objectively, by measuring H_2 excretion in exhaled breath. They studied whether these symptoms improve with long-term ingestion of FOS. H_2 was measured following a single 10-g dose of FOS after an overnight fast on day 1 of the study. The fifteen subjects were then randomly assigned to a control group (15 g/day sucrose) or a test group (5 g FOS three times daily, for a total of 15 g FOS/day) for 12 days. On day 14 of the study, all subjects again ingested 10 g FOS following an overnight fast. Subjects recorded GI symptoms in

daily diaries, with severity scores of 0 for absence of the symptom, 1 for mild, 2 for moderate, and 3 for severe. As expected, fermentation following ingestion of FOS was observed on days 1 and 14 of the study. There was no difference in H_2 production between the control group who had ingested sucrose for the prior 12 days and the group who had received FOS for the 12-day period, suggesting that there is no adaptation in the extent of fermentation that occurs in the colon. Although the mean symptom severity score (abdominal pain, eructation, flatulence, and bloating) for subjects in the FOS group was significantly higher than for controls, the symptoms were almost always rated between absent and mild. The authors noted that the one exception was for flatulence, with a mean score of 1.46 (between mild and moderate) for FOS compared to 0.30 in the control group. The differences in mean severity scores for diarrhea, nausea, and headache were not statistically significant in comparing for FOS and sucrose groups.

Yamamoto and Yonekubo (1993) reported results for the 7th Japanese Infant Formula Survey, conducted by Meiji Milk Products Co., Inc., from July through December 1987, to examine the safety and tolerance of FOS consumption in infants. The survey collected information for 20,742 infants up to 4.5 months of age on physical growth, nutritional intake, fecal properties and general health parameters for breast milk, infant formula-fed infants, and infants fed combinations of both breast milk and infant formula. Formula-fed infants represented 20.5 percent of the study population. (Based on FOS concentrations reported in Japanese infant formula and estimates of formula intake in the U.S., the mean and 90th percentile FOS intakes were estimated to be 3.0 and 4.2 grams FOS/day, respectively.) No statistically significant differences between breast-fed infants and those fed formula were observed for growth, mothers' perception of health of the baby, or other adverse effects included in the survey. Consistency of bowel movements in FOS-containing formula-fed infants were reported to be more like breast milk-fed infants, where soft-stools were reported by 77.8 percent of mothers of breast milk-fed babies compared to 70.0 percent of formula-fed babies (with the formula containing FOS).

Briet et al. (1995) conducted an escalating dose study to evaluate the GI tolerance of FOS in 14 healthy volunteers. FOS was consumed in hard candies containing 2.5 g FOS. The control product, also a hard candy, contained 2.5 g sucrose. The study was conducted as a cross-over design to identify the tolerance levels and laxative threshold when FOS was ingested in smaller doses throughout the day rather than in a bolus dose after a period of fasting. The first six-week period consisted of occasional (FOS consumed once per week and sucrose consumed once per week) consumption of the hard candies in doses ranging from 10 g/day of FOS or sucrose, and gradually increasing over six week to a consumption of up to 60 g/day. Intakes were escalated until Grade 3

diarrhea or other symptom occurred, which was defined as the threshold dose, or until the subject declined to consume any more candy. To prevent adaptation, subsequent FOS ingestion was always separated by at least 5 days. Following a 15-day washout, the second six-week period consisted of daily FOS or sucrose intake in gradually increasing doses until Grade 3 diarrhea or other symptom occurred, identified as the threshold dose Subjects maintained daily diaries to record and score symptoms. The 14 volunteers were six females and eight males ages 21 to 37 years. During the first period, the mean threshold and laxative doses of FOS were similar, at 54.3 g and 55.0 g, respectively. Flatulence (statistically significantly increased compared to sucrose) occurred at a mean concentration of 30 g. This was the lowest dose that produced symptoms during the first experimental period, in which subjects consumed FOS on an occasional basis. The threshold dose for borborygmi, bloating, and abdominal cramps was 40 g. During the second period, the threshold dose was defined by diarrhea in nine subjects, severe excess flatulence in one subject, and request to discontinue ingestion of the candy in four subjects. The mean threshold and laxative doses for this second period, 58.0 g and 60.9 g, respectively, were nearly identical with that observed in the first period. No statistically significant difference was observed between the occasional and regular consumption periods (periods one and two) for any of the GI symptoms. Flatulence was associated with the lowest dose, at 25 g.

Molis et al. (1996) reported the GI tolerance of FOS as part of a study to examine the extent of FOS absorption in the small intestine of healthy subjects. Six volunteers, three men and three women ages 20 to 27 years, ingested FOS or a placebo following daily test meals free of foods rich in fructans. Subjects kept symptom diaries, and were asked to record any significant GI symptoms. Following the 6-day placebo period, subjects were given postprandial FOS in gradually increasing doses for an initial 4-day period (in 3 divided doses per day), followed by a full dose of 20.1 g/day for days 4 through 11. The authors reported that none of the subjects experienced symptoms in the placebo and FOS periods.

Garleb et al. (1996) also examined tolerance to FOS as part of a study to determine the effect of FOS on serum chemistry profiles and fecal bifidobacteria levels. Twenty-seven male university students were given one of three dietary formulas (control, 5 g FOS/liter or 10 g FOS/liter) as the sole source of nutrition for a 14-day study period. Intake of the FOS-containing formulas resulted in average FOS intakes of 15 and 31 g/day, respectively. Subjects were questioned daily about the frequency and severity of GI symptoms. One subject in the 10 g/liter FOS group dropped out of the study after one day due to intolerance to the formula. No subjects reported severe GI symptoms, and there was no difference between treatment groups for nausea, cramping, marked

distension, vomiting, and regurgitation. Diarrhea was reported by slightly more subjects in the 10 g/liter FOS group (14 percent), compared to the control and 5 g/liter FOS groups (10 percent each in these two groups). The percent of subjects reporting mild or moderate excess flatus was highest in the first four days of the study in the 10 g/day FOS group, but subsided following adaptation to the diet. In addition to GI tolerance, this study demonstrated that there were no adverse effects on routine clinical chemistry parameters.

Campbell et al. (1997b) studied the metabolic effects of an enteral nutritional formula containing fish oil, FOS, xylooligosaccharides (XOS), gum arabic and antioxidant vitamins (UCNF). This formula was developed for treatment of inflammatory bowel disease, but was tested in this study on 30 healthy adult male subjects. The subjects ranged in age from 21 to 46 years old, with an average weight of 78 kg. Twenty subjects were randomly assigned to the study group to receive the test formula containing the FOS, other fibers, and fish oil for 14 days. The remaining 10 subjects were allocated to the control group to receive a formula containing protein, fat, and nonstructural carbohydrates during the study period. Feeding times simulated a three-meal-per-day schedule. The FOS content of the UNCF formula was 7.95 g/liter, and subjects consumed an average of 18.6 g FOS per day. The intake of XOS, consisting of other fibers that might contribute to GI symptoms, was 9.35 g per day. Subjects recorded and rated GI tolerance using daily questionnaires. Symptoms on the questionnaire included burping, constipation, cramping, diarrhea, distention, distress, flatulence, nausea, reflux, vomiting and other. Four subjects in the UNCF formula treatment group and two subjects in the control formula treatment group were removed from the study; five of these six removals were due to GI intolerance. The 18 subjects who consumed the UNCF formula reported mostly mild to moderate symptoms over the 14-day study period consisting primarily of flatulence, nausea, distention, cramping, and diarrhea. Two subjects in the treatment group reported severe flatulence and burping. In the control group, six subjects reported only mild flatulence. In addition to GI tolerance, the safety of FOS consumption was corroborated by the lack of adverse effect on routine clinical chemistry parameters.

In conclusion, FOS is well tolerated by most subjects at intakes of up to 20 g/day.

2. Clinical Data in Children and Infants

Numerous studies have been performed with adults demonstrating tolerance to FOS. A study in school-age children to assess the possible GI intolerance from use of oligofructose (DP 4-5) was reported by Carabin and Flamm (1999). Two schools participated in the study with a total of 43 healthy boys and girls, ages 10 to 13. A

noncontrolled diet was supplemented with oligofructose (up to 9 grams) mixed in chocolate drinks, apple juice, and gummy bears over the course of a day. Overall, the results demonstrated good tolerance to oligofructose. There were no recorded complaints of flatulence, abnormal stools, or diarrhea. Most complaints were broadly labeled as stomach cramps and were frequently noted in the controls as well as in the test groups. Subjects were also followed with hydrogen breath tests. Results showed that the breath hydrogen concentration is proportional to the amount of oligofructose intake and that liquid products caused higher breath hydrogen concentrations than the solid ones. Overall findings support the conclusion that doses of oligofructose up to 9 grams are well tolerated by children ages 10 to 13.

A nationwide survey of 20,742 infants (up to 4.5 months of age) in Japan provides safety and tolerance information on FOS consumption in infants (Yamamoto and Yunekubo 1993). In this survey, physical growth, nutritional intake, fecal properties and general health parameters for both breast milk- and infant formula-fed infants were reported. No adverse effects were reported for any of the health parameters surveyed. These infants were estimated to consume a mean and 90th percentile intake of 3.0 and 4.2 g/day of FOS, respectively.

TABLE 28

Summary of Studies Used to Derive Human Tolerance to FOS

Study	Description of Subjects	Dose and Duration	Results and Effects
Yamashita et al. (1984)	28 non-insulin-dependent diabetic subjects (13 males, 15 females) were divided into two groups: the FOS (Neosugar®) test group and the sucrose control group.	The FOS group received 8.0 g of FOS as a single dose daily for 14 days. The control group received 5.0 g of sucrose as a single dose daily for 14 days.	No gastrointestinal symptoms or other intolerances were reported in the study.
Hata and Nakajima (1985)	85 healthy subjects (51 men, 34 women) were randomly divided into 6 FOS dose groups (with increasing FOS) and a control sorbitol group.	6 FOS dose groups for men: 12 g, 17 g, 25 g, 33 g, 41 g, and 50 g 6 FOS dose groups for women: 10 g, 14 g, 20 g, 26 g, 34 g, and 41 g Subjects took their appropriate FOS dose as a single dose. The experiment was conducted roughly 3 times with at least a 1 week washout period between the tests.	In men and women, no symptoms of diarrhea occurred at the first two dose levels, 17 g and 14 g. In men, the first signs of diarrhea were noted at 25 g (9% experienced symptoms). At 33-41 g, 23% of the men experienced diarrhea, and at 50 g, 54% had diarrhea. In women, diarrhea was first observed at 26 g (14% experienced symptoms). At 34 g, still only 14% of the women experienced diarrhea, while at 41 g, 43% had it. No significant differences in GI symptoms or stool consistency were observed at FOS doses that were less than the maximum effective dose (dose that resulted in the onset of diarrhea) when compared to the sorbitol group. Furthermore, no significant differences resulted when the form of FOS was changed from being mixed with water to being added to milk or coffee.
Hidaka et al. (1986)	STUDY 1: healthy adult subjects STUDY 2: 23 senile persons aged 50 to 90 years STUDY 3: 21 senile persons aged 54 to 88 years STUDY 4: healthy adult subjects	STUDY 1: Subjects were given 25 g Neosugar as a single dose. STUDY 2: Subjects were given 8 g Neosugar daily for two weeks. STUDY 3: Subjects were given 1, 2, and 4 g Neosugar per day. STUDY 4: Subjects were given 8 g Neosugar every day for 2 months	STUDY 1: No GI or other intolerance reported. STUDY 2: 6 subjects with loose stool before the test, gradually improved and became normal after 8 days of administration. Subjects with moderate constipation showed improvement with Neosugar, as the number of bifidobacteria increased. STUDY 3: No GI or other intolerance reported STUDY 4: No GI or other intolerance reported

TABLE 28

Summary of Studies Used to Derive Human Tolerance to FOS

Study	Description of Subjects	Dose and Duration	Results and Effects
Stone-Dorshow and Levitt (1987)	15 healthy volunteers aged 21-65 years FOS used was Neosugar®	DAY 1: All 15 subjects ingested 10 grams of FOS. DAYS 2-13: 10 subjects ingested 5 g/meal of FOS (15 g/day); 5 control subjects ingested 5 g/meal of sucrose (15 g/day). DAY 14: All 15 subjects ingested 10 g of FOS.	The mean symptom score for subjects ingesting the FOS were significantly higher than for the control group ($p < 0.05$) for gaseous symptoms, such as abdominal discomfort, eructation, flatulence, and bloating. However, with the exception of flatulence, the subjects usually rated all of these symptoms between absent and mild. No significant difference in the severity of diarrhea, nausea, and headaches between the two groups. No significant diminution nor enhancement of any symptom occurred over the course of the study (i.e., no adaptation observed).
Yamamoto and Yonekubo (1993)	Authors' 7th nationwide survey in Japan of 20,742 healthy nursing infants (up to an age of 4.5 months after birth) to assess infants' physical growth, nutritional intake, fecal properties, and other health parameters as a function of their feeding method (breast milk, formula, or mixed nutrition). Basis for study was 1989 change in content of most formulas to slightly reduce protein and add FOS.	FOS consumption could reach maximum of 3.2 grams/day, based on five 200-mL feedings with 0.32 g of FOS/100 mL diluted formula each day. Mean and 90th percentile intakes were 2.7 and 4.5 g FOS/day.	20.5% were formula-fed. No statistically significant differences in body weight or body length observed. Breast milk-fed infants had significantly greater number of bowel movements than did the formula-fed infants, and a significantly higher incidence of soft stool. However, the formula-fed group showed a significantly higher incidence of soft stool in this survey than shown in previous surveys (attributed to addition of FOS). No differences were found among the groups for incidence of watery stool. No significant differences were found among the groups in relation to the color of stool (dark and pale yellow most common for breast-fed; green and yellow-green most common for formula). However, the percentage of pale yellow stool in the formula group for this survey significantly increased as compared to previous surveys. No significant differences were found among the groups in regards to the overall health of the infants.

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TABLE 28

Summary of Studies Used to Derive Human Tolerance to FOS

Study	Description of Subjects	Dose and Duration	Results and Effects
Briet et al. (1995)	14 healthy subjects (6 females, 8 males, 21-37 years old) participated in a study to assess tolerance to FOS consumed occasionally or daily. Study periods were separated by at least 15 days. FOS used was Actilight® as 2.5 g FOS in a hard candy Control subjects consumed sucrose in candy.	PERIOD 1: FOS/sucrose ingested throughout the day occasionally (once a week) PERIOD 2: FOS/sucrose was ingested throughout the day regularly (every day) In the two patterns of consumption, daily FOS/sucrose doses were increased until occurrence of diarrhea or a symptom graded as severe.	PERIOD 1: No threshold dose with sucrose reached (point at which they no longer wanted to ingest a candy); all subjects reached the threshold with FOS (13 experienced diarrhea and 1 severe abdominal pain) at mean FOS threshold and laxative doses of 54.3 g and 55.0 g, respectively. Excess flatus was the first symptom which had a severity significantly higher with FOS than with sucrose at a specific dose (> 30 g); for borborygmi, bloating, and abdominal cramps, this dose was > 40 g; with all symptoms, the percentage of subjects experiencing a given symptom increased with increased dosages of FOS. PERIOD 2: With sucrose, 8 subjects reached the threshold (2 toothache, 6 did not want any more candy); all subjects reached the threshold with FOS (9 diarrhea, 1 severe excess flatus, 4 did not want any more candy). The mean threshold and laxative doses of FOS were 58.0 g and 60.9 g, respectively (not significantly different from period 1). Specific symptom results were not significantly different from those in period 1 (flatus first at > 30 g, then borborygmi and bloating at > 40 g, and finally cramps and diarrhea at > 50 g).
Molis et al. (1996)	6 healthy subjects (3 women, 3 men, 20-27 years old) were studied in random order during a placebo and an FOS period (periods separated by > 1 week wash-out).	11-day FOS period, with first 4 days as adaptation period (subjects started with 5 g/day initially, reaching final intake of 20.1 g/day by day 4 through day 11, given in 3 divided doses).	None of the subjects experienced any gastrointestinal side effects during the FOS or placebo periods.

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TABLE 28

Summary of Studies Used to Derive Human Tolerance to FOS

Study	Description of Subjects	Dose and Duration	Results and Effects
Garleb et al. (1996)	27 healthy male university students were randomly assigned to one of three dietary formula groups (A, B, or C). Diet A: control formula; Diet B: formula containing 5 grams FOS/liter; Diet C: formula containing 10 g FOS/liter.	Subjects consumed their assigned diet three times daily for 14 days as their sole source of nutrition. Mean daily consumption of FOS for the groups was as follows: A, 0 g/day; B, 15 g/day; C, 31 g/day.	Tolerance to all dietary treatments was good. One dropout in group C due to intolerance. No significant difference in nausea, cramping, marked distension, vomiting, and regurgitation between the diet groups. Subjects consuming diet C complained slightly more about diarrhea (14%) than did those in the control or low FOS groups (10% in each group). More complaints of slight distension and flatus in both FOS groups compared to group A. The higher number of complaints was not statistically significant, but the authors noted that the higher number in Group C was clinically significant. They noted that most complaints occurred during the first 4 days on the diet before the subjects had adapted. No complaints of severe discomfort were recorded.
Campbell et al. (1997c)	30 healthy adult males (average age 29, average weight 78 kg) were randomly assigned to either a control formula (10 subjects) or an ulcerative colitis nutritional formula (UCNF) containing fish oil, FOS, XOS, gum arabic, and antioxidant vitamins (20 subjects) to study metabolic effects.	Subjects consumed their assigned diet three times daily for 14 days as their sole source of nutrition. For the UCNF group, the average daily intake of FOS was 18.63 g/day, XOS was 9.35 g/day, and gum arabic was 18.63 g/day. Control subjects received formula containing no FOS, XOS, or gum arabic.	18 subjects in the UCNF (FOS) group reported mild to moderate intolerance symptoms, primarily flatulence, nausea, diarrhea, cramping and diarrhea. 6 control subjects reported primarily mild intolerance symptoms consisting of flatulence. On occasion, 2 UCNF subjects reported severe intolerance symptoms of flatulence and bleeding.

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VII. GRAS SAFETY EVALUATION

An extensive database, consisting of both animal and human exposure and safety data, is available for determination of the safety of FOS for its proposed uses as an ingredient in foods at the levels specified in this document (see Table 1).

Studies on the metabolic fate of FOS indicate that it is virtually unabsorbed and undigested by endogenous enzymes. A very small amount is hydrolyzed by stomach acid and absorbed into the body as fructose and glucose. The majority (about 89%) of undigested FOS passes unchanged into the colon where it is fermented by the microflora into gases and short-chain fatty acids (SCFA).

The physiologic effects of FOS are similar to those of a fiber and include resistance to digestion by endogenous enzymes, fermentation by colonic microflora, shortened gastrointestinal transit time, increased fecal weight, reduction of fecal pH, predictable reduction in caloric value, reduction of plasma cholesterol and triglycerides, and reduction in glucose absorption.

Toxicological studies indicate that FOS is not mutagenic or teratogenic and does not produce significant adverse effects or carcinogenicity in animals following chronic administration at levels up to 15 percent (2,664 mg/kg/day) in the diet. The effects that were noted in animal studies (i.e., increased weight of the small intestines, cecum, and colon) are consistent with gastrointestinal disturbances caused by high levels of any non-digestible material.

The safety of FOS was corroborated by examining its impact on mineral absorption, nitrogen balance and colonic epithelial cells. FOS does not have a detrimental effect on mineral absorption. Indeed, β 2-1 fructans have been shown to have a positive impact on calcium and magnesium absorption and balance. The effect of FOS on nitrogen utilization and excretion indicates that there are no safety concerns, and that there is potential benefit due to the increase in nitrogen excretion in the feces, enhanced urea nitrogen transfer into the large intestine, and enhanced bacterial utilization of ammonia nitrogen. FOS has been shown to produce a trophic effect on colonocytes, and studies in rats have demonstrated that FOS can produce colon tumor inhibition.

The systemic effects produced by FOS include its modulation of both blood glucose and lipid levels. FOS ingestion does not increase plasma glucose or stimulate secretion of insulin. In addition, similar to other soluble dietary fibers, FOS has been shown to have both hypolipidemic and hypocholesterolemic effects. Therefore, there is no concern that FOS ingestion may adversely affect either glycemic control or blood lipid levels.

Historically, humans have been exposed to FOS through consumption of plants; at one time, intakes may have been as high as 9 g/day. Currently, it is estimated that FOS consumption is approximately 114 mg/day in the U.S. and as high as 579 mg/day in the EU.

Human tolerance to consumption of FOS has been established in a number of clinical trials. Study results indicate that FOS, ingested at up to 20 g/day in adults, appears to be safe and well tolerated. In addition, a study reporting the health effects of FOS consumption by infants in Japan reveals that it is well tolerated and has no adverse effects on health at estimated mean and 90th percentile consumptions of 3.0 and 4.2 g FOS/day, respectively. A clinical study of children ages 10 to 13 found that consumption of up to 9 g/day of oligofructose is well tolerated. Therefore, safety (and tolerance) of the β 2-1 fructans has been established, not only in adults, but in infants and children as well. Based on human clinical data, the AIL for FOS ingestion for the general population, excluding infants less than one year of age, is determined to be 20 g/day; the AIL for infants less than one year old is determined to be 4.2 g/day.

FOS is proposed for use as an ingredient in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup, and yogurt at the levels specified in Table 1. Estimates of the intake of FOS from its proposed uses are based on the USDA's 1994-96 Continuing Survey of Food Intakes by Individuals (94-96 CSFII). The EDIs of FOS for the general public, excluding infants less than one year old, associated with its proposed uses in 18 food categories range from 3.9 to 6.2 g/day (mean intake) and from 7.1 to 12.8 g/day (90th percentile intake) for consumers of these food products. The EDIs of FOS for infants less than one year old for the proposed use of FOS in the same 18 food categories are 1.6 and 3.1 g/day for the mean and 90th percentile consumers of these food products, respectively.

An EDI to AIL comparison reveals that the EDIs for FOS are all below the corresponding AILs of 20 g/day (for the general population) and 4.2 g/day (for infants less than one year old). Thus, it can be concluded, based on scientific procedures, and supplemented with evidence of safety from common use in food prior to January 1, 1958, that the proposed use of FOS, as a bulking or bifidogenic agent for the food types and at the use levels specified herein, is safe.

Under 21 CFR §170.30, general recognition of this safety requires that the scientific data and information upon which the determination of safety rests must ordinarily be published, and may be corroborated by unpublished studies and other data and information. The scientific data and information on which the safety determination of FOS is based are available in the published literature or are otherwise publicly available to experts qualified by training and experience to evaluate the safety of food and food additives. Thus, the data reviewed meet the common knowledge element required for all GRAS determinations.

Because FOS is GRAS for its intended use, it is excluded from the definition of a food additive, and thus may be marketed for this use without the need to promulgate a specific food additive regulation under 21 CFR.

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APPENDIX A
Mycotoxin Screen Certificate of Analysis

Japan Food Research Laboratories

Japan Food Research Laboratories

AUTHORIZED BY THE JAPANESE GOVERNMENT

HEAD OFFICE : 52-1, MOTOYOYOGI-CHO, SHIBUYA-KU, TOKYO
OSAKA BRANCH : 3-1, TOYOTSU-CHO, SUITA-SHI, OSAKA
NAGOYA BRANCH : 5-13, 4-CHOME, OSU, NAKA-KU, NAGOYA
KYUSHU BRANCH : 1-12, SHIMOGOFUKU-MACHI, HAKATA-KU, FUKUOKA-SHI
TAMA BRANCH : 11-10, 6-CHOME, NAGAYAMA, TAMA-SHI, TOKYO

ANALYSIS CERTIFICATE

No. 199101329-002
October 28, 1999

Requested by: MEIJI SEIKA KAISHA, LTD.
4-16 Kyobashi 2-chome, Chuo-ku
Tokyo 104-0031
Japan

Sample: Furanosidase

Description: Lot No. PFF (M) -10100

Received: October 13, 1999

This is to certify that the following result(s) have been obtained according to our analysis on the above-mentioned sample(s) submitted by the client.

RESULTS

Moisture [Vacuum oven method]:	2.1 g/100 g
Protein*1:	39.5 g/100 g
Fat [Method with Soxhlet extractor]:	7.4 g/100 g
Fiber:	12.6 g/100 g
Ash:	4.3 g/100 g
Non-fibrous carbohydrates*2:	34.1 g/100 g
Arsenic (as As_2O_3):	0.3 ppm
Heavy metals (as Pb):	7.0 ppm
Lead:	not detected (LD*3 0.05 ppm)
Cadmium:	0.02 ppm
Mercury:	not detected (LD 0.01 ppm)
Aflatoxin B_1:	not detected (LD 5 ppb)
Aflatoxin B_2:	not detected (LD 5 ppb)
Aflatoxin G_1:	not detected (LD 5 ppb)
Aflatoxin G_2:	not detected (LD 5 ppb)

- continued -

Sterigmatocystin:	not detected (LD 0.05 ppm)
Antibiotic activity*4:	negative
Aerobic plate count:	1.4×10^7/g
Coliform bacteria:	positive/0.1 g
Escherichia coli:	negative/2.22 g
Coagulase positive *Staphylococci* :	negative/0.01 g
Salmonella :	negative/25 g

*1 N×6.25

*2 100−(Moisture+Protein+Fat+Fiber+Ash)

*3 LD: Minimum limit of determination

*4 By Annex A. Appendix (Determination of Antibiotic Activity) in the Joint FAO/WHO Expert Committee on Food Additives [Specifications for identity and purity of certain food additives (FAO Food and Nutrition Paper 49)].

Noriko Imaizumi
Principal Investigator
Japan Food Research Laboratories

End Submission

000131

GTC Nutrition Company

AM

14252 W. 44th Ave., Suite F
Golden, CO 80403

2000 SEP -1 P 12: 41

2000 SEP -1 P 12: 41

August 31, 2000

Dr. Paulette Gaynor
Office of Premarket Approval, HFS-215
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Dear Dr. Gaynor:

We wish to notify you that GTC Nutrition Company has determined that short chain fructooligosaccharides (scFOS) is "generally recognized as safe" ("GRAS") for use as an ingredient in acidophilus milk, baby foods, bars, beverages, biscuits, cakes, confectionery, cookies, crackers, flavored/unflavored milk, hard candy, ice cream, jams and jellies, muffins, ready-to-eat cereals, sorbet and sherbet, soup and yogurt, at the levels specified in the attached GRAS notification document. Accordingly, scFOS is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act.

GTC Nutrition Company is hereby submitting the attached document to make its GRAS determination. As directed by the agency, the information is formatted in accordance with proposal 21CFR 170.36(c) (62 Fed. Reg. 18937 (April 17, 1997)).

In the previous letter dated May 1, 2000 the agent for this GRAS determination was identified as ENVIRON Corporation, please direct all inquiries to GTC Nutrition Company who will act as their own agent. The data and information that serve as the basis for this GRAS notification will be sent to FDA upon request or are available for the FDA's review and copying at reasonable times at the office of Paula Karabell, Technical Director, GTC Nutrition Company, 14252 West 44th Ave Suite F, Golden, CO. 80403, telephone: (303) 216-2489, ext 22, facsimile: (303) 216-2477.

In the discussion with Dr. Linda Kahl and Dr. Paulette Gaynor on August 29, 2000, it was requested the lead analysis be more defined to determine a more concise measurement. Samples of the short chain fructooligosaccharides have been sent for analysis and the data will be sent to FDA when received.

Also discussed was Section I. Gras Exemption Claim, pages 5 and 13. Instead of re-working these pages, Section I was re-typed, same pages as before to make it easier to insert into the whole document. (Please find attached)

Sincerely,

Paula Karabell
Technical Director

I. GRAS EXEMPTION CLAIM

A. Name and Address of Notifier

GTC Nutrition Company
14252 W. 44th Ave, Suite F
Golden, CO 80403

Contact: Ms. Paula Karabell
Telephone: (303) 216-2489
Facsimile: (303) 216-2477

2000 SEP -1 P 12:41

B. Name of GRAS Substance

The substance that is the subject of this Generally Recognized As Safe (GRAS) determination is short chain fructooligosaccharide (scFOS or FOS), also referred to as Neosugar. FOS is marketed and sold under the trade names NutraFlora®, Meioligo®, and Actilight®.

C. Intended Use

FOS is a carbohydrate that is not hydrolyzed in the human intestinal tract and thus is reduced in caloric value compared to other digestible carbohydrates. It can be used in food products as a bulking agent, in place of sugar, with a resultant reduction in the energy content of the food. In addition, since it passes unchanged into the colon, it serves as a substrate for colonic bacteria. One beneficial effect that results is a selective fermentation of the FOS by bifidobacteria. The enhancement of bifidobacterial growth may alter the colonic flora in favor of a normal healthy composition.

GTC Nutrition Company intends to add FOS to acidophilus milk, nutritional bars, baby food, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt at the levels specified in Table 1.

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TABLE 1
Proposed Food Use Categories and Use Levels

Proposed Food Use Categories	Foods included in Category	Proposed FOS Use Level	% Weight of FOS in food	Serving Size
Acidophilus Milk Milk with culture	Acidophilus-milk (1% fat and 2% fat milk)	5.0 g/serving	2.0%	240 ml [a,g] (approx 244 g)
Bars Nutritional Bars	Meal replacement bars, granola bars, and high energy sports bars	3.0 g/bar	4.6 - 13.6%	22-65 g [b]
Baby Foods	Baby foods, including cereals (dry and prepared) and ready-to-serve mixed dishes, desserts, fruits, vegetables, meats and yogurt drinks	0.25 mg/serving	0.1-3.6%	7-125 g [a, c]
Beverages Nutritional Beverage	Instant breakfast beverages, diet beverages, meal replacements, nutrient supplements, protein beverage powders, and electrolyte-replacement beverages (includes ready-to-drink and powders)	3.0 g/serving (244 g beverage, various amount of powders make one 244 g serving)	1.2%	240 ml [a, g] (approx 244 g)
Biscuits	Zwieback and teething cookies	0.25 g/serving	3.6%	7 g [a]
Cakes	Cakes, coffee cakes, croissants, breakfast tarts, scones, sweet breads, sweet rolls, pastries, and doughnuts	2.0 g/serving	1.6-3.6%	55-125 g [a, d, g]
Confectionery	Candy bars and confectionery	2.0 g/serving	5.0%	40 g [a]
Cookies	All types of cookies and brownies	1.0 g/serving	2.5-3.3%	30-40 g [a, e]
Crackers	Animal, graham, saltine, snack, cheese, and sandwich-type crackers, crispbread and rice cakes	0.5 g/serving	1.7-3.3%	15-30 g [a, f]

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Proposed Food Use Categories	Foods included in Category	Proposed FOS Use Level	% Weight of FOS in food	Serving Size
Flavored and unflavored milks	Chocolate milk, hot cocoa, malted milk, chocolate milk prepared with chocolate syrup (included ready-to-drink and powders); unflavored milk (includes fluid, reconstituted from dry, and dry), soy milk, milk used in the preparation of beverages and milk shakes	1.0 g/serving (244 g milk; various amounts of powders make one 244 g serving)	0.4%	240 ml [a,g] (Approx 244 g)
Hard candy	Regular and dietetic hard candy	1.0 g/serving	6.7%	15 g [a]
Ice Cream Ice Cream and frozen yogurt	Ice cream - unspecified flavors, chocolate, non-chocolate flavors, frozen yogurt, ice milk, and frozen milk desserts, including novelties such as bars and cones	1.0 g/ serving	1.5%	1/2 cup [a, g] (approx 66 g)
Jams and Jellies Fruit preparations	Jam, jellies, marmalades, fruit butters, and fruit in yogurt	0.25 g/ounce	0.9%	1 tbsp [a] (approx 20 g)
Muffins	All muffin flavors, including cornbread muffins	2.0 g/serving	3.6%	55 g [a]
Ready-to-Eat cereals	Includes all types of ready-to-eat breakfast cereals	2.0 g/serving	3.3-15.4%	13-61 g [b]
Sorbet and Sherbet	Citrus and non-citrus flavors of sorbet and sherbet	1.0 g/serving	1.4%	1/2 cup [a, g] (approc 74 g)
Soup	Prepared soups - canned (ready-to-eat), condensed, dry mix, and unknown preparation	1.0 g/serving (245 g soup; various amounts of condensed soups or powders make one 245 g servings)	0.4%	245 g [a]

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Proposed Food Use Categories	Foods included in Category	Proposed FOS Use Level	% Weight of FOS in food	Serving Size
Yogurt	Plain, flavored and fruited yogurt, and yogurt used in dressings	3.0 g/serving	1.3%	225 g [a]

[a] Serving size based on 21 CFR §101.12 CFR "Reference Amounts Customarily Consumed per Eating Occasion"

[b] Serving size based on actual product data

[c] 7 g = other cereal and grain products; 15 g = cereals, dry, instant; 55 g = eggs/egg yolks; 60 g = strained type foods; 70 g = vegetables for toddlers; 110 g = junior type foods or cereals, prepared; 125 g = fruits for toddlers; 170 g = dinners, soups or stews for toddlers; assumed 60 g for unspecified foods

[d] 55 g = light cakes; 80 g= medium weight cakes; 125 g = dense cakes

[e] 30 g = cookies; 40 g = brownies

[f] 15 g = plain crackers; 30 g = snack crackers

[g] Serving weight cased on data from the USDA Nutrient Database for Standard Reference, Release 13, 1999.

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D. Basis for GRAS Determination

1. Introduction

In this document, we demonstrate that FOS intake, resulting from its intended use, is safe and is also GRAS, under the Federal Food, Drug and Cosmetic Act (FDCA or the Act). To accomplish this, first, the safety of FOS intake under its intended conditions of use is established. Then, this intake level is determined to be GRAS by demonstrating that the safety of FOS for its intended use is generally recognized by GTC Nutrition Company, and experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the FDCA is set forth under 21 CFR§ 170.30. This regulation states that general recognition of safety may be based on the view of GTC Nutrition Company and 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 through: (1) scientific procedure (§ 170.30(b)); or (2) experience based on common use in food, in the case of a substance used in food prior to January 1, 1958 (§170.30(c)). A scientific procedures GRAS determination under §170.30(b) requires the same quantity and quality of scientific evidence as is required to obtain approval of the substance as a food additive. This current GRAS determination employs both scientific procedures and common use in food prior to January 1, 1958.

In addition to requiring scientific evidence of safety, a GRAS determination also requires that his scientific evidence of safety be generally known and accepted. This so-called "common knowledge" element of a GRAS determination consists of the following 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 a consensus exists among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a GRAS determination based on scientific procedures, supplemented with evidence of safety from common used in food prior to January 1, 1958, are applied below in an assessment of whether FOS used in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt is safe and is GRAS.

Once FOS is determined to be GRAS for its intended use, it is permitted to be used for that purpose because it is not (by definition) a food additive, and therefore does not require promulgation of a specific food additive regulation under 21 CFR prior to marketing.

2. Safety of FOS for its Proposed Use

a) Traditional Approach for Evaluation the Safety of Food Additives

The regulatory criterion by which the safety of a food additive is judged that "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" (21 CFR§170.3(i)). This regulation specifies that three factors be considered in determining safety. These are:

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

the cumulative effect of the substance in the diet, taking into account any chemically- or pharmacologically-related substance or substances in such diet;

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.

After consideration of these factors, the FDA usually establishes an acceptable daily intake (ADI) for the additive. The ADA represents the maximum amount of the additive that can be safely consumed by humans on a daily basis for a lifetime. The FDA has specified than an ADI is usually established by application of a safety factor to the highest no-observed-adverse-effect level (NOAEL) identified in the most sensitive animal species studied. Except where evidence is submitted that justifies use of a different safety factor, a safety factor in applying animal experimentation data to man of 100 to 1 is used; that is, tolerance for the use of a human food ingredient will not exceed $1/100^{th}$ of the maximum amount demonstrated to be without harm to experimental animals.

b) Approach for Evaluating the Safety of Macroingredients

Macroingredients are a class of food additives that are intended to be replacements for conventional macroingredients such as fats, proteins and carbohydrates and thus are intended for use at relatively high levels in food (USFDA 1993). Macroingredients have the

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potential to be consumed in gram quantities per day (whereas traditional food additives are generally consumed at levels below 1 gram/day) because they are intended to replace energy-dense traditional food constituents that form a substantial part of the diet (Borzelleca 1992). The current trend is toward increased consumption of macroingredients because of a public health emphasis on reducing both total caloric intake and dietary fat intake, and because of consumer demand for more and varied products with fewer calories and less fat (Rulis 1990; FDA 1991). Thus, both the food industry and the FDA have intensified their efforts to develop a more appropriate approach to assess the safety of macroingredients.

One of the key features that differentiates the safety assessment approach for macroingredients from that for traditional food additives is the potentially large human exposure to macroingredients. If macroingredients are tested in animal studies at substantial multiples of the anticipated human exposure, similar to what is done for the testing of traditional food additives, spurious results would occur. These results would not reflect toxicological effects due to the macronutrient, but rather perturbations resulting from physiological or nutritional imbalances created by the large doses of the macronutrient administered (Borzelleca 1992; IFBC 1990; USFDA 1993; Verschuren 1988; WHO 1987; Feron et al. 1990). Therefore, macroingredients require an alternative safety assessment approach that utilizes information about the physical, chemical, and biological properties of the macronutrient.

These biological properties typically include the digestibility, absorption, and metabolic fate of the macronutrient. This information allows for the prediction of potential target organs and toxic effects (Munro 1994). Initial short-term toxicity testing with rodents can be used to identify any unanticipated systemic toxicity. However, testing should then proceed to more appropriate models, including humans, to confirm safety and to assess specific endpoints such as palatability, nutritional status and tolerance (Borzelleca 1992; Munro 1994). Exposures to the macronutrient in human trials should be at multiples of the estimated daily intake (EDI). Human tolerance to the macronutrient can then be used as the basis for identifying an ADI (Borzelleca 1992). Characteristics, such as the similarity of a macronutrient to a traditional food, metabolic conversion into normal body constituents, and lack of overt toxicity, allow the use of human studies, supported by other data establishing safety, to be used in the development of an ADI or acceptable intake level (AIL) for a macroingredient.

Therefore, the traditional ADI/safety factor approach outlined in the previous section is generally not appropriate for use with food additives that will be consumed in relatively large quantities, such as FOS. For these macroingredients an alternative approach is generally needed to establish an AIL. The FDA's Red Book II (USFDA 1993) states:

> The common characteristic of macro-additives is that they will be consumed in large quantities compared to conventional food additives and, as a consequence, they will present testing problems that require "customized" approaches. For example, it may not be feasible to calculate safety factors in the conventional way, that is, as a fraction of the highest oral dose that has no adverse effects in animals. Other means of providing margins of safety for macro-additives will have to be used; these may include information derived from metabolic, pharmacokinetics, and human clinical studies.

Therefore, a safe intake level for FOS, used as a macroingredient, will be derived by employing an AIL approach, rather than the traditional ADI/safety factor approach. This AIL approach for FOS is described in greater detail in the following section.

c) **Acceptable Intake Level (AIL) for FOS**

An extensive database, consisting of both animal and human exposure and safety data, is available for determination of the safety of FOS for its proposed uses as an ingredient in foods at the levels specified in this document (see Table 1). Studies on the metabolic fate of FOS indicate that it is virtually unabsorbed and undigested by endogenous enzymes. A very small amount is hydrolyzed by stomach acid and absorbed into the body as fructose and glucose. Approximately 0.12% of ingested FOS is recovered unchanged in the urine. About 89% of undigested FOS passes unchanged into the colon where it is fermented by the microflora into gases and short-chain fatty acids (SCFA). Studies have demonstrated that the effect of FOS on the colonic microflora is not a health concern; indeed, it has been show to help produce a normal, healthy microflora composition with improved bifidobacteria and lactobacilli numbers.

The physiologic effects of FOS are similar to those of a fiber and include resistance to digestion by endogenous enzymes, fermentation by colonic microflora, shortened gastrointestinal transit

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time, increased fecal weight, reduction of fecal pH, predictable reduction in caloric value, reduction of plasma cholesterol and triglycerides, and reduction in glucose absorption.

The safety of FOS was corroborated by examining its impact on mineral absorption, nitrogen balance and colonic epithelial cells. FOS does not have a detrimental effect on mineral absorption. Indeed, β 2-1 fructans have been shown to have a positive impact on calcium and magnesium absorption and balance. The effect of FOS on nitrogen utilization and excretion indicates that there are no safety concerns, and that there is potential benefit due to the increase in nitrogen excretion in the feces, enhanced urea nitrogen transfer into the large intestine, and enhanced bacterial utilization of ammonia nitrogen. Furthermore, FOS has been shown to produce a trophic effect on coloncytes. Finally, studies in rats have demonstrated that FOS can produce colon tumor inhibition.

The systemic effects produced by FOS include its modulation of both glucose and lipid levels. FOS ingestion does not increase plasma glucose or stimulate secretion of insulin. In addition, similar to other soluble dietary fibers, FOS has been shown to have both hypolipidemic and hypocholesterolemic effects. Therefore, there is no concern that FOS ingestion may adversely affect either glycemic control or blood lipid levels.

Animal toxicology studies of FOS include a mutagenicity battery, acute and subchronic studies, teratology studies and a carcinogenicity bioassay. The results of toxicological studies indicate that FOS is not mutagenic or teratogenic and does not produce significant adverse effects or carcinogenicity in animals following chronic administration at levels up to 15 percent (2,664 mg/kg/day) in the diet. The effects that were noted in animal studies (i.e., increased weight of the small intestines, cecum, and colon) are consistent with gastrointestinal disturbances caused by high levels of any non-digestible material.

Historically, humans have been exposed to FOS through the consumption of plants. Intake of FOS may have been higher in the past due to the use of plants such as Jerusalem artichoke as sustenance crops or as a substitute for the potato. Consumption of FOS may have been as high as 9 g/day at one time. Currently, it is estimated that FOS consumption is approximately 114 mg/day in the U.S. and 579 mg/day in the EU.

Human tolerance to consumption of FOS has been established in a number of clinical trials. These data were used to determine the AIL for FOS. Study results indicate that FOS, ingested at up to 20 g/day in adults, appears to be safe and well tolerated. In addition, a study reporting the health effects of FOS consumption by infants in

Japan reveals that is it well tolerated and has no adverse effects on health at estimated mean and 90th percentile consumptions of 3.0 and 4.2 g FOS/day, respectively. A clinical study of children ages 10-13 found that consumption of up to 9 g/day of oligofructose is well tolerated. Therefore, safety (and tolerance) of the β2-1 fructans has been established, not only in adults, but in infants and children as well.

In conclusion, the AIL for FOS ingestion for the general population, excluding infants less than one year of age, is determined to be 20 g/day; the AIL for infants less than one year old is 4.2 g/day.

d) Estimated Daily Intake (EDI) of FOS from it Proposed Use

FOS is proposed for use as an ingredient in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt at the levels specified in Table 1.

Estimates of the intake of FOS from its proposed uses are based on the 1994-1996 Continuing Survey of Food Intakes by Individuals (94-96 CSFII), the most recent data available from the national food consumption survey conducted by the U.S. Department of Agriculture (USDA). The 94-96 CSFII was a three-year survey in which data were collected from a stratified area probability sample of individuals residing in households in the U.S. Households represented a cross-section of the population of the 50 states and the District of Columbia.

Using the 94-96 CSFII survey data, the EDIs of FOS for the general public, excluding infants less than one year old, associated with its proposed uses in 18 food categories range from 3.9 to 6.2 g/day (mean intake) and from 7.1 to 12.8 g/day (90th percentile intake) for consumers of these food products. The EDIs of FOS for infants less than one year old for the proposed use of FOS in the same 18 food categories are 1.6 and 3.1 g/day for the mean 90th percentile consumers of these food products, respectively.

The EDIs for both the general public and infants assume that FOS will be used at the proposed use level in all 18 food categories to which FOS is intended to be added. As such, the EDIs derived herein are considered highly conservative estimates of potential FOS intake.

e) **Safety Evaluation Following Chronic and Acute Intake of FOS**

Evaluation of the safety of FOS is accomplished through a review of the extensive database of the safety of FOS, including history of human exposure, animal and human studies, and a comparison of the AIL for FOS to its EDI. It the EDI is less than (or approximates) the AIL, then the proposed use can be considered to be safe.

The AIL is derived from clinical studies of FOS tolerance. Results of these studies indicate that up to 20 g FOS/day in the general population, excluding infants less than one year old, and up to 4.2 g/day in infants less than one year of age, is safe and well tolerated.

The EDI for the general population, excluding infants less than one year old, is estimated to be as high as 6.2 g/day for the mean consumer, and as high as 12.8 g/day for the 90th percentile consumer. Even at the 90th percentile consumption level, the EDI is below the AIL of 20 g/day. The EDIs for infants less than one year old are estimated to be as high as 1.6 g/day for the mean consumer and 3.1 g/day for the 90th percentile consumer. Both of these EDIs are below the AIL for infants of 4.2 g/day.

In conclusion, the EDIs of FOS resulting from consumption of the food products that will contain FOS are all below the corresponding AILs for FOS of 20 g/day (for the general population) and 4.2 g/day (for infants less than one year old). It can thus be concluded, based on scientific procedures, and supplemented with evidence of safety from common used in food prior to January 1, 1958, that the proposed use of FOS, as a bulking or bifidogenic agent for the food types at the use levels specified herein, is safe.

3. General Recognition of the Safety of FOS

This document presents the information used to determine the GRAS status of FOS for use as an ingredient in acidophilus milk, nutritional bars, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored milk, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt. This determination of safety is based upon a critical review of both toxicological and clinical safety studies.

A review of the following information was completed in the evaluation of the GRAS status of FOS: production process, background information (i.e., composition and uses), consumer exposure data, and toxicological studies in both animals and humans. The sources of the reviewed information included pertinent studies and published literature provided by GTC Nutrition Company, or identified in literature searches conducted through online bibliographic retrieval systems, including Medline® and Dialog®.

In the previous section, the proposed use of FOS as an ingredient in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt was determined to be safe through the use of scientific procedures, and supplemented with evidence of safety from common use in food prior to January 1, 1958. Under 21 CFR §170.30, general recognition of this safety requires that the scientific data and information upon which the determination of safety rests must ordinary be published, but may be corroborated by unpublished studies and other data and information. The scientific data and information on which the safety determination of FOS is based are available in the published literature or are otherwise publicly available to experts qualified by training and experience to evaluate the safety of food and food additives. Thus the data reviewed must meet the common knowledge element required for all GRAS determinations.

Determination of the GRAS status of FOS as a food ingredient has been made through the deliberation of GTC Nutrition Company and experts, Dr. Vasilios H. Frankos and Dr. Claire L. Kruger, scientists qualified by training and experience to evaluate the safety of food ingredients. GTC Nutrition Company and these experts have carefully reviewed all the available data on the metabolism, acute and chronic toxicity of FOS in animals, as well as consideration of the human exposure and tolerance to FOS along with human studies evaluating the safety of administered intakes of FOS, and have concluded that:

> There is no evidence in the available information on FOS that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when it is used at levels that are now current or that might reasonably be expected from the proposed application. Therefore, FOS is GRAS for the uses and levels proposed by GTC Nutrition Company.

It is in the opinion of GTC Nutrition Company and these experts that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, FOS, to be used as an ingredient in acidophilus milk, nutritional bars, baby foods, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt is GRAS, at an intake level up to and including 20 g/day for the general population and 4.2 g/day for infants less than one year

old. Because FOS is GRAS for its intended use, it is excluded from the definition of a food additive, and thus may be marketed for use without the need to promulgate a specific food additive regulation under 21 CFR.

E. Availability of Information

The data and information that serve as the basis for this GRAS notification will be sent to the FDA upon request or are available for the FDA's review and copying at reasonable times of the office of Paula Karabell, Technical Director, GTC Nutrition Company, 14252 West 44th Ave Suite F, Golden, CO 80403, telephone: (303) 216-2489, ext 22, facsimile: (303) 216-2477.

GTC Nutrition Company

AM

14252 W. 44th Ave., Suite F
Golden, CO 80403

September 15, 2000

Dr. Paulette Gaynor
FDA/CFSAN
Office of Premarket Approval, HFS-215
FDA/CFSAN
200 C Street, SW
Washington, DC 20204

2000 SEP 19 P 12:01

RE: GRN44 - Short chain fructooligosaccharides

Dear Dr. Gaynor:

As discussed in a phone discussion, August 29, with you and Dr. Linda Kahl, it was requested to have a more concise lead analysis conducted on short chain fructooligosaccharides. Samples were analyzed for lead using EPA Method 6020A. The detection limit using this method is less than 0.18 ppm. The results of the samples are attached as well as a revised copy of Table 2, page 20 reflecting lead analysis.

If there are any questions please contact Paula Karabell, Technical Director, GTC Nutrition Company, telephone: (303) 216-2489 ext 22, facsimile: (303) 216-2477.

Sincerely,

Paula Karabell
Technical Director

2. **Finished Product Specifications**
The manufacturing specifications for FOS are listed in Table 2 below.

TABLE 2
Product Specifications

Item	NutraFlora® Powder	NutraFlora® G Syrup	NutraFlora® Syrup
Moisture % (w/w)	<5.0	<25.0	<25.0
Carbohydrate Composition (% dry basis)			
Glucose + Fructose + Sucrose Fructooligosaccharides GF_2 GF_3 GF_4	<5.0 >95.0 35.0 ± 6.0 50.0 ± 6.0 10.0 ± 4.0	<35.0* and 10 ± 2** >55.0 25.0 ± 3.0 25.0 ±3.0 5.0 ±3.0 * glucose + fructose **sucrose	<5.0 >95.0 42.0 ± 5.0 45 ± 5.0 8.0 ± 3.0
Appearance	White powder	Clear syrup	Clear syrup
Granular Size	40 mesh pass	Not Applicable	Not Applicable
Color (nanometers)	Not Applicable	<0.15	<0.15
Ash (% w/w)	<0.1	<0.1	<0.1
pH (10% solution)	5.0 - 7.0	6.0 ±1.0	6.0 ± 1.0
Total lead	<0.18 ppm	<0.18 ppm	<0.18 ppm
Arsenic (As_20_3)	Not detected at 1 ppm	Not detected at 1 ppm	Not detected at 1 ppm
Foreign taste and odor	Free	Free	Free
Foreign substance	Free	Free	Free
Microbiological results (counts/g)			
Mesophilic bacteria	0 counts/gm	<300 ml	<300 ml
Mold and Yeast	0 counts/gm	<20 ml	<20 ml
Coliforms	Negative	Negative	Negative

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Environmental Chemistry Services, Inc.

Date: *12-Sep-00*

CLIENT: GTC Nutrition Company
Lab Order: 0008099
Project:
Lab ID: 0008099-04A

Client Sample ID: ScFOS powder-Lot #938353
Tag Number:
Collection Date: 8/30/2000
Matrix: POWDER

Analyses	Result	Units	RL	DF	Qual	Date Analyzed
LEAD-TOTAL			**SW6020**			Analyst **SS**
Lead	ND	mg/Kg	0 18	1		9/12/2000 10:59 00 AM

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Qualifiers:

ND - Not Detected at the Reporting Limit
J - Analyte detected below RL and above MDL
B - Analyte detected in the associated Method Blank
* - Value exceeds Maximum Contaminant Level
S - Spike Recovery outside accepted recovery limits
R - RPD outside accepted recovery limits
E - Value above quantitation range
RL-Reporting Limit

Environmental Chemistry Services, Inc.

Date: *12-Sep-00*

CLIENT: GTC Nutrition Company
Lab Order: 0008099
Project:
Lab ID: 0008099-01A

Client Sample ID: G Syrup-Lot #279
Tag Number:
Collection Date: 8/30/2000
Matrix: SYRUP

Analyses	Result	Units	RL	DF	Qual	Date Analyzed
LEAD-TOTAL			**SW6020**			Analyst **SS**
Lead	ND	mg/Kg	0.18	1		9/12/2000 10:43:00 AM

Qualifiers:

ND - Not Detected at the Reporting Limit
J - Analyte detected below RL and above MDL
B - Analyte detected in the associated Method Blank
* - Value exceeds Maximum Contaminant Level
S - Spike Recovery outside accepted recovery limits
R - RPD outside accepted recovery limits
E - Value above quantitation range
RL-Reporting Limit

000158

Environmental Chemistry Services, Inc.

Date: *12-Sep-00*

CLIENT: GTC Nutrition Company
Lab Order: 0008099
Project:
Lab ID: 0008099-02A

Client Sample ID: P Syrup-Lot #228
Tag Number:
Collection Date: 8/30/2000
Matrix: SYRUP

Analyses	Result	Units	RL	DF	Qual	Date Analyzed
LEAD-TOTAL			SW6020			Analyst SS
Lead	ND	mg/Kg	0 18	1		9/12/2000 10 49 00 AM

Qualifiers:

ND - Not Detected at the Reporting Limit
J - Analyte detected below RL and above MDL
B - Analyte detected in the associated Method Blank
* - Value exceeds Maximum Contaminant Level
S - Spike Recovery outside accepted recovery limits
R - RPD outside accepted recovery limits
E - Value above quantitation range
RL-Reporting Limit

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GTC NUTRITION

SPECIALTY INGREDIENTS

SU

September 15, 2006

RECD SEP 21 2006

Robert Martin
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRN No. 000044

Dear Mr. Martin,

This letter is in reference to the substance short-chain fructooligosaccharide (scFOS or FOS) as discussed in GRN No. 000044. In GTC Nutrition's original GRAS exemption claim sent to the FDA in 2000, a statement is made in regard to the Acceptable Intake Level (AIL) of the general use of FOS that is not included in the affirmation letter response the FDA returned to GTC Nutrition. GTC Nutrition has determined that FOS is GRAS for general use in foods, beverages and supplements at levels up to 20g/day in the general population excluding infants less than one year of age, and at levels up to 4.2g/day in infants less than one year of age in accordance to current good manufacturing practices. Please refer to Table 1 below for the typical use levels of fructooligosaccharide in foods, beverages and supplements. The data and information that serve as a basis for this notification will be sent to the FDA upon request by utilizing the contact information below.

Table 1 – Typical Use Levels of Fructooligosaccharide

Food Category	Standard Serving Size	Typical Use Level of FOS per serving (g)[1]	Proposed Use Level Recommendation of FOS per serving (%)
Acidophilus Milk	240mL (244g)	1	0.4
Bars	40-70g	1	1.4-2.5
Breakfast Cereals	40-55g	1	1.8-2.5
Beverages and Juices	240mL (244g)	1	0.4
Cakes	55g	1	1.8
Cheese	30-110g	1	0.9-3.3
Cream	15-30g	1	3.3-6.7
Confectionary	40g	1	2.5

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Table 1 – Typical Use Levels of Fructooligosaccharide (continued)

Food Category	Standard Serving Size	Typical Use Level of FOS per serving (g)[1]	Proposed Typical Use Level Recommendation of FOS per serving (%)
Cookies	30g	1	3.3
Crackers	15-30g	1	3.3-6.7
Dessert Toppings and Fillings	30g	1	3.3
Hard Candy	15g	1	6.7
Ice Cream	½ cup (68g)	1	1.5
Infant Foods (0-12 months)	7-60g	0.25	0.4-3.6
Jams and Jellies	1 tbsp (20g)	1	5.0
Meat, Poultry, Fish, Analogs and Substitutes	15-85g	1	1.2-6.7
Milk, flavored and unflavored	240mL (244g)	1	0.4
Milk, evaporated and condensed	30mL (32-39g)	1	2.6-3.1
Muffins and Quick Breads	50-55g	1	1.8-2.0
Sauces, Gravies and Condiments	30-125g	1	0.8-3.3
Snacks	30g	1	3.3
Sorbet and Sherbet	85g	1	1.2
Soup	245g	1	0.4
Toddler Foods (12-24 months)	15-125g	1	0.8-6.7
Yogurt	225g	1	0.4

[1]Based on the consumption of 3 servings per day

*The recommended daily intake of FOS to obtain health benefits is 3g for the general population, excluding infants less than 1 year of age.

**The maximum daily intake of FOS, when used in foods, beverages and supplements, for the general population, one year of age or greater, is 20g and 4.2g for infants less than one year of age in accordance to good manufacturing practices.

Sincerely,

Luke R. Kazmierski
Quality Assurance and Regulatory Affairs Specialist
GTC Nutrition
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Golden, CO 80401
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GTC NUTRITION

SPECIALTY INGREDIENTS

RECD FEB 6 2007

January 26, 2007

Robert Martin
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRN No. 000044

Dear Mr. Martin,

This letter is in reference to the substance short-chain fructooligosaccharide (scFOS or FOS) as discussed in GRN No. 000044. In GTC Nutrition's original GRAS exemption claim sent to the FDA in 2000, a statement is made in regard to the Acceptable Intake Level (AIL) of the general use of FOS that is not included in the affirmation letter response the FDA returned to GTC Nutrition. GTC Nutrition has determined that FOS is GRAS for general use in foods at levels up to 20g/day in the general population excluding infants less than one year of age, and at levels up to 4.2g/day in infants less than one year of age in accordance to current good manufacturing practices. Please refer to Table 1 below for the typical use levels of fructooligosaccharide in foods. The data and information that serve as a basis for this notification will be sent to the FDA upon request by utilizing the contact information below.

Table 1 – Typical Use Levels of Fructooligosaccharide

Food Category	Standard Serving Size	Proposed Typical Use Level Recommendation of FOS per serving (%)
Acidophilus Milk	240mL (244g)	0.4
Bars	40-70g	1.4-2.5
Breakfast Cereals	40-55g	1.8-2.5
Beverages and Juices	240mL (244g)	0.4
Cakes	55g	1.8
Cheese	30-110g	0.9-3.3
Cream	15-30g	3.3-6.7
Confectionary	40g	2.5

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Table 1 – Typical Use Levels of Fructooligosaccharide (continued)

Food Category	Standard Serving Size	Proposed Typical Use Level Recommendation of FOS per serving (%)
Cookies	30g	3.3
Crackers	15-30g	3.3-6.7
Dessert Toppings and Fillings	30g	3.3
Hard Candy	15g	6.7
Ice Cream	½ cup (68g)	1.5
Infant Foods (0-12 months)[1]	7-60g	0.4-3.6
Jams and Jellies	1 tbsp (20g)	5.0
Meat, Poultry, Fish, Analogs and Substitutes	15-85g	1.2-6.7
Milk, flavored and unflavored	240mL (244g)	0.4
Milk, evaporated and condensed	30mL (32-39g)	2.6-3.1
Muffins and Quick Breads	50-55g	1.8-2.0
Sauces, Gravies and Condiments	30-125g	0.8-3.3
Snacks	30g	3.3
Sorbet and Sherbet	85g	1.2
Soup	245g	0.4
Toddler Foods (12-24 months)	15-125g	0.8-6.7
Yogurt	225g	0.4

[1]This category does not include infant formula

*The recommended daily intake of FOS to obtain health benefits is 3g for the general population, excluding infants less than 1 year of age

**The maximum daily intake of FOS, when used in foods, beverages and supplements, for the general population, one year of age or greater, is 20g and 4 2g for infants less than one year of age in accordance to good manufacturing practices

Sincerely,

Luke R. Kazmierski
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